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Direct Inhibitory Effects of 17β-Estradiol on hCG-Stimulated cAMP and Testosterone Responses of Canine Testis

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ABSTRACT

The direct effects of 17β-estradiol (E2) on testicular function were investigated using *in situ* perfusion of canine testis. During perfusion of E2 (10^{-8}—10^{-4}M), we measured the concentrations of cAMP, testosterone (T), androstenedione and 5α-dihydrotestosterone (DHT) in the spermatic venous effluent after hCG (500 IU) injection into the spermatic artery.

The cAMP concentration in the spermatic venous effluent was 122 ± 3.7 (SE) pmoles/ml, increasing after hCG to a peak level of 259.1 ± 33.0 (SE) pmoles/ml at 45 min and decreasing thereafter. T increased gradually from the pretreated level of 12.2 ± 4.4 (SE) mg/ml to 90.9 ± 30.9 (SE) ng/ml at 90 min after hCG injection. Androstenedione production was also stimulated by hCG. Although the pattern of changes of DHT in the venous effluent was similar to that of T, a statistical significant increase was not observed after hCG injection. Continuous infusion with E2 at the concentrations of 10^{-8}—$^{-4}$ M decreased hCG-induced cAMP increase in a dose-dependent fashion. The peak level of cAMP after hCG was shown to be increasingly depleted (50 and 80%) by the increasing doses of 10^{-6} and 10^{-4} M of exogenous E2. The responses of T and androstenedione were also suppressed by E2 and at 10^{-4} M of E2 no increase was observed, although the response of cAMP to hCG remained. These findings indicate that E2 can directly suppress the hCG-stimulated steroidogenesis and cAMP production in *in situ* canine testis.

Direct inhibitory effects of estrogens on androgen secretion have been studied by determining the effects of estrogen treatment on androgen secretion in intact or hypophysectomized animals or on testicular tissue in vitro[10,15,19]. Many experiments have suggested that estrogens inhibit different testicular enzymes essential for androgen production[7,10,13]. Inhibition of steroidogenesis from direct interaction with these enzymes could reduce responsiveness to LH following administration of pharmacological doses of estrogens.

Although it is now apparent that at least in the rat, estrogen treatment decrease testosterone (T) secretion primarily by inhibiting LH secretion, this does not exclude the possibility that estrogens directly affects the testis.

Recently, estrogen receptors have been identified in testicular tissue[8,9]. The presence of estrogen receptor could provide a mechanism by which estrogens could directly affect the testis and would further suggest that estrogen play a physiological role in controlling testicular function.

In the present studies, we have attempted to determine the influence of estrogen on androgen secretion in perfused dog testes under controlled conditions of hCG stimulation. In this

experimental model, emphasis was especially placed on determining the effects of estrogen on hCG stimulated cAMP and T secretion.

MATERIALS AND METHODS

In situ perfusion

Eighteen healthy mongrel, mature dogs weighing between 15—22 kg were anesthetized with iv sodium pentobarbitol, laparotomized and the left spermatic vein and artery exposed. The left spermatic artery was dissected and connected to the outlet channel of a withdrawal infusion pump by a polyethylene tube containing T-piece.

The perfusion medium used was Krebs-Ringer bicarbonate buffer containing 0.1% bovine serum albumin and 0.1% glucose (KRBG-BSA) with or without 17β-estradiol (E2). Three different doses of E2 were added in 1 ml ethanol and diluted with KRBG-BSA giving a final ethanol concentration of 1 μl/ml and E2 concentrations of 10^{-8}, 10^{-6} and 10^{-4} M. Animals were placed into four experimental groups consisting of four dogs each and the left testis from each dog was perfused in situ with KRBG-BSA bubbled with an atmosphere of 95% O_2—5% CO_2 at a constant flow of 3.6 ml/min through the spermatic artery. After 30 min of perfusion with or without E2, hCG (1,000 IU) in 500 μl physiological saline was injected via a T-piece into the spermatic artery. Animals, served as control, were given 500 μl physiological saline into the spermatic artery instead of hCG.

Samples from the spermatic venous effluent were collected into EDTA-coated tubes at every 15 min interval for up to 120 min after the injections. All samples of venous effluent were prepared by centrifugation at 2,000 rpm at 4°C for 20 min and stored at −20°C until analysis.

Steroids radioimmunoassays

T, DHT, androstenedione were extracted from 200 μl of perfusate twice with 5 ml of ethylether by shaking for 1 min. The combined ethylether phase was evaporated to dryness under a stream of air. The residue was spotted onto silica gel thin layer chromatographic plates for separation of T, DHT and androstenedione. The plates were developed by the solvent system of chloroform/ethyl acetate/petroleum ether: 50/45/5 (vol/vol/vol). T, DHT and androstenedione standards were run as markers at each edge and were located with an UV light or Allen's reagent. The appropriate area of silica was scrapped with razor blades and then extracted with methanol. Aliquots of methanol extracts were pipetted into 10 × 75 mm glass tubes for T, DHT and androstenedione determinations. These steroid concentrations were measured by radioimmunoassay methods[12,17]. Samples from each dogs were processed in the same assay. The within assay coefficients of variation were 7.9% for T, 8.1% for DHT and 8.5% for androstenedione. The inter assay coefficients of variation were 8.2% for T, 10.1% for DHT and 9.2% for androstenedione.

cAMP determination

All samples were diluted 1:10 with 0.05 M acetate buffer (pH 6.0) and one hundred microliter of aliquot was used in the cAMP radioimmunoassay[3,16].

Date analysis

The radioimmunoassay dose-response curves were analyzed by an iterative least squares methods for logistic curve fitting developed by Rodbard et al[14]. The unpaired T test was used to determine significant differences between experimental and appropriate control groups.

RESULTS

Administration of hCG induced a marked increase in effluent cAMP concentration; fifteen minutes after the administration, cAMP level was 9-fold higher than the initial level and reached a peak level of 259.1 pmoles/ml at 45 min and decreased gradually thereafter. The injection of saline into the testicular artery caused no change in cAMP concentration for up to 120 min (Fig. 1). The effluent androstenedione concentration fell from 3.56 ± 0.55 (SE) ng/ml to 0.99 ± 0.16 (SE) ng/ml during the initial 30 min perfusion, but a significant increase in androstenedione was evident at 15 min after hCG administration and reached a peak level of 7.22 ± 0.47 (SE) ng/ml 60 min after hCG administration (Fig. 2, upper panel). The T concentration in the spermatic venous blood was 41.1 ± 4.4 (SE) ng/ml and declined to a low steady level of 12.4 ± 4.4 (SE) ng/ml during the initial 30 min perfusion. Saline injection caused no change in T concentration, whereas hCG produced a sig-

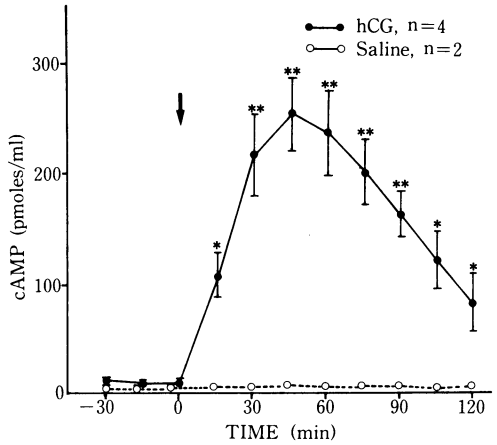

Fig. 1. Concentrations of cAMP in the spermatic venous effluent after a single injection of 500 IU hCG (●—●) or physiological saline (○---○) into the spermatic artery. Each point represents the mean ± SE of determination from the same experiment, and values after physiological saline injection represent the mean of two determinations: Arrow represents the administration of hCG or physiological saline. Asterisks represent the statistical significance vs. the time zero (*,$p<0.05$;**, $p<0.01$). n, Number of animals used.

nificant increase in T which increased gradually and reached a plateau level of 90.9 ± 30.9 (SE) ng/ml at 90 min (Fig. 2, lower panel). As is shown in Fig. 3, a high concentration of DHT was observed from 90 to 120 min after hCG administration.

The inhibitory actions of E2 on hCG stimulated cAMP, androstenedione and T production were evaluated by continuous infusion of E2 at three concentrations (10^{-8}, 10^{-6} and 10^{-4} M). As compared to results with KRBG-BSA alone, no significant inhibition of hCG stimulated cAMP release was found in the presence of 10^{-8} M E2, whereas an inhibition was found with 10^{-6} or 10^{-4} M E2; 10^{-6} M E2 resulted in an approximate 50% fall in venous effluent cAMP at 45 min after hCG and 10^{-4} M E2 led to 80% depletion of the cAMP response to hCG (Fig. 4).

The responses of androstenedione and T to hCG were not affected at 10^{-8} M E2, but 10^{-6} M E2 resulted in a significant decrease in both androstenedione and T production from 45 to 120 min after hCG when compared to controls without E2 and 10^{-4} M E2 almost completely suppressed the stimulation of both androstenedione and T production, although the lowered cAMP response to hCG still remained (Fig. 5).

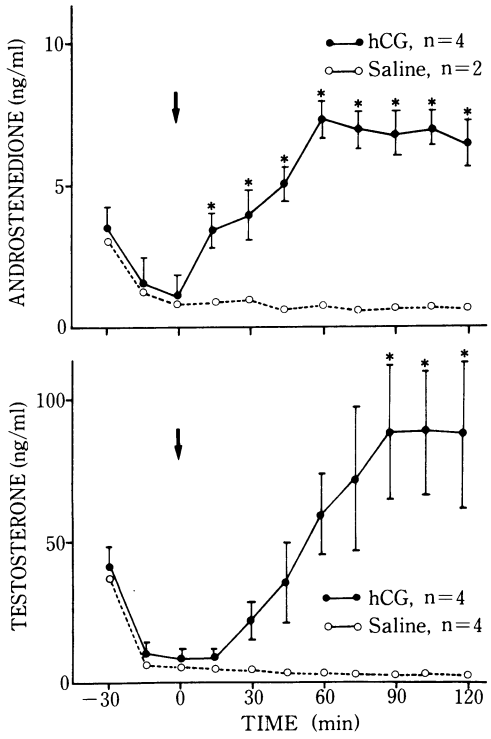

Fig. 2. Concentrations of androstenedione and T in the spermatic venous effluent after a single injection of 500 IU hCG (●—●) or physiological saline (○---○) into the spermatic artery. Each point represents the mean ± SE of determination from the same experiment; and values after physiological saline injection represent the mean of two determinations. Arrows represent the administration of hCG or saline. Asterisks represent the statistical significance vs. the time zero (*,$p<0.05$). n, Number of animals used.

DISCUSSION

We have show that E2 perfusion the response of Leydig cell to hCG judged by cAMP and T production. In the absence of E2, cAMP concentrations increased for 60 min after hCG administration when the level was 20 times more than that before treatment. The response of cAMP by the canine testis to *in situ* hCG administration agrees with *in vitro* experiments by Dufau et al, who showed that gonadotropins stimulated cAMP production in rat testicular fragments[4,10].

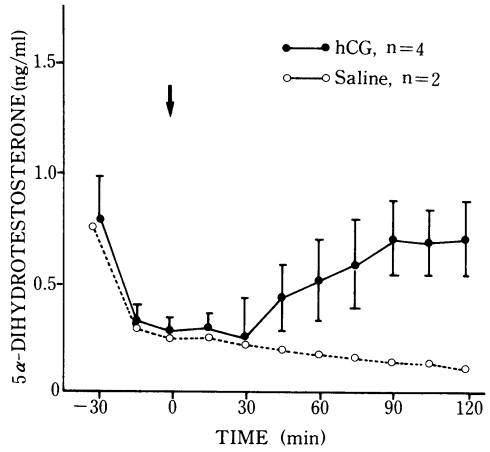

Fig. 3. Concentrations of DHT in the spermatic venous effluent after a single injection of 500 IU hCG (●—●) or physiological saline (○—○) into the spermatic artery. Each point represents the mean ± SE of determination from experiment, and values after physiological saline injection represent the mean of two determinations. Arrow represents the administration of hCG or physiological saline. n, Number of animals used.

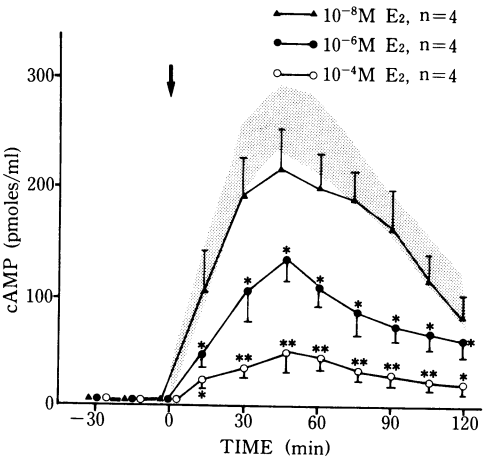

Fig. 4. Effect of E2 on the cAMP release induced by the administration of hCG. E2 was added to the perfusate at concentrations of 10^{-8} (▲—▲), 10^{-6} (●—●) and 10^{-4} (○—○) M. Arrow represents the injection of 500 IU hCG into the spermatic artery. For this and subsequent figures, each point represents the mean ± SE. The shaded area represents the control data ± ISE. Asterisks represent the statistical significance vs. the corresponding time of the appropriate control group (*,$p<0.05$;**, $p<0.01$). n, Number of animals used.

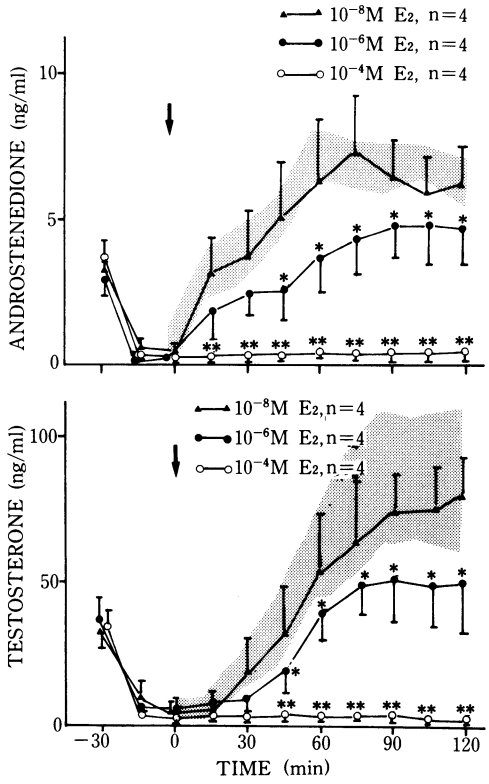

Fig. 5. Effect of E2 on the androstenedione and T release induced by the administration of hCG.

Compared to cAMP, T stimulation with hCG was less and delayed. T rose gradually after hCG and in 90 min the venous effluent T concentration was 8-fold higher than the pretreated level. hCG also stimulated androstenedione production by the canine testis in situ, confirming the previous reports[2,18,20].

hCG enhanced the concentration of DHT in spermatic venous effluent, although the response of DHT was not so evident as that of T. Folman et al showed the production of DHT was not due to a direct secretion by Leydig cells but, rather; to the transformation of T into DHT outside the Leydig cells[5]. These data show the independence of the 5α-reductase activity of testicular tissue from hCG stimulation.

The in situ perfusion study suggests that E2 has direct effects on testicular functions and the testis is a target tissue for this hormone. The lowest dose of E2 (10^{-8} M) showed no inhibition on hCG stimulated T synthesis as reflected

in the venous effluent T concentration in the venous effluent 90 min after hCG in comparison to the testes without E2 and 10^{-4} M of E2 completely blocked the hCG stimulated T response. Our data confirm the report of Moger et al, that very high concentrations of E2 were required to inhibit LH stimulated T production by rat testis *in vitro*[11]. A possible direct action of estrogen may be on some enzymes which catalyze the synthesis of T, since Brinkmann et al showed that E2 inhibited the activities of two microsomal enzymes (17α-hydroxylase and C17-C20 lyase) in the testis of normal rats[1]. In this study we further observed that the estrogen-induced inhibition of steroidogenesis at high doses of E2 (10^{-6} and 10^{-4} M) was accompanied by a decrease in cAMP production. Low dose of E2 (10^{-8} M) was unable to deplete both cAMP and T production. Hsueh et al demonstrated a dose-related direct inhibitory effect of a synthetic estrogen, diethylstilbestrol, on steroidogenic function of Leydig cell in hypophysectomized rats treated with FSH. The decrease in testicular T production was accompanied by decrease in LH/hCG binding capacity of the rat testis, whereas, there was no significant change in *in vitro* cAMP production in testis of estrogen-treated rats[6]. Our data suggests that E2 at high concentration may be acting on steps of cAMP formation as well as steps distal to hCG-induced cAMP formation. The observed decrease in hCG-induced cAMP production at high concentration of E2 could be a consequence of decreased LH/hCG receptor, but could also depend on the suppression of adenylate cyclase activation in Leydig cell. Further experiments are needed to clarify this point.

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