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CHANGES IN PLASMA STEROID LEVELS AFTER SINGLE ADMINISTRATION OF hCG OR LHRH AGONIST ANALOGUE IN DOG AND RAT

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Summary—The present study was designed to investigate the effect of acute administration of gonadotropin on testicular steroid secretion in dog and rat. Animals received a subcutaneous injection of 25 IU/kg of hCG or 1.5 μg/kg of [D-Trp^6, des-Gly-NH$_2^{10}$]LHRH ethylamide (LHRH-A). Testosterone is the predominant steroid measured, in dog plasma, under basal conditions. After LHRH-A injection, testosterone levels are not significantly changed while dehydroepiandrosterone and androst-5-ene-3β,17β-diol (Δ^5-steroids) levels are stimulated by almost 20-fold ($P < 0.01$). When dogs were injected with hCG, we also observed a marked stimulation of dehydroepiandrosterone levels (20-fold; $P < 0.01$) accompanied by a small increase of plasma testosterone concentration (2-fold, $P < 0.01$). In rats injected with either hCG or the LHRH analogue, an increment of plasma testosterone (7-fold, $P < 0.01$) is detected in the first hour while plasma dehydroepiandrosterone levels are slightly stimulated. Moreover, in rats injected with hCG, low plasma steroid levels are present between 4–12 h after injection due to testicular desensitization. This marked decrease is then followed by a second peak of steroid secretion 24 h later. Acute testicular steroidogenic responsiveness to hCG on the dog is, however, different: after stimulation, the levels of plasma dehydroepiandrosterone are maintained at a plateau and slowly decline after 24–48 h. Our data indicate that in dogs, stimulation of testicular steroidogenesis leads to an increase of plasma Δ^5-steroid levels while the same stimuli cause, in the rat, a stimulation of Δ^4-androgen, particularly testosterone.

INTRODUCTION

It is well established that testicular steroidogenesis is stimulated by acute administration of hCG or LHRH which stimulates endogenous plasma LH. Detailed studies on testicular steroid response to a hCG administration in the adult rat have shown that plasma 17-hydroxyprogesterone, androstenedione as well as testosterone levels are strongly stimulated while the concentration of other steroid precursors (pregnenolone, 17-hydroxypregnenolone, progesterone) or metabolites (dihydrotestosterone and 5α-androstane-3α,17β-diol) did not change significantly [1]. By contrast, we have observed that plasma 5α-androstane-3α.17β-diol levels are markedly increased under the same stimuli in immature rats [2]. The presence of 5α-reductase in Leydig cells of immature rats is responsible for the secretion of this 5α-steroid metabolite and it has been demonstrated that, in the rat, the activity of this enzyme becomes extremely low after puberty [3]. Recently, we have reported that plasma 17-hydroxyprogesterone and testosterone levels can be stimulated up to 5–6-fold in rhesus monkey after a single LHRH analogue administration [4]. On the other hand, in humans, we and others have demonstrated that the stimulation of testicular steroidogenesis is reflected by a 4-fold increase of plasma estradiol levels while the change in testosterone levels is less than 50% [5, 6]. From these results, it appears that a detailed study on testicular steroidogenesis has to be performed in each species before determination of the exact parameter of steroidogenesis to measure after gonadotropin stimulation.

The testicular biosynthesis of steroid involves two pathways from pregnenolone: the 4-ene and the 5-ene pathway. In the 4-ene pathway, pregnenolone is converted into progesterone, 17-hydroxyprogesterone, androstenedione and testosterone while in the 5-ene pathway, testosterone is formed through 17-hydroxypregnenolone, dehydroepiandrosterone and androst-5-ene-3β, 17β-diol. In agreement with the above data, several recent observations indicate that the preferred testosterone biosynthetic pathway varies between species [7–10]. Hence, androgens are synthesized in the testis of rat and mouse through the 4-ene pathway while in the man, monkey, cat, rabbit and dog, they are formed through the 5-ene pathway.

The present study has been designed to investigate the action of gonadotropin on testicular steroid secretion in the dog. The changes in the pattern of plasma steroid levels observed in the dog after gonadotropin administration were then compared with those measured in the rat after stimulation with the same drug. Our data demonstrate that plasma dehydroepiandrosterone and androst-5-ene-3β,17β-diol levels markedly increase in dog after gonadotropin stimu-

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lation while the steroidogenesis stimulation in the rat is reflected by an augmentation of testosterone levels.

EXPERIMENTAL

Six adult mongrel dogs (2–3-years old, 15–20 kg) were housed 1 per cage under controlled conditions of light (14 h light–10 h darkness light on at 0500 h) and fed Purina Dog Chow and tap water *ad libitum*. On the control pretreatment day, blood samples were withdrawn at 0800, 1100 and 1600 h. On the day of the drug administration, blood samples were also collected at 0900, 1000, 1100, 1200, 1300, 1400, 1500, 1600 and 2000 h. For animals injected with hCG, blood samples were also drawn for the 2 post-treatment days at 0800, 1100 and 1600 h.

Adult male Sprague–Dawley rats ($n = 8$), 300–325 g upon arrival, were obtained from Charles River Inc., St Constant, Quebec. Animals were housed 2 per cage in a temperature (20–22°C)—and light (14 h light–10 h darkness, lights on at 0500 h)—controlled room and given food and water *ad libitum*. Insertion of a catheter into the right superior vena cava was performed under Surital (sodium thiamylal, 50 mg/kg, b.wt.) anesthesia 1 day before the drug injection. Blood samples (1 ml) were drawn each 30 min after LHRH agonist or hCG administration for a period of 6 h through the catheter. For animals receiving hCG, blood samples were also collected 24, 36 and 48 h after injection.

Hormones

The LHRH agonist [D-Trp^6, des-Gly-NH$_2^{10}$]LHRH ethylamide was purchased from Bachem Inc., Torrance, California. hCG (2910 IU/mg) was kindly supplied by Dr J. P. Raynaud, Roussel-UCLAF, Romainville, France. All animals received a subcutaneous injection of 25 IU/kg of hCG or 1.5 μg/kg of [D-Tryp^6, des-Gly-NH$_2^{10}$]LHRH ethylamide.

Steroid assays

Plasma pregnenolone, progesterone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, dehydroepiandrosterone, androst-5-ene-3β,17β-diol, testosterone, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, dihydrotestosterone and estradiol were measured as described [11, 12]. In brief, plasma was extracted with diethyl ether and steroids were separated on LH-20 columns before RIA measurement. The data were analyzed with a program derived from model II of Rodbard and Lewald [13]. Statistical significance was measured according to the multiple-range test of Duncan Kramer [14].

RESULTS

As shown in Fig. 1, testosterone (2.4 ± 0.35 ng/ml) is the predominant steroid measured in dog plasma. The levels of pregnenolone, 17-hydroxypregnenolone, progesterone, 17-hydroxyprogesterone, andro-

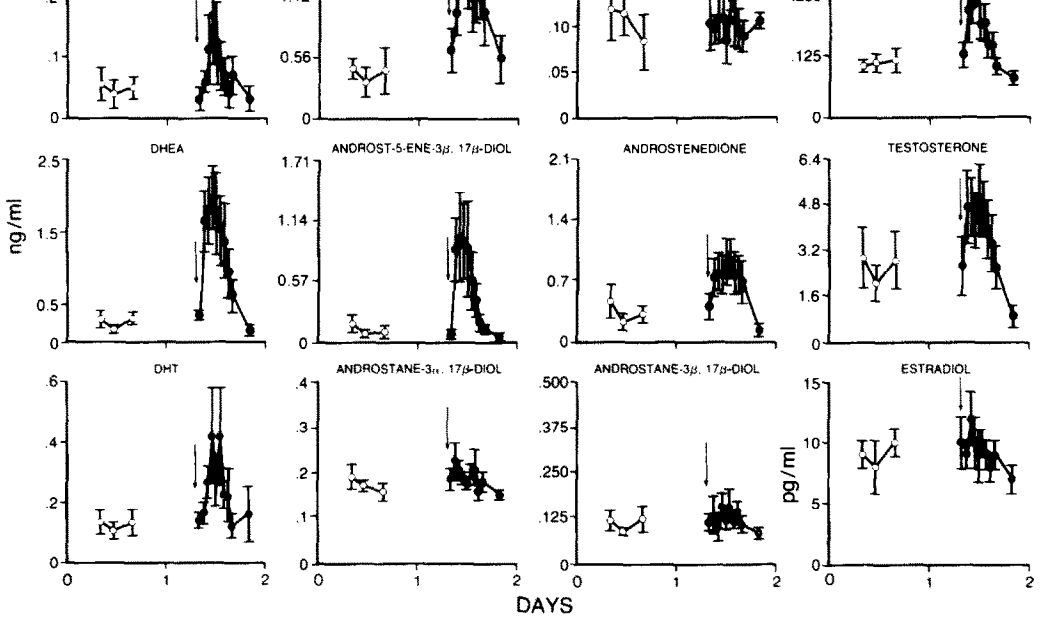

Fig. 1. Effect of single administration of 1.5 μg/kg, b.wt., of [D-Trp^6, des-Gly-NH$_2^{10}$]LHRH ethylamide on serum pregnenolone, 17-hydroxypregnenolone (17-OH-pregnenolone), progesterone, 17-hydroxyprogesterone (17-OH-progesterone), dehydroepiandrosterone (DHEA), androst-5-ene-3β,17β,-diol, androstenedione, testosterone, dihydrotestosterone (DHT), androstane-3α,17βdiol, androstane-3β,17β-diol and estradiol in normal adult dog. Steroid levels were measured on one pretreatment and on treatment day. Data are expressed as the mean ± SEM of values obtained from six normal adult dogs.

stenedione, dehydroepiandrosterone, androst-5-ene-3β,17β-diol and estradiol do not exceed 0.5 ng/ml. After subcutaneous injection of LHRH-A, a change of plasma pregnenolone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, dehydroepiandrosterone, androst-5-ene-3β,17β-diol, androstenedione, testosterone, and dihydrotestosterone levels occurs rapidly with a maximal stimulation between 2–4 h later. However, at this time interval, only 17-hydroxypregnenolone, 17-hydroxyprogesterone, dehydroepiandrosterone, androst-5-ene-3β,17β-diol and androstenedione levels show a significant increase (from 410 ± 90, 118 ± 9, 259 ± 37, 111 ± 24 and 322 ± 65 to 1405 ± 216 ($P < 0.01$), 310 ± 55 ($P < 0.01$), 1979 ± 522 ($P < 0.01$), 982 ± 403 ($P < 0.01$), and 902 ± 256 pg/ml, respectively). Thereafter, the stimulated steroid levels decline and reach control value 12 h after the LHRH agonist administration.

Figure 2 illustrates the effect of hCG on plasma steroid levels in the dog. Since, in many species, the half-life of hCG is longer than that reported for the LHRH agonist, blood samples were collected during the two post-treatment days. Between 4–9 h after hCG administration, we observed a maximal stimulation of 17-hydroxypregnenolone, dehydroepiandrosterone, androst-5-ene-3β,17β-diol, androstenedione, testosterone, dihydrotestosterone and 5α-androstane-3α,17β-diol while the peak of 17-hydroxyprogesterone was only detected 24 h later. Although the levels of testosterone were stimulated by only 2-fold ($P < 0.01$), an increment of 5–20-fold ($P < 0.01$) was obtained for 17-hydroxyprogesterone, androstenedione, dehydroepiandrosterone, androst-5-ene-3β,17β-diol and dihydrotestosterone. Two days after the hCG administration, 17-hydroxyprogesterone, androstenedione, dehydroepiandrosterone and testosterone levels were still elevated while those of other steroids measured returned to normal values.

In agreement with previous findings [1], it can be seen in Fig. 3 that progesterone and testosterone with concentrations of 3.4 ± 0.2 and 2.2 ± 0.4 ng/ml, respectively, are the major steroids measured in rat plasma. 60–120 min after LHRH agonist administration, increases were seen in progesterone (from 3.4 ± 0.2 to 8.0 ± 1.4, $P < 0.01$), 17-hydroxyprogesterone (from 0.2 ± 0.1 to 3.8 ± 0.9, $P < 0.01$) androstenedione (from 0.6 ± 0.2 to 4.0 ± 0.8 ng/ml, $P < 0.01$), and testosterone (from 2.2 ± 0.4 to 15.5 ± 0.4 ng/ml, $P < 0.01$) while the dihydrotestosterone only raised the maximal levels (2.8 ± 0.5 ng/ml, $P < 0.01$) 240 min later. The concentration of dehydroepiandrosterone in plasma was slightly stimulated after LHRH-A injection (from 0.096 ± 0.08 to 0.251 ± 0.047 ng/ml, $P < 0.01$) although the levels of 17-hydroxypregnenolone, androst-5-ene-3β,17β-diol, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol remained almost unchanged. In contrast, plasma pregnenolone levels were decreased by almost 70% ($P < 0.01$).

After the subcutaneous injection of hCG, a sharp

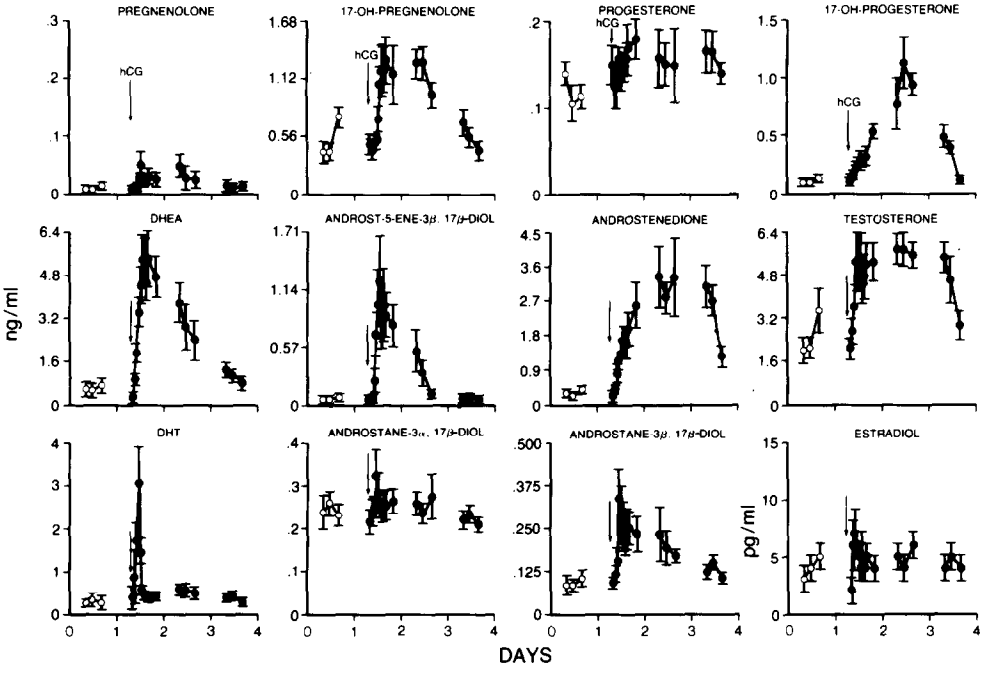

Fig. 2. Effect of single administration of hCG (25 IU/kg, b.wt.) on serum steroids (see Fig. 1) in normal adult dogs. Steroid levels were measured on one pretreatment, one treatment and two post-treatment days. Data are expressed as the mean ± SEM of values obtained from six normal adult dogs.

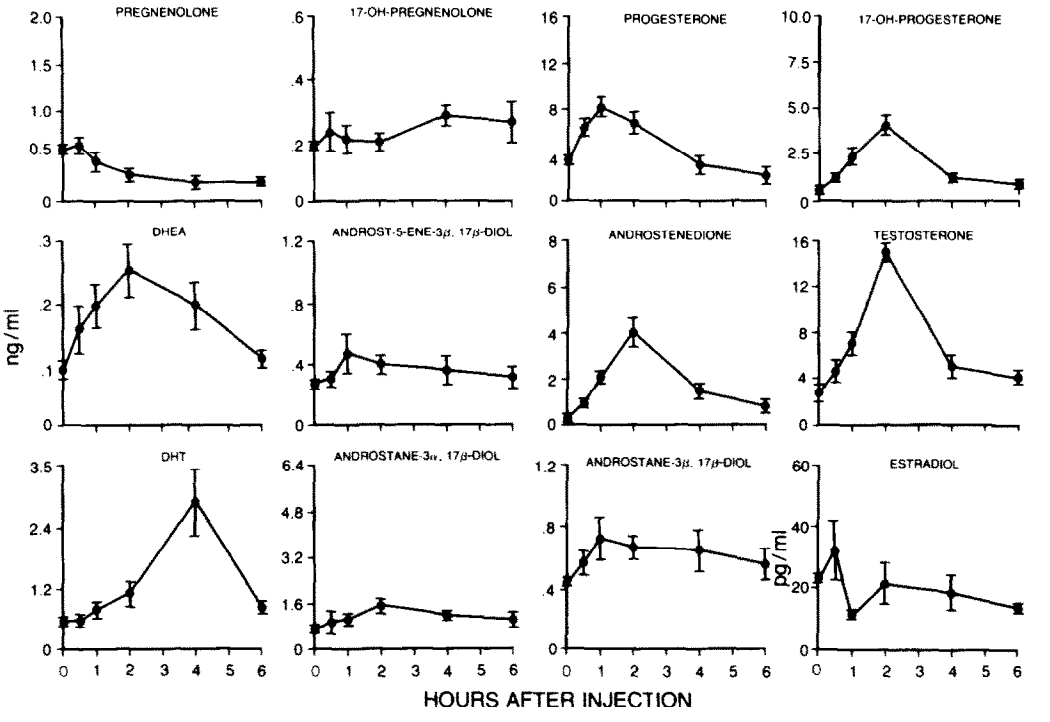

Fig. 3. Effect of single administration of [D-Trp^6, des-Gly-NH$_2^{10}$]LHRH ethylamide (1.5 μg/kg, b.wt.) on serum steroids (see Fig. 1) in normal adult rat. Steroid levels were measured 30, 60, 120, 240 and 360 min after the LHRH agonist injection. Data are expressed as the mean ± SEM of values obtained from 8 normal adult rats.

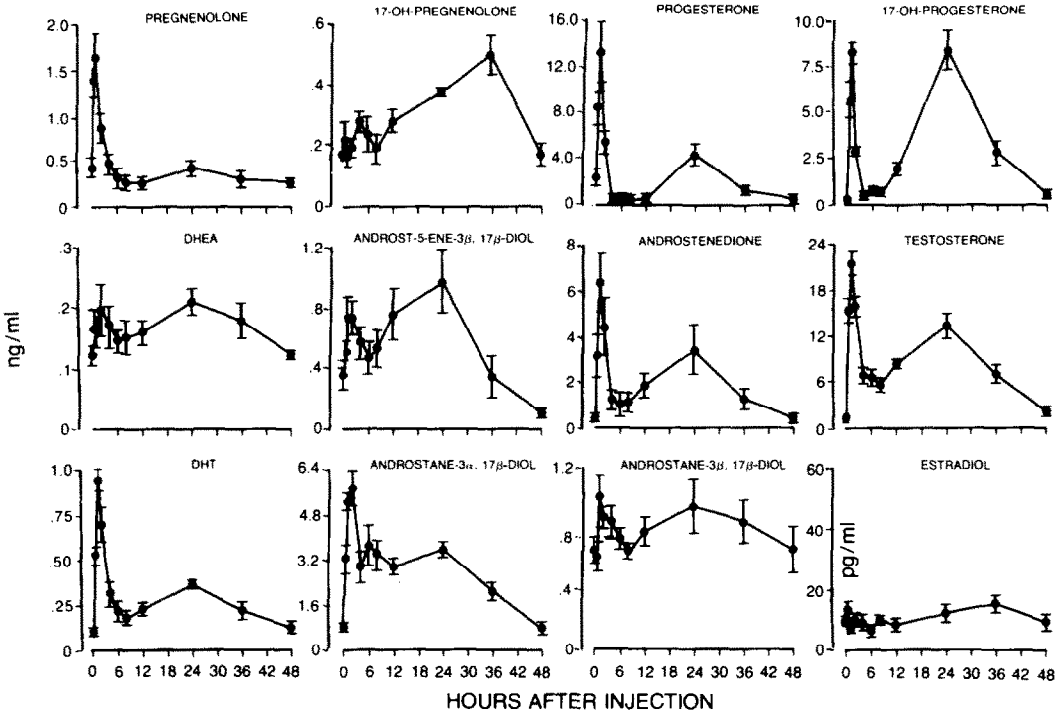

Fig. 4. Effect of single administration of hCG (25 IU/kg, b.wt.) on serum steroids (see Fig. 1) in normal adult rat. Steroid levels were measured on the treatment and two post-treatment days. Data are expressed as the mean + SEM of values obtained from 8 normal adult rats.

increase in plasma pregnenolone, progesterone, 17-hydroxyprogesterone, androstenedione, testosterone, dihydrotestosterone and 5α-androstane-3α,17β-diol (Fig. 4) was observed within the first 2 h. Thereafter, the concentration of these steroids rapidly decreased to reach a nadir 4–12 h later. Interestingly, 24 h after the injection of hCG, 17-hydroxyprogesterone, androstenedione and testosterone concentrations were significantly higher than the non-stimulated levels as well as those observed between 4–12 h after the hCG administration. The plasma steroid levels decreased during the next 24 h to almost reach control values 48 h later.

DISCUSSION

The present study indicates that the action of gonadotropin on testicular steroidogenesis in the dog leads to increased plasma concentrations of androgen, particularly, dehydroepiandrosterone and androst-5-ene-3β,17β-diol while testosterone levels is less affected. When compared with data obtained in rats, the pattern of gonadotropin response is completely different. In fact, testosterone is, in the rodents, the predominant C19 steroid (androgen) measured in plasma following hCG or LHRH agonist injection. While it is difficult to determine from the present data, the mechanism by which dehydroepiandrosterone and androst-5-ene-3β,17β-diol levels accumulated in dog plasma, it seems that the conversion of Δ^5-steroids into Δ^4-steroids might be a rate-limiting step in dog testicular androgen production.

From the time-course study of pregnenolone metabolism in canine dog testes *in vitro* [7], it has been demonstrated that the pathway of androgen biosynthesis is the Δ^5-pathway:

pregnenolone→17-hydroxypregnenolone
→dehydroepiandrosterone
→androst-5-ene-3β,17β-diol→testosterone.

In agreement with this observation, the infusion of radioactive pregnenolone to dogs has also shown that the Δ^5-pathway is the major route of androgen production [15]. Moreover, the analysis of spermatic vein blood of dogs has indicated that dehydroepiandrosterone and androst-5-ene-3β,17β-diol are two intermediates of testosterone synthesis [16]. From the present study, the small response of testosterone to gonadotropin in the dog is in striking contrast to the response observed for Δ^5-androgen and seems to result from regulation at the step of the enzyme 3β-hydroxysteroid dehydrogenase. Alternatively, facilitated release of dehydroepiandrosterone from the testis to plasma could also explain our observation. The questions of enzymatic activity in dog testis and why such differences in biosynthesis of testosterone route exist remain to be answered.

In vitro studies of testicular steroidogenesis in the rat have shown that the two pathways are predominant:

pregnenolone→17-hydroxypregnenolone
→17-hydroxyprogesterone
→androstenedione→testosterone

and

pregnenolone→progesterone
→17-hydroxyprogesterone
→androstenedione→testosterone [9].

The present data which show a rapid augmentation of plasma C21 and C18 steroid concentrations in rat after gonadotropin are in agreement with these *in vitro* studies. While a blockade at the level of 17-hydroxylase and 17,20-desmolase has been already demonstrated in rat testicular steroidogenesis following chronic treatment with an LHRH agonist or hCG administration [17], it appears that the rapid response to a single stimulus causes a stimulation of plasma testosterone and that the conversion of cholesterol into pregnenolone is likely to be the rate-limiting step.

One interesting observation of the present study is the striking difference of the pattern of plasma steroid concentrations after hCG administration in rats and dogs. In agreement with previous finding [20], we have observed, in rats, two peaks of androgen secretion after hCG injection. Such a pattern of steroid production following hCG administration had also been reported in man and ram [18, 19]. Acute testicular steroidogenic responsiveness to hCG in the dog is, however, different: after stimulation, the levels of plasma dehydroepiandrosterone are maintained at a plateau and slowly decline after 24–48 h.

In the rat, it is well known that hCG induces steroidogenic refractoriness of Leydig cells to further gonadotropin stimulation [17, 20, 21]. This inhibitory effect is a complex process related to modification of the coupling system between the binding sites and the adenylate cyclase and alterations of 17-hydroxylase and 17,20-desmolase as well as cholesterol metabolism. The delayed testosterone secretion is likely due to the partial recovery after desensitization and the presence of hCG at a concentration still able to stimulate steroidogenesis. However, the pattern of steroid secretion observed in dog might suggest that such mechanisms do not occur in this species.

In summary, our results directly demonstrate that dog testis is able to respond acutely to gonadotropin stimulation with increased dehydroepiandrosterone and androst-5-ene-3β,17β-diol secretion. Moreover, while a biphasic steroid secretory response of hCG is observed in the rat, acute administration of hCG in the dog causes a prolonged stimulation of steroidogenesis.

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