Comparative effects of chronic administration of the non-steroidal antiandrogens flutamide and Casodex on the reproductive system of the adult male rat

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Abstract. The effects of chronic blockade of androgen action by the antiandrogens flutamide and Casodex on serum and pituitary concentrations of LH and FSH, serum and testicular androgen levels, reproductive organ weights, and on spermatogenesis were compared in the adult rat. Animals were treated for 3 and 8 weeks with vehicle, Casodex (20 mg · kg⁻¹ · (day)⁻¹, flutamide (20 mg · kg⁻¹ · (day)⁻¹) and GnRH antagonist (150 µg/day, Decetrelx). Treatment with GnRH antagonist suppressed gonadotropin and testosterone production, reduced the weights of testes, epididymides and seminal vesicles, and inhibited germ cell development. Flutamide administration markedly elevated serum and pituitary levels of gonadotropins as well as serum and testicular androgen concentrations. Casodex-induced elevation of gonadotropin concentrations was less pronounced and serum and testicular levels of androgens did not change significantly. The reduction of seminal vesicle weights was similar after Casodex and GnRH antagonist treatment, whereas flutamide was less effective. Testicular weight and spermatogenesis (assessed by light microscopical and flow-cytometric analysis) remained unaffected by Casodex and flutamide. It is concluded, that 1. Casodex, in contrast to flutamide, is a peripherally selective antiandrogen, and 2. Casodex influences release of gonadotropins into circulation less than flutamide. Therefore this antiandrogen might be useful clinically for selectively blocking androgen actions in the accessory sex glands.

The therapeutic value of antiandrogens in androgen-dependent diseases, e.g. acne, hirsutism, exaggerated sexual behaviour, prostatic hyperplasia, etc. is well established (1). Either steroidal (cyproterone acetate) or non-steroidal (flutamide) antiandrogens are in clinical use. Whereas cyproterone acetate has additional gestagenic properties (2,3) and suppressed pituitary gonadotropin secretion, flutamide is a pure antiandrogen (4). In the male, apart from counteracting the effects of androgens on peripheral androgen target organs, flutamide also prevents the negative feedback action of androgens on the secretion of luteinizing hormone and follicle-stimulating hormone (5).

Consequently, a drastic rise of serum gonadotropin concentrations results from flutamide administration (6). The elevation of LH levels is accompanied by a rise of testosterone production to an extent that during long-term administration, antiandrogenic effects of flutamide are counteracted by the high amounts of testosterone (7). Recently, however, a new non-steroidal antiandrogen, Casodex, which acts selectively on the peripheral androgen target organs has been developed (8). This antiandrogen did not significantly alter serum testosterone levels but pronouncedly reduced the weights of the seminal vesicles and prostate gland in rats and dogs (9). Therefore, this antiandrogen might prove useful for chronic treatment of androgen-dependent disease.

Furthermore, on the basis of its peripheral antiandrogenic effects, we hypothesized that Casodex could be used to prevent androgen action within the testes and, therefore, for studying the effects of...
selective androgen withdrawal on germ cell development. This important question still remains unresolved. The approach of immunization against testosterone failed, as intratesticular testosterone levels remained unaffected (10). Elimination of Leydig cells, the testicular source of androgens, using ethane dimethanesulphonate (11) must be considered with caution because of evidence for direct effects of this drug on germ and Sertoli cells (12,13). We demonstrated recently, that following a 2-week treatment phase with Casodex germ cell numbers were slightly reduced in rats (14).

The present studies were undertaken to compare the effects of long-term administration (3 and 8 weeks) of Casodex and flutamide on the pituitary-testicular axis, reproductive organ weights and spermatogenesis in adult rats. Treatment with a gonadotropin-releasing hormone antagonist, known to suppress endogenous androgen production (15-17), served to compare the effects of removal of androgen with that of blocking androgen action.

Material and Methods

Animals
Adult male Wistar rats weighing 380-400 g (age 15-18 weeks) were obtained from Central Institute for Laboratory Animal Breeding (Hannover, Germany) and were kept in groups of 2 and 3 under conditions of controlled temperature and a 12:12 hour light:dark cycle, with free access to rat chow and tap water. Handling and treatment of the experimental animals were performed according to the regulations of the German Federal Law on the Care and Use of Laboratory Animals.

Compounds
Casodex (ICI 176, 334: (2RS)-4-cyano-3-(4-fluorophenyl-sulphonyl)-2-hydroxy-2-methyl-3-(trifluoro methyl) propionanilide, ICI Pharmaceuticals, Macclesfield, UK) was dissolved in sesame oil. Flutamide (4-nitro-3-trifluoromethyl-isobutryanilide; Schering Corp, Bloomfield, NJ) was dissolved in sesame oil:ethanol (2:1, v/v). The GnRH antagonist Detirelix (RS 68439, Syntex Research, Palo Alto, CA) was dissolved in propylene glycol:water (1:1, v/v).

Experimental protocols
Four groups of 10 rats each received the following treatments sc: Vehicle (controls); GnRH antagonist (150 μg/day); Casodex (20 mg · kg⁻¹ · (day)⁻¹) and flutamide (20 mg · kg⁻¹ · (day)⁻¹), respectively. At this dose Casodex and flutamide enhanced GnRH antagonist-induced testicular involution to a similar extent in a previous study (14). Blood samples were collected from the retro-orbital sinus prior to treatment and thereafter during week 2. After a treatment period of 3 weeks half of the animals in each group were sacrificed. Trunk blood was collected and the testes, epididymides, seminal vesicles (including the coagulating glands) and pituitaries were excised and weighed. The other half of the animals continued to receive the same treatments until week 8. An additional blood sample was collected by eye puncture during week 4. Trunk blood, testis, accessory organs and pituitaries were collected. At both time points one testis was immersed in liquid nitrogen immediately after weighing and then preserved at −80°C for determination of intratesticular androgen levels and flow-cytometric analysis of testicular cells. The other testis was fixed in Bouin’s solution for histological examination.

Hormone measurements
Serum rat LH and FSH were measured by double-antibody radioimmunoassay with reagents supplied by NIDDK (Bethesda, MD). The standard preparations used were LH-RP-1 and FSH-RP-2; tracers were prepared from LH-1-6 and FSH-1-6, and the antisera were anti-rLH-S-9 and anti-rFSH-S-11. Each hormone was analysed in a single assay. The detection limit for both assays was 1.6 mg/l and the intra-assay coefficients of variation were 3.8 and 3.5% for LH and FSH, respectively.

Serum testosterone was measured by a solid-phase, double-antibody RIA technique, using a commercially available iodinated tracer (testosterone-3-(O-carboxymethyl)oximino-2-[¹³¹I]iodohistamine, Amersham International, Braunsweg, Germany) and an antiseraum raised in rabbit against testosterone-3-(carboxymethylxime)-BSA. The bound/free separation was performed by addition of a solution of solid-phase anti-rabbit immunoglobulins (Immunonseal Second Antibody, Biorad, Munich, Germany). The recovery after ether extraction was monitored by addition of trace amounts of [1,2,3H]testosterone (NET-187, NEN, Boston, MA) and the final results were corrected accordingly. The sensitivity was 2 pg/tube (0.07 nmol/l). In 10 consecutive assays the intra-assay coefficients of variation (mean ± SEM) were 8.42±1.42, 4.2±0.59 and 4.37±0.63% for control sera with low, middle and high testosterone concentrations, respectively. The corresponding inter-assay coefficients of variations were 16.62, 6.26 and 3.85%, respectively. The same method was used for determination of intratesticular testosterone concentrations. To this end, part of testicular tissue was homogenized in phosphate buffer (100 g/l), ether extracted, and measured in the testosterone RIA without chromatography. Since the tests contains significant amounts of dihydrotestosterone (18) and testosterone cross-reacts with dihydrotestosterone, the term "testicular androgen" is used for expression of the results.
**Evaluation of spermatogenesis**

For histological analysis, testes were immersion-fixed in Bouin's fluid, dehydrated and embedded in historesin (LKB, Bromma, Sweden); 2-μm sections were cut and stained with periodic acid Schiff's reagent and hematoxylin. Germ cells were enumerated in stage VII seminiferous tubules (19) containing representative germ cells (A-spermatogonia, preleptotene and pachytene spermatocytes, step 7 round and step 19 elongated spermatids).

The cell counts were corrected for section thickness (20) and for tubular shrinkage on the basis of Sertoli cell counts. Enumeration of germ cells in stage VII was chosen because this stage has been shown to be particularly sensitive to removal of gonadotrophic hormones (21) and is believed to be most dependent on testosterone (22).

On this ground any antiandrogenic action of Casodex or flutamide within the testis should become detectable. In addition, the diameter of the seminiferous tubules was measured in cross-sections of stage VII tubules (N=20/animal) by means of a semi-automatic image analysis system (MOP-Videoplan, Zeiss, Oberkochen, Germany).

As a second measure, the testicular cell numbers were determined by flow cytometry as previously described in detail (14) using the PAS II sorter (Partec GmbH, Münster, Germany, 23). This type of analysis yields different cell populations expressed as 'C' values. '1CC' represents elongated spermatids; '1C' round spermatids; '2C' indicates cells with a diploid DNA content (e.g. G1-spermatogonia, G2-Sertoli-cells), and '4C' stands for cells during mitotic or primary meiotic division following DNA synthesis and before division (e.g. leptotene, zygotene, pachytene and diplotene primary spermatocytes, spermatogonial and non-germinal cells during the G2 phase) (24,25).

**Statistical analysis**

One-way and two-way analysis of variance were used to detect overall significance and followed by Tukey's test to determine significant differences between groups and different time points at the 5% probability level. Pituitary gonadotropin levels were log-normalized prior to statistical evaluation. Data are expressed as mean ± sem.

**Results**

**Organ weights**

Fig. 1 shows the weight of testes, epididymides, seminal vesicles + coagulating gland and pituitary glands. In the GnRH antagonist-treated group testes weight after 3 weeks was one third (0.6±0.06 g) and was further reduced after 8 weeks to one sixth (0.34±0.009 g) of controls. Epididymal weight in animal treated with Casodex and flutamide was lowered to a similar extent and was lower (p<0.05) than in controls. The GnRH antagonist group had the lowest epididymal weights. Dissimilar to the flutamide group, testes weight in the Casodex group after 8 weeks, was slightly, but significantly lower than in control. The weights of seminal vesicles + coagulating glands were lower (p<0.05) in control-treated than in flutamine-treated animals. Treatment with Casodex did not affect pituitary and body weight. After 8 weeks, pituitary weight in the GnRH antagonist group was lower than in the controls. The gain in body weight in the flutamide (92±10 g) and GnRH antagonist

![Figure 1](https://via.placeholder.com/150)

**Fig. 1.**

Weight of (A) testes, (B) epididymides, (C) seminal vesicle + coagulating gland (CG) and (D) pituitaries in rats receiving vehicle (CONT), GnRH antagonist (150 μg/day; ANT), Casodex (20 mg · kg⁻¹ · day⁻¹; CAS) and flutamide (20 mg · kg⁻¹ · (day)⁻¹; FL) for 3 (open bars) and 8 (solid bars) weeks. * significantly different from CONT. Values are mean ± SEM, N=5.

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(90±16 g) groups was lower than in the Casodex (146±7 g) and controls (150±4 g) groups.

**Pituitary and serum LH and FSH**

Table 1 shows the pituitary concentrations of LH and FSH. Following 3 weeks of GnRH antagonist administration, pituitary LH and FSH concentrations were 50 and 30%, respectively, compared with controls. In the Casodex-treated group LH concentrations were elevated 2-fold but were rather variable. With flutamide a 4-fold increase in pituitary LH concentrations was observed. Pituitary FSH levels were not significantly influenced by the antiandrogens.

After 8 weeks of GnRH antagonist treatment, pituitary LH and FSH concentrations were 30 and 20%, respectively, compared with controls. Both antiandrogens significantly raised pituitary LH levels. A significant elevation of FSH concentrations was seen with Casodex only.

After 3 weeks of GnRH antagonist treatment, the serum concentrations of LH and FSH were markedly suppressed (Fig. 2). Following Casodex administration, LH levels increased 3-fold (p>0.05) and more than 10-fold with flutamide (p<0.05). Flutamide also increased the concentrations of FSH (p<0.05). After 8 weeks gonadotrophic hormone concentrations were suppressed in the GnRH antagonist-treated group throughout the entire treatment phase. Among Casodex-treated animals, which had slightly higher FSH concentrations in week 0, significant elevations of FSH were observed during weeks 4 and 8. Concentrations of LH became increased after 8 weeks only (p<0.05). Flutamide markedly raised FSH and LH levels at all time points (p<0.05).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>LH (ng/mg)</th>
<th>FSH (ng/mg)</th>
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</thead>
<tbody>
<tr>
<td>3 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1020±69</td>
<td>460±70</td>
</tr>
<tr>
<td>GnRH antagonist</td>
<td>528±30</td>
<td>128±9*</td>
</tr>
<tr>
<td>Casodex</td>
<td>2175±531*</td>
<td>608±98</td>
</tr>
<tr>
<td>Flutamide</td>
<td>4069±987*</td>
<td>766±11</td>
</tr>
<tr>
<td>8 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1268±63</td>
<td>291±11</td>
</tr>
<tr>
<td>GnRH antagonist</td>
<td>387±50*</td>
<td>52±3*</td>
</tr>
<tr>
<td>Casodex</td>
<td>2870±216*</td>
<td>620±105*</td>
</tr>
<tr>
<td>Flutamide</td>
<td>3376±345*</td>
<td>429±54</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (N=5/group). GnRH antagonist: 150 μg/day; Casodex: 20 mg · kg⁻¹ · (day)⁻¹ and flutamide: 20 mg · kg⁻¹ · (day)⁻¹. * significantly different from controls (p<0.05).

**Table 1.**

Pituitary concentration of LH and FSH in rats receiving vehicle (controls), GnRH antagonist, Casodex and flutamide for 3 and 8 weeks.

**Fig. 2.**

Serum concentration of (A) FSH, (B) LH and (C) testosterone in rats receiving vehicle (○), GnRH antagonist (150 mg/day, □), Casodex (20 mg · kg⁻¹ · (day)⁻¹, Δ) and flutamide (20 mg · kg⁻¹ · (day)⁻¹, ◇) for 8 weeks. The solid symbols denote another group of rats sacrificed after 3 weeks of treatment. * significantly different from controls. Values are mean ± SEM, N= 5/group.
Serum testosterone and testicular androgens

Serum testosterone is shown in Fig. 2. In the GnRH antagonist group serum testosterone levels remained close to the detection limit of the assay. During Casodex administration, testosterone concentrations were stimulated 2- to 3-fold, but without statistical significance, at all time points. Following flutamide, the concentrations of testosterone were 10- to 20-fold increased, with the highest values present during weeks 3-8 (p<0.05).

The testicular concentrations of androgens (Fig. 3) were below 10% of controls after 3 and 8 weeks of GnRH antagonist treatment. Unlike flutamide which raised testicular androgen levels 5- to 6-fold (p<0.05), the 1.8- to 2-fold increase in androgen concentrations following Casodex administration did not attain statistical significance.

Spermatogenesis

Qualitatively normal spermatogeneration was present in the Casodex and flutamide groups. Data for quantitative enumeration are shown in Fig. 4. In the Casodex as well as in the flutamide group, the numbers of round and elongated spermatids were slightly but significantly different from those in controls. After 8 weeks, higher numbers of type A-spermatogonia were achieved in the Casodex and flutamide groups. In the GnRH antagonist-treated group, the elongated spermatids were absent and a marked reduction in the number of round spermatids and preleptotene and pachytene spermatocytes was recorded.
Table 2.
Flow cytometric enumeration of germ cells (10⁶/testis) in rats receiving vehicle (controls), GnRH antagonist, Casodex and flutamide for 3 and 8 weeks.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>GnRH antagonist</th>
<th>Casodex</th>
<th>Flutamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C</td>
<td>65.2±6.2</td>
<td>48.2±3.0*</td>
<td>55.6±4.9</td>
<td>58.4±6.2</td>
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<td>4C</td>
<td>30.5±2.4</td>
<td>12.6±1.2*</td>
<td>23.5±1.8</td>
<td>23.7±2.0</td>
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<tr>
<td>1C</td>
<td>106±9.1</td>
<td>17.4±4.5*</td>
<td>89.9±5.9</td>
<td>91.8±8.4</td>
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<tr>
<td>1CC</td>
<td>82.1±8.5</td>
<td>0±0</td>
<td>70.2±8.1</td>
<td>68.3±5.3</td>
</tr>
<tr>
<td>8 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C</td>
<td>92.4±8.1</td>
<td>53.8±4.7*</td>
<td>82.9±6.0</td>
<td>76.8±3.6</td>
</tr>
<tr>
<td>4C</td>
<td>40.8±1.8</td>
<td>11.7±1.0*</td>
<td>37.3±1.2</td>
<td>32.5±3.0</td>
</tr>
<tr>
<td>1C</td>
<td>123.1±3.3</td>
<td>3.5±0.6*</td>
<td>126.±4.8</td>
<td>112.6±4.5</td>
</tr>
<tr>
<td>1CC</td>
<td>145.2±10</td>
<td>0±0</td>
<td>153±7.7</td>
<td>134±10.7</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (N=5/group). GnRH antagonist: 150 µg/day; Casodex: 20 mg · kg⁻¹ · (day)⁻¹; flutamide: 20 mg · kg⁻¹ · (day)⁻¹. * significantly different from controls (p<0.05).

The diameter of stage VII seminiferous tubules (Fig. 4) was significantly lower in the GnRH antagonist group (p<0.05). None of the antiandrogens significantly influenced seminiferous tubule diameters. The outcome of flow-cytometric analysis is given in Table 2. In the GnRH antagonist group, 1CC cells were absent and 1C, 2G and 4C cells were significantly lower than in the control groups. At both time points neither the spermatids (1CC and 1C cells) nor 2C and 4C cells in the Casodex and flutamide group were significantly different from those in control.

Discussion

The findings obtained during the present investigation support previous work (8,9,14) that Casodex is a so-called peripherally selective antiandrogen (8), i.e. Casodex blocks the peripheral target organ androgen receptor, but to a lesser extent the central (brain) androgen receptor. Throughout an administration period of 8 weeks no significant elevation of serum concentrations of testosterone and intratesticular levels of androgens was observed. Furthermore, the weight of the seminal vesicles was reduced to a similar extent as when GnRH antagonists was given for an equally long treatment phase. Since GnRH antagonist virtually abolished testicular androgen production (15-17), the similarity of the effects of the seminal vesicles strongly suggests that Casodex completely blocked androgen action in that organ. The weight and function of the seminal vesicles is particularly dependent on androgens (1,26).

Different from Casodex, flutamide induced a pronounced elevation of serum and testicular androgen levels and was considerably less effective in maintaining the decrease of seminal vesicle weights. The reduced ability of flutamide to do so was already apparent after 3 weeks. Even until 2 weeks of treatment, flutamide was found less effective than Casodex in that respect (14). Since flutamide caused a manifold higher elevation of LH secretion compared with Casodex, it is most likely that this compensatory rise of LH and androgens compromised the effects of flutamide on seminal vesicle weight in the present study. This view is entirely consistent with previous studies (1,7). It has also been reported that Casodex has a longer half-time in the circulation and a higher in vivo potency when compared with flutamide (9). Whether the increased half-time of Casodex has contributed to its effects on the seminal vesicles in the present study remains unknown.

In the present study, pituitary concentrations of LH were consistently higher following administration of flutamide in comparison to Casodex. Since flutamide did not prevent the effects of androgens on LH in cultured pituitary cells (27), but elevated GnRH content in the median eminence (5,6), this antiandrogen probably acts at the hypothalamic level. In the present investigation, Casodex elevated pituitary LH within 3 and 8 weeks, although the response was variable and became statistically significant after 8 weeks only. Inspite of increased pituitary LH, serum levels of LH were elevated significantly after 8 weeks only, but not after 4 weeks. It has been reported that Casodex does not enter the central brain tissue but has access to the pituitary (28). It, therefore, appears possible that the elevation of pituitary gonadotropin levels reflects a diminished feedback of androgens at the pituitary level. On the other hand, in vitro studies revealed that androgens actually blocked the release of gonadotropic hormones from the pituitary (29). Taken together, these observations suggest that androgens exert their negative feedback action both at the hypothalamic and pituitary levels. On the basis of the higher serum concentrations of LH in flutamide- than in Casodex-treated animals, one
might assume that the hypothalamus is the more important site of androgen feedback action on the release of gonadotropins under in vivo conditions.

Epididymal weights were markedly lowered by GnRH antagonist, but to a significantly lesser extent by the antiandrogens. This is most likely due to the fact that the testes in the Casodex- and flutamide-treated groups still produced spermatozoa, which are known significantly to contribute to the epididymal weight. In terms of reducing epididymal weight both Casodex and flutamide were equally effective. The high testosterone levels induced by flutamide most probably counteract its effects on androgen target organs (see above). In consequence of that, epididymal weight appeared to respond to a lesser extent when exposed to a given amount of androgens than the seminal vesicles. Concordant with this assumption, a higher sensitivity to androgens of the seminal vesicles and prostates than of the epididymis has been reported in rats (31) and monkeys (32).

Unlike our previous study (14) where Casodex induced a slight germ cell loss within 2 weeks, no such effect was observed after 3 and 8 weeks in the present investigation. Both light microscopic and flow-cytometric analysis of testicular cells were used. In some instances the results obtained by the histological and cytometrical approach, did not fully coincide. However, both methods failed to reveal any significant cell loss induced by the treatments in relation to the control group. Undoubtedly Casodex reaches the testis and can block androgen action when intratesticular androgen levels are below normal (14). In the intact testis, androgen levels are very much higher than in the circulation (33). It cannot be decided from the present work whether or to what extent Casodex could block the testicular androgen receptor. Since testosterone is a major stimulator of spermatogenesis (34,35) and Casodex did not affect spermatogenic function, it is quite likely that Casodex was unable to compete with the high intratesticular androgen levels at the receptor. Alternatively, since FSH also strongly supports germ cell production (36-39), a partial testicular androgen receptor blockade might have been insufficient to cause detectable effects on the spermatogenesis in the presence of normal FSH concentrations. In the dog, a 42 days treatment phase with 4 mg/kg of Casodex induced testicular atrophy in 60% of the animals (9).

From the present experiments it is concluded that 1. the peripherally selective antiandrogenic action of Casodex persists during long-term treatment, and 2. Casodex influences the release of gonadotropins into circulation less than flutamide. Therefore, this antiandrogen might become useful clinically for blocking androgen effects on accessory sex glands.

Acknowledgments

This work was performed while Dr R. K. Chandolia was the recipient of a German Academic Exchange Service (DAAD) grant. We are indebted to Dr B. Furr (ICI, Macclesfield, UK), Drs B. H. Vickery and J. J. Nestor, Jr (Syntex Corp, Palo Alto, CA), Schering Corp (Bloomfield NJ) and Essex Pharma (München, Germany) for provision of Casodex, Dutirelix and flutamide, respectively. We gratefully acknowledge the technical assistance of R. Sandhowe, M. Möller-Hubert, M. Heuermann and G. Stelke and the language assistance of S. Nieschlag, MA.

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Received January 14th, 1991.
Accepted June 5th, 1991.

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