J. steroid Biochem. Vol. 35, No. 5, pp. 575–581, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

REGULATION OF THE PITUITARY 5α-REDUCTASE ACTIVITY BY GONADOTROPIN RELEASING HORMONE AND TESTOSTERONE IN THE ADULT MALE RAT

H. VANDERSTICHELE, W. EECHAUTE and E. LACROIX*
Laboratory of Normal and Pathological Physiology, University of Gent, B-9000 Gent, Belgium

(*Received* 2 *October* 1989)

Summary—Intact or castrated adult male rats were treated for nine days with GnRH (10 μg/day), the synthetic GnRH goserelin (100 μg/day) or the GnRH-antagonist Org 30276 (250 or 500 μg/day). In some series, 1 mg testosterone propionate was administered alone, or in combination with goserelin or Org 30276. The *in vitro* metabolism of [1α,2α-^{3}H]testosterone by pituitary and hypothalamic homogenates was investigated in combination with the estimation of plasma concentrations of testosterone and gonadotropins. No qualitative or quantitative differences were observed in hypothalamic testosterone metabolism or in the pituitary 17β-hydroxysteroid dehydrogenase activity. Testosterone administration to intact male rats decreased the pituitary 5α-reductase activity and LH, while administered to castrated rats, it was able to suppress totally the castration-induced increase of the 5α-reductase activity and of the gonadotropin secretion. The drastic decrease of the plasma levels of testosterone, observed after a prolonged treatment with GnRH, goserelin or Org 30276 was not accompanied by an increased pituitary 5α-reductase activity. Injected to castrated rats, it was observed that the castration-induced increase of the pituitary 5α-reductase was further stimulated by GnRH, totally suppressed by goserelin and partially suppressed by Org 30276. Concomitant administration of goserelin or Org 30276 and testosterone propionate to castrated rats resulted in a further decrease of the pituitary 5α-reductase activity, compared to the castrated, GnRH-analogue treated rats. These data indicate that the pituitary 5α-reductase enzyme system is controlled by both direct steroidal and indirect GnRH-mediated mechanisms.

INTRODUCTION

The pituitary and several structures of the central nervous system can metabolize gonadal steroids; however, the physiological significance of these processes is still largely unknown [1].

In the pituitary of the male rat, testosterone is mainly metabolized by a 5α-reductase-3α/β-hydroxysteroid dehydrogenase enzyme system to 5α-reduced derivatives. Castration increases strongly the 5α-reductase activity of the pituitary, while the castration effect is reversed by the administration of testosterone. This suggests that testosterone is involved in the control of the 5α-reductase activity of the pituitary [2, 3]. However, it is still unsettled whether the feedback results from a direct effect of testosterone on the pituitary, or whether it is mediated through the hypothalamus or both.

The purpose of the present experiments was to investigate the effects of a prolonged administration of GnRH, of the synthetic GnRH-analogue goserelin (ICI 118630) and of the GnRH-antagonist Org 30276, on the pituitary and hypothalamic 5α-reductase-activity of intact or castrated adult male rats, in correlation with plasma levels of testosterone and gonadotropins.

EXPERIMENTAL

Rats of the Wistar strain, inbred in the laboratory, were kept under natural lighting and at constant temperature, and had food and water *ad libitum*.

Intact or castrated 90-day-old male rats were injected twice daily, for a period of 9 days with either 5 μg GnRH (i.p.) or 50 μg goserelin (i.p.), dissolved in 0.4 ml 0.9% NaCl; or with 125 μg or 250 μg Org 30276 (i.m.), dissolved in 0.4 ml sesame oil. In some series, 1 mg testosterone propionate was administered i.m. twice daily. The first injection to the castrated rats was given a few hours after operation. The animals were sacrificed 24 h after the last injection.

*To whom correspondence should be addressed.

Abbreviations: Testosterone: 17β-hydroxy-4-androsten-3-one. 5α-Dihydrotestosterone: 17β-hydroxy-5α-androstan-3-one. 5α-Androstanediol: 5α-androstane-3α,17β-diol + 5α-androstane-3β,17β-diol. Androstenedione: 4-androstene-3,17-dione. 5α-Androstanedione: 5α-androstane-3,17-dione. 5α-Androsterone: 3α-hydroxy-5α-androstan-17-one. Testosterone-3-CMO-BSA: testosterone-3-*O*(carboxymethyl)oxine-BSA.

Heparinized blood was collected after decapitation and centrifuged at 4°C; plasma was kept at −20°C until assay.

Immediately after decapitation, the pituitary and the brain were removed and cooled on ice. The isolated hypothalamic tissue included the dorsal and ventral hypothalamus, the median eminence and the preoptic area. Pooled pituitaries or hypothalamic tissue (corresponding to 25 mg of tissue for each incubation) were homogenized and incubated at 37°C for 2 h in the presence of [1α,2α-^{3}H]testosterone, under continuous shaking and a stream of 95% oxygen and 5% carbon dioxide. The reaction was stopped by the addition of 10 volumes of a mixture of acetone–methanol (v/v).

Analytical procedure

The amount of metabolites produced from ^{3}H-labelled testosterone was measured by an analytical procedure described in detail elsewhere [4]. Briefly, the content of the vials was first filtered through Whatman No. 2 filter paper. After reducing the filtrate under nitrogen, 3 ml of water was added and the metabolites were extracted twice by means of 50 ml dichloromethane. This extraction procedure resulted in more than 97% recovery of the steroids. The extract was evaporated under nitrogen and 5 μg of each of the following steroids were added to the residue: androstenedione, 5α-androstanedione, 5α-androsterone, testosterone, 5α-dihydrotestosterone, 5α-androstane-3α,17β-diol.

Fractionation and estimation

The metabolites were chromatographed on a Sephadex LH 20 column (i.d. 1 cm, height 42 cm) (Pharmacia, Upssala, Sweden) using benzene–dichloromethane–methanol (60:35:5, by vol) as elution liquid. Three fractions were collected (11–22 ml, 23–38 ml, 39–78 ml): fraction 1 contained androstenedione + 5α-androstanedione; fraction 2 contained testosterone + 5α-dihydrotestosterone + 5α-androsterone; and fraction 3: 5α-androstane-(3α + 3β),17β-diol and an unidentified metabolite. Further separation was obtained by paper chromatography in petroleumether–methanol–water (100:70:30, by vol) (fractions 1 and 2) or in benzene–n-heptane–methanol–water (70:30:80:20, by vol) (fraction 3). The radioactive zones were detected with a Packard radiochromatogram scanner and eluted with ethanol. A portion was analyzed by liquid scintillation counting; the remaining part was used for identification studies.

Identification was made on the basis of:

1. Identical mobility of the radioactive metabolites with authentic steroids during paper chromatography.

2. Constant specific activity of the crystals isolated after repeated crystallizations from n-heptane–dichloromethane (8:2, v/v) or from n-heptane–chloroform (8:2, v/v) of the radioactive metabolites mixed with their authentic steroids. Correction for experimental losses of the metabolites was done on the basis of the recovery calculated for the unlabelled steroid added before the separation procedure and eluted in the corresponding system.

The metabolism experiments have shown that in the incubation conditions used, pituitary and hypothalamus converted testosterone mainly to 5α-reduced metabolites, especially 5α-androstanediol, and to minor amounts of 17-keto-derivatives [5]. The 5α-reductase activity was deduced from the sum of 5α-dihydrotestosterone, 5α-androstanediol, 5α-androstanedione and 5α-androsterone. The 17β-hydroxysteroid dehydrogenase activity was deduced from the sum of androstenedione, 5α-androstanedione and 5α-androsterone.

Plasma testosterone was estimated as follows: the plasma (1 ml) was extracted with ether (15 ml), the extract was evaporated to dryness under a stream of nitrogen and the residue resolved with 1 ml phosphate buffered saline (PBS; pH = 7.2). The amount of testosterone in the PBS solution was measured by radioimmunoassay using an antiserum raised in rabbits against testosterone-3-CMO-BSA as antigen. The anti-testosterone serum was highly specific: the cross-reactivity of a great number of steroids (n = 58) was less than 0.5%; for 5α-dihydrotestosterone a cross-reactivity of 24% was found. The inter-assay coefficient of variation was 3.2%. LH and FSH were measured by radioimmunoassay on 100 μl plasma using specific antisera against rat-LH and rat-FSH respectively. Inter- and intra-assay coefficients of variation, calculated from a single plasma pool, were found to be respectively 5 and 12% for rat-LH and 3 and 8% for rat-FSH.

Materials

All reagents used were of analytical grade (Merck, Darmstadt, G.F.R.). The GnRH-analogue (pyro)-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 × 2ac·OH, with the same structure as natural GnRH, was purchased from UCB, Brussels, Belgium. The synthetic GnRH D-Ser(Bu^t)^6-AzGly^{10} (goserelin: ICI 118630) was kindly provided by Dr Furr. B. J. A., Imperial Chemical Industries, PLC, Pharmaceutical division, Macclesfield, Cheshire. The GnRH-antagonist N-Ac-D-p-Cl-Phe^{1,2}, D-Trp^3, D-Arg^6, D-Ala^{10} (Org 30276) and testosterone propionate (TP) were supplied by Organon International, through Organon, Oss, The Netherlands. Unlabelled steroids were from Makor Chemicals (Jerusalem, Israel). [1α,2α-^{3}H]testosterone (51 Ci/mmol) was purchased from the radiochemical centre Amersham, England. The purity of the labelled steroids was checked by paper chromatography in the system petroleumether–methanol–water (100:70:30, by vol) and in the system benzene–n-heptane–methanol–water (70:30:80:20, by vol)

RESULTS

Influence of castration and testosterone propionate (Tables 1 and 4)

As expected, ten days after castration, there was a nearly complete disappearance of testosterone from the plasma, together with a 4–6-fold increase of the plasma concentrations of both LH and FSH; and a 2–3-fold increase of the pituitary 5α-reductase activity. The postcastration increase of the 5α-reductase activity and of the plasma concentrations of FSH and LH could be entirely prevented by the concomitant administration of a daily dose of 2 mg testosterone propionate; the LH concentrations were even significantly lower than in the intact, control rats.

The administration of the same dose of testosterone propionate to intact, adult male rats, resulted in normal concentration of FSH and a significant decrease of both LH concentration as well as pituitary 5α-reductase activity.

Influence of Gonadotropin Releasing Hormone (GnRH) (Tables 1 and 4)

Daily administration of 10 μg (i.p.) GnRH to intact male rats for 9 days resulted in a more than 80% decrease of the plasma concentrations of testosterone, with a significant increase of the FSH, but not of the LH concentrations. The 5α-reductase activity of the pituitary was not modified by the treatment.

GnRH administered to castrated rats in the same dose and according to the same treatment schedule partially blocked the increase of the LH concentrations in the plasma after castration, but did not affect the increase of the FSH concentrations. Moreover, it enhanced significantly the increase of the 5α-reductase activity of the pituitary observed after castration.

Influence of goserelin (Tables 2 and 4)

Administered in a daily dose of 100 μg (i.p.) for 9 days, the GnRH-analogue goserelin produced in intact male rats, a decrease of the plasma testosterone concentration of more than 70%. In these conditions, the concentrations of LH were not modified, while those of FSH were slightly but significantly increased; in addition, the 5α-reductase activity of the pituitary was slightly ($P < 0.05$) decreased compared to the controls.

When goserelin was administered to castrated rats, it sharply reduced the increase of the FSH and LH concentrations and prevented quite entirely the increase of the pituitary 5α-reductase activity observed after castration.

Concomitant daily administration of 2 mg testosterone propionate and 100 μg goserelin to castrated rats, resulted in a further decrease of the plasma LH and FSH concentrations and of the pituitary 5α-reductase activity, compared to castrated goserelin-treated rats. In these conditions, the LH concentrations and the 5α-reductase activity were even significantly lower than in uncastrated control rats.

Table 1. Influence of administration of 10 μg GnRH or 2 mg testosterone propionate on pituitary weight and plasma concentrations of steroids and gonadotropins

	Intact			Castrated		
	Control	Testosterone	GnRH	Control	Testosterone	GnRH
Pituitary (mg/organ)	8.3 ± 0.20 (68)	10.1 ± 0.16 (36)*	8.2 ± 0.31 (24)	9.5 ± 0.78 (20)	9.0 ± 0.20 (36)	7.3 ± 0.30 (20)
Testosterone (ng/ml)	8.3 ± 1.27 (12)	41.6 ± 1.70 (36)*	1.1 ± 0.15 (14)*	<0.2 (9)*	35.8 ± 0.92 (12)*†	<0.2 (10)*
LH (ng/ml)	46.3 ± 9.5 (14)	8.5 ± 0.4 (18)*	40.7 ± 7.1 (10)	226.9 ± 17.5 (9)*	10.3 ± 1.1 (22)*†	140.4 ± 10.1 (10)*†
FSH (ng/ml)	226.6 ± 15.3 (14)	231.6 ± 6.6 (18)	402.5 ± 27.3 (10)*	888.0 ± 58.6 (9)*	260.3 ± 8.3 (24)†	868.9 ± 26.5 (10)*

Intact or castrated male rats were injected i.p. for 9 days with 2 mg testosterone propionate or 10 μg GnRH. Blood from the rats used in the metabolism studies was collected. The results are expressed as the mean ± SEM (number of individual samples). Significant differences vs intact, control rats: *$P < 0.01$. Significant differences vs castrated, control rats: †$P < 0.01$.

Table 2. Influence of administration of goserelin to intact, or castrated male rats, on pituitary weight and plasma concentrations of steroids and gonadotropins

	Intact		Castrated		
	Control (24)	Goserelin (24)	Control (24)	Goserelin (24)	Goserelin + TP (24)
Pituitary (mg/organ)	7.88 ± 0.23	9.33 ± 0.52	8.22 ± 0.27	8.07 ± 0.29	8.75 ± 0.17
Testosterone (ng/ml)	4.98 ± 0.60	1.39 ± 0.10*	<0.2*	<0.2*	46.32 ± 2.59*†‡
LH (ng/ml)	59.9 ± 5.5	70.0 ± 4.2	329.5 ± 25.4*	94.7 ± 10.4*†	9.9 ± 1.0*†‡
FSH (ng/m)	156.8 ± 8.8	229.5 ± 12.2*	1111.6 ± 52.4*	566.6 ± 30.2*†	334.6 ± 15.1*†‡

Goserelin was adminstered for 9 days in a daily dose of 100 μg. Testosterone propionate was administered in a daily dose of 2 mg. The results are expressed as the mean ± SEM (number of individual samples). Blood from the rats used in the metabolism experiments was collected. Significant differences vs intact, control rats: *$P < 0.01$. Significant differences vs castrated, control rats: †$P < 0.01$. Significant differences vs castrated, goserelin-treated rats: ‡$P < 0.01$.

Influence of ORG 30276 (Tables 3 and 4)

The administration of the GnRH-antagonist Org 30276 in a daily dose of 250 μg (i.m.) for 9 days resulted in a more than 97% decrease of the plasma testosterone concentrations to castration levels, while the concentrations of FSH and LH were reduced by more than 70 and 57%, respectively. However, the 5α-reductase activity of the pituitary was not modified.

The same treatment in castrated rats, prevented entirely the castration-induced increase of the FSH and LH concentrations, but blocked only partially the castration-induced increase of the pituitary 5α-reductase activity ($P < 0.02$). When the daily dose of Org 30276 administered to the castrated rats was increased to 500 μg, the FSH and LH concentrations were further depressed to values which were significantly lower than in the uncastrated controls; however, in these conditions, there was no additional effect on the partial inhibition of the pituitary 5α-reductase activity.

Concomitant administration of a daily dose of 2 mg testosterone propionate and 250 μg Org 30276 to castrated rats prevented entirely the increase of the pituitary 5α-reductase activity induced by the castration. The FSH and LH concentrations in the plasma, were slightly, but significantly higher than those measured in the castrated-Org 30276-treated animals.

No qualitative nor quantitative differences were observed concerning the testosterone metabolism by hypothalamic homogenates, neither between intact or castrated animals, nor between control and testosterone-, GnRH-, goserelin- or Org 30276-treated rats. In addition, none of the experimental procedures influenced the 17β-hydroxysteroid dehydrogenase activity in both hypothalamic or pituitary homogenates.

DISCUSSION

The main purpose of the present experiments was to investigate whether the regulation of the pituitary 5α-reductase activity by testosterone resulted from a direct effect of testosterone on the pituitary gland; or from an indirect hypothalamic and GnRH mediated influence, or both. In addition, the aim of this study was also to investigate to what extent that the pituitary 5α-reductase activity can be correlated with the plasma concentrations of LH and FSH.

The results show that a prolonged treatment of intact male rats with different GnRH-analogues results in a depression of the testicular function, characterized by sharply decreased plasma concentrations of testosterone. The mechanisms through which this decrease is obtained, seem, however, to differ.

The decrease of the plasma concentrations of testosterone to castration levels observed after the administration of Org 30276, which is considered to be a GnRH-antagonist [6–8], is probably mainly due to the sharp decrease of the plasma concentrations of FSH and LH. This reduction of the gonadotropin release can be attributed to a block of the GnRH receptors of the pituitary gonadotrophs by Org 30276, as shown by Heber *et al.*[9] and Puente and Catt[10], interfering with the action of the endogenous GnRH. Confirmation of such an antagonistic effect on the gonadotropin release is found in the results obtained in castrated rats, in which the high

Table 3. Effects of administration of Org 30276 to intact, or castrated male rats on pituitary weight and on plasma concentrations of steroids and gonadotropins

	Intact		Castrated			
	Control (24)	Org 30276 250 μg (24)	Control (24)	Org 30276 250 μg (24)	Org 30276 500 μg (24)	Org 30276 + TP 250 μg 2 mg (24)
Pituitary (mg/organ)	8.9 ± 0.4	8.4 ± 0.25	8.5 ± 0.2	8.5 ± 0.3	7.7 ± 0.20	8.5 ± 0.20
Testosterone (ng/ml)	4.7 ± 0.86	<0.2*	<0.2*	<0.2*	<0.2*	54.7 ± 2.12*†‡
LH (ng/ml)	31.6 ± 3.3	13.6 ± 0.96*	352.0 ± 24.2*	25.6 ± 1.9†	11.8 ± 1.5*†	36.8 ± 1.75†‡
FSH (ng/ml)	248.5 ± 17.0	71.1 ± 49.0*	938.7 ± 39.9*	200.4 ± 9.8†	149.5 ± 3.8*†	285.2 ± 13.9†‡

Intact or castrated adult male rats were injected i.m. for 9 days with a daily dose of 250 or 500 μg Org 30276, and/or 2 mg testosterone propionate. The results are expressed as the mean ± SEM. Number of individual samples in parenthesis. Significant differences vs intact, control rats: *$P < 0.01$. Significant differences vs castrated, control rats: †$P < 0.01$. Significant differences vs castrated, Org 30276-treated rats: ‡$P < 0.01$.

Table 4. Influence of a prolonged administration of GnRH, goserelin or Org 30276 on the pituitary 5α-reductase activity of intact or castrated male rats

	Intact	Castrated
Testosterone propionate		
Control	8.01 ± 0.42 (17)	17.29 ± 1.22 (13)*
TP: 2 mg	5.49 ± 0.33 (9)*	6.64 ± 0.38 (9)†
GnRH		
Control	7.0 ± 0.54 (7)	22.4 ± 1.08 (5)*
GnRH: 10 μg	8.4 ± 1.02 (6)	31.2 ± 1.26 (5)*†
Goserelin		
Control	6.40 ± 0.23 (5)	18.20 ± 1.44 (6)*
goserelin: 100 μg	5.43 ± 0.24 (5)	7.53 ± 0.37 (6)†
goserelin: 100 μg + TP: 2 mg	ND	4.53 ± 0.16 (6)*†‡
Org 30276		
Control	8.68 ± 0.47 (6)	19.62 ± 1.65 (6)*
Org 30276: 250 μg	8.82 ± 0.73 (6)	14.35 ± 1.58 (5)*
Org 30276: 500 μg	ND	14.54 ± 0.33 (6)*
Org 30276: 250 μg + TP: 2 mg	ND	6.97 ± 0.28 (6)†*‡

Pituitary homogenates were incubated for 2 h at 37°C with 24 ng [1α, 2α-^{3}H]testosterone + 300 ng testosterone. The metabolites are expressed as ng metabolites produced per mg protein in the incubation vessel. Each incubation contained pituitaries pooled from 4 rats. (Number of incubations). The results are expressed as the mean ± SEM. Significant differences vs intact, control rats: *$P < 0.01$. Significant differences vs castrated, control rats: †$P < 0.01$. Significant differences vs castrated, GnRH-analogue-treated rats: ‡$P < 0.01$.

postcastration plasma concentrations of FSH and LH were reduced under Org 30276 to lower levels than found in the normal rats.

GnRH and goserelin, the latter considered to be a GnRH agonist [11–13], seem to influence the testicular function through a more complicated mechanism. As a result of their administration the LH and FSH concentrations are not reduced, but, at least for FSH, significantly increased while testosterone in the plasma is decreased by more than 80%. These data are in agreement with other reports, which state that the low plasma testosterone concentrations observed after prolonged administration of GnRH-analogues to intact male rats, concurs with higher FSH concentrations [14, 15] and normal [15, 16] or increased [14, 17, 18] LH concentrations. Accordingly, the sharp reduction of the plasma testosterone concentrations cannot be explained by a reduction of the LH and FSH, as is probably the case for Org 30276, but results seemingly from a direct effect of GnRH and goserelin on the testes. A direct inhibitory effect of GnRH-analogues on the testes has indeed been observed in hypophysectomized rats [19–24], in rats treated with an anti-LH-serum [25, 26] and on testicular cells *in vitro* [26–28].

The complete blockade of the postcastration gonadotropin increase by Org 30276 suggests that testosterone normally exerts its negative feedback mainly indirectly through the hypothalamus, blocking GnRH release and the GnRH-mediated gonadotropin output by the pituitary gonadotrophs. It also suggests that the postcastration stimulation of the gonadotropin release cannot be attributed to a direct effect of the testosterone withdrawal at the pituitary level; but that it is mediated by an increase of the hypothalamic release of GnRH.

These data, however, do not exclude the possibility of an additional direct effect of testosterone at the pituitary level.

Indeed, the direct testosterone effect seems to be different according to whether or not the pituitary gland is stimulated by GnRH in that condition.

During partial GnRH blockade, as observed after the administration of goserelin to castrated rats, testosterone seems to exert an additional negative feedback at the pituitary level. Indeed, in these conditions, the plasma concentrations of FSH and particularly of LH, are further depressed by the concomitant administration of testosterone. Furthermore, in conditions of nearly complete GnRH blockade, as observed by the administration of Org 30276 to castrated rats, testosterone seems to exert a positive feedback on the pituitary, as the plasma concentration of both FSH and LH are increased by the concomitant administration of testosterone. This seemingly paradoxical positive feedback of testosterone on the pituitary is in agreement with the findings of others, who have observed that the plasma concentrations of FSH, which were substantially reduced during a treatment with a GnRH-antagonist, could be restored by testosterone [29, 30].

The fact that the LH and FSH concentrations, notwithstanding the sharp reduction of the testosterone levels, are not or only moderately increased by these analogues, suggests that either the remaining testosterone concentrations are sufficient to maintain a negative feedback, at least on the LH release mechanisms, or that the administered GnRH and goserelin have additional effects at the hypothalamo–hypophyseal level. The latter view is supported by the observations in the castrated rats, in which the castration-induced increase of the LH and FSH concentration is suppressed to a more or less extent, particularly by the administration of goserelin.

The data obtained concerning the pituitary 5α-reductase activity, provides experimental evidence that the testosterone feedback on that enzyme system also occurs mainly indirectly through the hypothalamic GnRH release, with the possibility of an additional direct negative feedback on the pituitary. They indicate that the withdrawal of testosterone is unable by itself to increase 5α-reductase activity in the absence of an adequate GnRH stimulus.

Indeed, the observation that the castration-induced increase of the pituitary 5α-reductase activity is more or less suppressed by goserelin and Org 30276 and can be augmented by the exogenous administration of natural GnRH, indicates that this castration increase is mediated to a large extent by an augmented release of GnRH from the hypothalamus. Further evidence is given by the experiments on intact rats treated with Org 30276, in which the decrease of the plasma concentration of testosterone to castration levels was not associated with an increase of the pituitary 5α-reductase activity.

The lack of an increase of the 5α-reductase activity, notwithstanding the sharp reduction of the plasma concentrations of testosterone after the administration of GnRH or goserelin to intact rats, suggests that either the remaining levels of testosterone are sufficient to maintain a normal negative feedback control on the 5α-reductase or that these GnRH-analogues exert a central blocking effect, or both.

The observation that the complete (goserelin) or partial (Org 30276) inhibition of the castration-induced increase of the pituitary 5α-reductase activity could be further augmented by the concomitant administration of testosterone propionate, indicates that testosterone has also a direct inhibitory effect on the pituitary 5α-reductase activity.

Globally, the present experiments provide evidence that the regulation of the pituitary 5α-reductase activity by testosterone depends on both an indirect GnRH-mediated and a direct effect on the pituitary, as has been suggested [31, 32] for the regulation of the secretion of gonadotropins.

The absence of a parallelism between the plasma concentrations of LH/FSH and the pituitary 5α-reductase activity under several experimental conditions, does not allow to conclude whether the regulation of the pituitary 5α-reductase activity and the gonadotropin release are causally linked. The absence of a parallelism is particularly striking in the experiments with castrated rats in which the administration of Org 30276 depresses the plasma gonadotropin concentrations far below the control values, but only partially blocks the increase of the 5α-reductase activity, even after augmenting the dose from 250 to 500 μg per day. While the concomitant administration of testosterone propionate in these conditions has a stimulating effect on the gonadotropin release, it has an inhibiting effect on the 5α-reductase activity.

It also appears from the experiments that the regulation of the pituitary 5α-reductase activity is very selective, as none of the experimental procedures did alter the pituitary 17β-hydroxysteroid dehydrogenase or hypothalamic 5α-reductase/17β-hydroxysteroid dehydrogenase activity.

In conclusion, the present experiments show that the pituitary 5α-reductase enzyme system is controlled by both direct steroidal and indirect GnRH-mediated mechanisms.

Acknowledgement—We wish to thank Professor P. Franchimont of the Université de Liège (Belgium) for the determinations of the plasma FSH and LH.

REFERENCES

1. Celotti F., Naftolin F. and Martini L. (Eds): *Metabolism of Hormonal Steroids in the Neuroendocrine Structures*. Serono Symposia publications, Vol. 13 (1984) Raven Press, New York.
2. Celotti F., Massa R. and Martini L.: Metabolism of sex steroids in the central nervous system. In *Endocrinology* (Edited by L. J. De Groot). Grune & Stratton, New York, Vol. 1 (1979) pp. 41–53.
3. Martini L.: The 5α-reduction of testosterone in the neuroendocrine structures. Biochemical and physiological implications. *Endocr. Rev.* **3** (1982) 1–25.
4. Eechaute W., Lacroix E. and Leusen I.: Metabolism of [4-^{14}]testosterone and precursors by homogenates of rat testes after chronic LHRH-treatment. *J. Steroid Biochem.* **20** (1984) 1201–1205.
5. Vanderstichele H., Eechaute W., Lacroix E. and Leusen I.: The effects of neonatal androgenization of male rats on testosterone metabolism by the hypothalamus–pituitary–gonadal axis. *J. Steroid Biochem.* **26** (1987) 493–497.
6. Nekola M. V. and Coy D. H.: Direct and indirect inhibition of ovulation in rats by an antagonist of luteinizing hormone-releasing hormone. *Endocrinology* **116** (1985) 756–760.
7. Debeljuk L. and Schally A. V.: Antifertility effects of a potent LH-RH antagonist in male and female rats. *Int. J. Fert.* **31** (1986) 284–292.
8. Marshall G. R., Bint Akhtar F., Weinbauer G. F. and Nieschlag E.: Gonadotrophin-releasing hormone (GnRH) overcomes GnRH antagonist-induced suppression of LH secretion in primates. *J. Endocr.* **110** (1986) 145–150.
9. Heber D., Dodson R., Swerdloff R. S., Channabasavaiah K. and Stewart J. M.: Pituitary receptor site blockade by a gonadotropin-releasing hormone antagonist *in vivo*: mechanism of action. *Science* **216** (1982) 420–421.
10. Puente M. and Catt K. J.: Inhibition of pituitary–gonadal function in male rats by a potent GnRH antagonist. *J. Steroid Biochem.* **25** (1986) 917–925.
11. Dutta A. S., Furr B. J. A., Giles M. B., Valcaccia B. and Walpole A. L.: Potent agonist and antagonist analogues of luliberin containing an azaglycine residue in position 10. *Biochem. Biophys. Res. Commun.* **81** (1978) 382–390.
12. Maynard P. V. and Nicholson R. I.: Effects of high doses of a series of new luteinizing hormone-releasing hormone analogues in intact female rats. *Br. J. Cancer* **39** (1979) 274–279.
13. van Kroonenburgh M. J. P. G., Beck J. L., Vemer H. M., Rolland R., Thomas C. M. G. and Herman C. J.: Effects of a single injection of a new depot formulation of an LH-releasing hormone agonist on spermatogenesis in adult rats. *J. Endocr.* **111** (1986) 449–454.
14. Cusan L., Auclair C., Belanger A., Ferland L., Kelly P. A., Seguin C. and Labrie F.: Inhibitory effects of long term treatment with a luteinizing hormone-releasing hormone agonist on the pituitary-gonadal axis in male and female rats. *Endocrinology* **104** (1979) 1369–1376.
15. Belanger A., Auclair C., Seguin C., Kelly P. A. and Labrie F.: Down-regulation of testicular androgen biosynthesis and LH receptor levels by an LHRH agonist: role of prolactin. *Molec. Cell. Endocr.* **13** (1979) 47–53.
16. Frager M. S., Pieper D. R., Tonetta S. A., Duncan J. A. and Marshall J. C.: Pituitary gonadotropin-releasing hormone receptors. Effects of castration, steroid replacement, and the role of gonadotropin-releasing hormone in modulating receptors in the rat. *J. Clin. Invest.* **67** (1981) 615–623.
17. Pelletier G., Cusan L., Auclair C., Kelly P. A., Désy L. and Labrie F.: Inhibition of spermatogenesis in the rat by treatment with (D-Ala^6, des-Gly-NH$_2^{10}$)LHRH ethylamide. *Endocrinology* **103** (1978) 641–643.
18. Komatsu M., Iwahana H. and Mitsuhashi S.: Effects of an LHRH agonist on endocrine function, LHRH receptor and LH/hCG receptor in the pituitary-gonadal axis of male rats. *Endocr. Jap.* **33** (1986) 185–195.
19. Belanger A., Auclair C., Ferland L., Caron S. and Labrie F.: Time-course of the effect of treatment with a potent LHRH agonist on testicular steroidogenesis and

gonadotropin receptor levels in the adult rat. *J. Steroid Biochem.* **13** (1980) 191–196.
20. Bambino T. H., Schreiber J. R. and Hsueh A. J. W.: Gonadotropin-releasing hormone and its agonist inhibit testicular luteinizing hormone receptor and steroidogenesis in immature and adult hypophysectomized rats. *Endocrinology* **107** (1980) 908–917.
21. Cao Y.-Q., Sundaram K., Bardin C. W., Rivier J. and Vale W.: Direct inhibition of testicular steroidogenesis and gonadotrophin receptor levels by [(imBzl)-D-His^6,Pro^9-NEt]GnRH and [D-Trp^6, Pro^9-NEt]GnRH, potent agonists of GnRH. *Int. J. Androl.* **5** (1982) 158–170.
22. Bourne G. A., Dockrill M. R., Regiani S., Marshall J. C. and Payne A. H.: Induction of testicular gonadotropin-releasing hormone (GnRH) receptors by GnRH: effects of pituitary hormones and relationship to inhibition of testosterone production. *Endocrinology* **110** (1982) 727–733.
23. Marchetti B. and Labrie F.: Further characterization of the direct inhibitory effect of LHRH agonists at the testicular level in the rat. *J. Steroid Biochem.* **20** (1984) 339–342.
24. D'Agata R., Cassorla F., Munabi A. K., Mericq V., Gelato M.C., Albertson B. D. and Loriaux D. L.: Direct effect of the luteinizing hormone releasing hormone analog D-Trp^6-Pro^9-Net-LHRH on rat testicular steroidogenesis. *Steroids* **43** (1984) 201–207.
25. Séguin C., Bélanger A., Labrie F. and Hansel W.: Study of the direct action of luteinizing hormone-releasing hormone agonists at the testicular level in intact rats treated with an antiluteinizing hormone serum. *Endocrinology* **110** (1982) 524–530.
26. Carmichael R. and Belanger A.: Study of the direct effect of LHRH agonist on testicular 17-hydroxylase and 5α-reductase activities in non-hypophysectomized adult rats treated with an anti-luteinizing hormone serum. *Steroids* **43** (1984) 1–12.
27. Hsueh A. J. W. and Erickson G. F.: Extra-pituitary inhibition of testicular function by luteinising hormone releasing hormone. *Nature* **281** (1979) 66–67.
28. Browning J. Y., D'Agata R., Steinberger A., Grotjan H. E. and Steinberger E.: Biphasic effect of gonadotropin-releasing hormone and its agonist analog (HOE766) on *in vitro* testosterone production by purified rat Leydig cells. *Endocrinology* **113** (1983) 985–991.
29. Rea M. A., Weinbauer G. F., Marshall G. R., Nieschlag E.: Testosterone stimulates pituitary and serum FSH in GnRH antagonist-suppressed rats. *Acta Endocr.* **113** (1986) 487–492.
30. Bhasin S., Fielder T. J., Swerdloff R. S.: Testosterone selectively increases serum follicle-stimulating hormonal (FSH) but not luteinizing hormone (LH) in gonadotropin-releasing hormone antagonist-treated male rats: evidence for differential regulation of LH and FSH secretion. *Biol. Reprod.* **37** (1987) 55–59.
31. Kalra S. P. and Kalra P. S.: Neural regulation of luteinizing hormone secretion in the rat. *Endocr. Rev.* **4** (1983) 311–351.
32. Chappel S. C., Ulloa-Aguirre A. and Coutifaris C.: Biosynthesis and secretion of follicle-stimulating hormone. *Endocr. Rev.* **4** (1983) 179–211.