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EVALUATION OF A PERIPHERALLY SELECTIVE ANTIANDROGEN (CASODEX) AS A TOOL FOR STUDYING THE RELATIONSHIP BETWEEN TESTOSTERONE AND SPERMATOGENESIS IN THE RAT

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Summary—The endocrine profile and the effects on spermatogenesis of the new antiandrogen, Casodex ((2RS)-4-cyano-3-(4-fluorophenylsulphonyl)-2-anilide, CAS) were evaluated in the adult rat. In the first experiment rats were administered CAS at daily doses of 10, 20 and 40 mg/kg for 14 days. For comparison groups receiving flutamide (FL, 10 mg/kg) and ethane dimethane sulphonate (EDS) were included. Unlike FL, administration of CAS (10 and 20 mg/kg) did not significantly raise serum concentrations of gonadotropic hormones and testosterone. With 40 mg/kg CAS gonadotropin secretion, but not testosterone levels, were elevated on day 15. Administration of CAS lowered the weight of the seminal vesicles and coagulating glands comparable to the administration of the Leydig cell toxin EDS. In contrast to FL a significant loss of germ cells in stage VII of spermatogenesis was observed with CAS. In a second experiment the ability of FL and CAS to block testicular androgen action was compared in rats with reduced testicular androgen production induced by a gonadotropin-releasing hormone antagonist. Both antiandrogens markedly enhanced spermatogenic involution as revealed by quantitative flow cytometric analysis of germ cell numbers.

The study demonstrates that (a) CAS is a peripherally selective antiandrogen and (b) CAS might provide a feasible approach to study androgen dependence of spermatogenesis in the presence of normal FSH levels.

INTRODUCTION

The pivotal role of testosterone, besides follicle-stimulating hormone (FSH), for mammalian spermatogenesis is well established. According to the prevailing concept the combination of testosterone and FSH is required for normal spermatogenesis [1, 2]. The common approach to study the relative importance of testosterone and FSH for the spermatogenic process comprises experimental induction of a hypogonadotropic state followed by substitution with the hormones of interest [3]. One important results of these studies was the observation that the efficacy of FSH is apparently influenced by the presence of intratesticular androgen. Selective removal of androgens or blockade of androgen action provides an alternative possibility to investigate the effects of testosterone and FSH on germ cell development without a need for hormone substitution. The avoidance of hormone replacement is highly desirable since exogenous hormone administration will not restore the physiological pattern of the respective hormone.

Immunization against testosterone did not prove suitable for the study of intratesticular regulation of spermatogenesis since it neutralises only circulating but not intratesticular testosterone [4]. Elimination of Leydig cells, the testicular source of androgens, by the cytotoxic drug ethane dimethane sulphonate (EDS) induces spermatogenic involution [5]. However, this drug might in addition directly affect germ cells [6, 7]. As a consequence of androgen withdrawal hypersecretion of FSH and luteinizing hormone (LH) occurs. Ideally, androgen action should be prevented in the peripheral target organs only, without a central effect, thus rendering gonadotropic hormones unaltered.

Steroidal antiandrogens are not suited as experimental tools for this purpose since these compounds have progestational and glucocorticoid activities [8, 9]. Non-steroidal antiandro-

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gens centrally antagonize androgen action and consequently cause a compensatory rise of gonadotropin secretion and testosterone production [10, 11]. The increased androgen production counteracts the effectiveness of these drugs. It has been reported recently, however, that the non-steroidal antiandrogen Casodex (CAS) acts selectively on the peripheral androgen-dependent organs [12, 13]. According to these studies no elevation of LH and testosterone secretion occurred during administration of CAS. Therefore, the present work was undertaken in rats, to evaluate the potential of CAS for studying hormonal regulation of spermatogenesis.

EXPERIMENTAL

Animals

Adult male Wistar rats, weighing between 400 and 420 g, were obtained from the 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 h light:dark cycle, with free access to rat chow and tapwater. 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-fluorophenylsulphonyl)-2-anilide, ICI Pharmaceuticals, Macclesfield, England) and Flutamide (4-nitro-3-trifluromethyl-isobutyranilide; Schering Corp., Bloomfield, N.J., U.S.A.) were dissolved in sesame oil and sesame oil:ethanol (1:1), v/v), respectively and administered subcutaneously. Ethane dimethane sulphonate (EDS) was given intraperitoneally in dimethylsulphoxide:water (1:3, v/v). The GnRH antagonist Detirelix ([*N*-Ac-D-Nal(2)^1, D-pCL-Phe^2, D-Trp^3, D-hArg(Et$_2$)^6, D-Ala^{10}]-GnRH, Syntex Research, Palo Alto, Calif., U.S.A.) was dissolved in propylene glycol:water (1:1, v/v) and injected subcutaneously.

Experimental protocols

Study 1. Six groups (8 rats/group) were subjected to the following treatments: Vehicle (CONT); Casodex (10 mg/kg/day, CAS1); Casodex (20 mg/kg/day, CAS2); Casodex (40 mg/kg/day, CAS4); Flutamide (10 mg/kg/day, FL1) and EDS (100 mg/kg, single injection).

Study 2. Four groups (8 rats/group) received the following treatments: CONT; GnRH antagonist (200 μg/rat/day, ANT); ANT + Flutamide (20 mg/kg/day, FL2) and ANT + CAS2.

Blood samples were collected from the retro-orbital sinus prior to treatments and thereafter on days 5 and 10. On day 15 both studies were terminated. The animals were sedated with carbon dioxide and decapitated. Trunk blood was collected for determination of LH, FSH and testosterone concentrations. The testes, epididymides, seminal vesicles (including the coagulating glands) and pituitaries were excised and weighed. One testis was immersed in liquid nitrogen immediately after weighing and then preserved at −80°C. The other testis was fixed in Bouin's solution for histological examination in study 1. In study 2 frozen testicular tissue was used for flow cytometric analysis of germ cells.

Hormone measurements

Serum rat LH and FSH were measured by double antibody radioimmunoassay (RIA) with reagents supplied by NIADDK (Bethesda, Md, U.S.A.). 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 ng/ml and the intra-assay coefficient of variation was 3.9 and 3.0% for LH and FSH respectively. Serum testosterone concentrations were measured by RIA as described previously [14]. For determination of intratesticular androgen concentration, testes were homogenised in phosphate buffer (1 g/3 ml) and after ether extraction the RIA was performed [15] but without further chromatography.

Evaluation of spermatogenesis

For histological analysis, testes from study 1 were immersion-fixed in Bouin's fluid, dehydrated, and embedded in historesin (LKB, Bromma, Sweden). Two micrometre sections were cut and stained with periodic acid Schiff's reagent and haematoxylin. Germ cells were enumerated in stage VII seminiferous tubules [16] 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 [17] and for tubular shrinkage on the basis of Sertoli cell counts. This type of analysis was chosen for study 1 since stage VII is particularly sensitive to gonadotropic hormone deprivation [18] and is believed to be highly dependent on testosterone [19]. Therefore antiandrogen actions of CAS within the testis should be traceable by this approach. In addition, the diameter of seminiferous tubules was measured in cross sections of stage VII tubules ($n = 20$/animal) using a semiautomatic image analysis system (MOP-Videoplan, Zeiss, Oberkochen, Germany).

Since ANT inhibits germ cell development in all stages of spermatogenesis, the histological approach was not used in study 2. Instead the testicular cell numbers were determined by flow cytometry. Approximately 20 mg testicular tissue was minced with surgical blades and finally dispersed with a low speed homogenizer for 3–4 s in 4 ml of 0.1 mol/l citric acid containing 0.5% Tween-20 (C 100 T). After incubation for 20 min at room temperature, the single cells were fixed by adding 46 ml ethanol (96%). After fixation for 24 h, 10 ml of the sample were centrifuged and the pellet resuspended in 1 ml pepsin 0.5% for 5 min. Subsequently, the testicular cells were stained with 9 ml of 4,6-diamidino-phenyl-indole (DAPI)/sulforhodamine 101-staining solution [20]. Flow cytometric analysis and quantification of DNA-stained cells was performed using the PAS II sorter (Partec GmbH, Münster, Germany) [21]. The different cell populations based on their DNA content are expressed as 'C' values. '1CC' represents elongated spermatids, '1C' round spermatids. '2C' cells indicates with a diploid DNA content (e.g. G_1-spermatogonia, G_1-Leydig-cells, G_1-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 G_2 phase) [22, 23].

Statistical analysis

Data were analysed using one-way and two-way analysis of variance followed by Tukey's test to determine significant differences between groups and different time points at the 5% probability level. Data are expressed as mean ± SEM.

RESULTS

Study 1

Organ weights. Weights of the testes, epididymides, seminal vesicles plus coagulating glands, and pituitaries are depicted in Table 1. Except for the EDS-treated group no significant reduction of testis weights was observed. Epididymal weights in the CAS and FL groups were reduced significantly compared to CONT and were further lowered following EDS treatment. The weights of seminal vesicles were also reduced in all animals. This effect was more pronounced in the CAS and EDS groups in relation to the FL group. There was no significant variation in the organ weights among CAS groups. Pituitary and body weights were not influenced by the antiandrogens.

Hormone concentrations. Serum FSH levels (Fig. 1A) within all CAS groups showed non-significant variation throughout the experimental period, while these levels became progressively elevated in the FL group. In the EDS-treated group FSH levels were elevated on day 5 but dropped on days 10 and 15. Concentrations of serum LH (Fig. 1B) were not significantly different within animals receiving CAS. For unknown reasons, in animals of the CAS4 group higher basal concentrations of LH than in CAS1, CAS2 and CONT were encountered. By day 15 the LH levels were increased proportionally to the dose of CAS. This effect only attained statistical significance in the CAS4 when compared to CONT. In the FL and EDS groups, LH levels were 3- and 4-fold elevated at all time points.

The concentration of serum testosterone among the CAS groups was not statistically different from the control group at any time point (Fig. 1C). In the FL group serum testosterone levels showed a significant increase at all points and on day 15 the levels were 6-fold higher than control animals. In the EDS group the serum testosterone was below or around the

Table 1. Weight of organs after 2 weeks in vehicle (CONT), Casodex (CAS), Flutamide (FL1) and ethane dimethanesulphonate (EDS) treated rats

	Testes (g)	Epididymides (g)	Seminal vesicles (g)	Pituitaries (mg)
CONT	1.904 ± 0.058	1.276 ± 0.048	0.664 ± 0.034	10.06 ± 0.28
CAS1	1.845 ± 0.076	0.034 ± 0.033*	0.268 ± 0.016*	9.89 ± 0.72
CAS2	1.766 ± 0.07	0.884 ± 0.032*	0.266 ± 0.017*	9.45 ± 0.64
CAS4	1.801 ± 0.047	0.916 ± 0.028*	0.252 ± 0.01*	10.40 ± 0.45
FL1	1.891 ± 0.068	0.955 ± 0.026*	0.369 ± 0.012*	9.96 ± 0.55
EDS	1.146 ± 0.092*	0.721 ± 0.038*	0.223 ± 0.023*	8.46 ± 0.34*

Values are mean ± SEM ($n = 8$/group). CAS1: 10 mg/kg/day; CAS2: 20 mg/kg/day; CAS4: 40 mg/kg/day; FL1: 10 mg/kg/day; EDS: 100 mg/kg once. *Significantly different from control ($P < 0.05$).

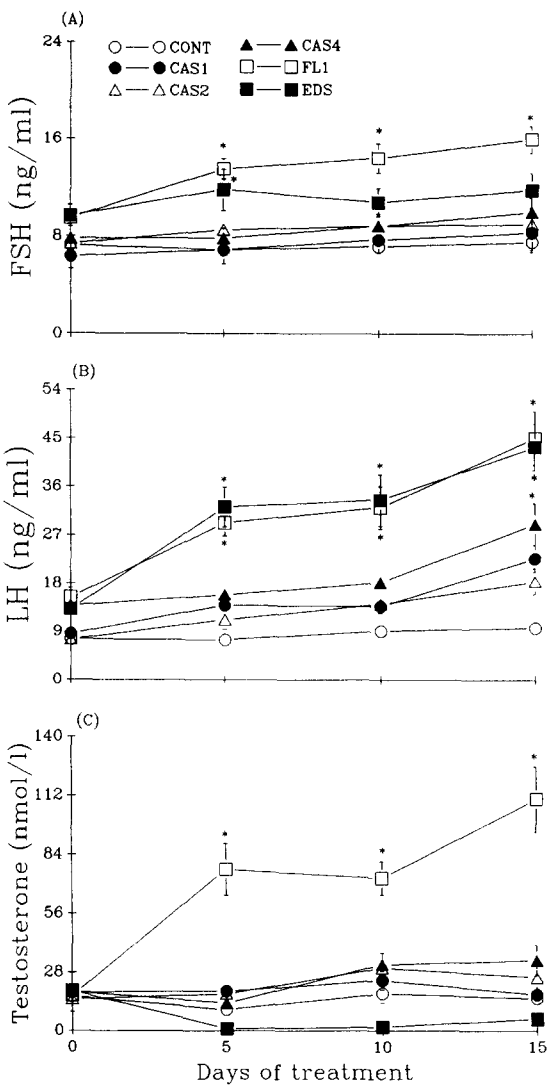

Fig. 1. Serum concentration of FSH (A) LH (B) and testosterone (C) during 2 weeks of treatment with vehicle (CONT); Casodex 10 mg/kg/day s.c. (CAS1); Casodex 20 mg/kg/day s.c. (CAS2); Casodex 40 mg/kg/day s.c. (CAS4); Flutamide 10 mg/kg/day s.c. (FL1); and ethane dimethanesulphonate 100 mg/kg once i.p. (EDS). The values with * significantly different from controls ($P < 0.05$). Values are means ± SEM, $n = 8$.

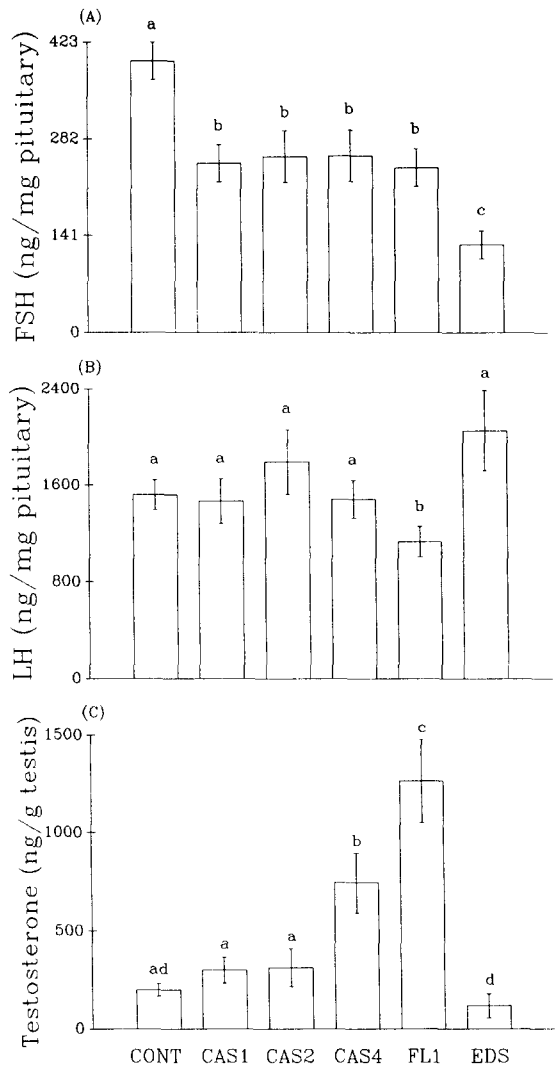

Fig. 2. Pituitary concentration of FSH (A) LH (B) and intratesticular testosterone (C) after 2 weeks of treatment with vehicle (CONT); Casodex 10, 20 and 40 mg/kg (CAS1, CAS2 and CAS4); Flutamide 10 mg/kg (FL1) and ethane dimethanesulphonate 100 mg/kg (EDS). The concentrations with different superscript are significantly ($P < 0.05$) different from each other. The values are means ± SEM, $n = 8$.

detection limit of the assay. Testicular concentrations of androgen (Fig. 2C) were slightly but not significantly raised by CAS1 and CAS2 in comparison to CONT while with CAS4 a pronounced elevation occurred. Following administration of FL testicular androgens increased further. In the EDS-treated group testicular androgen production had started to recover.

The concentrations of pituitary LH (Fig. 2B) were significantly lower in FL group than CONT while in all other groups the levels were within control range. Concentrations of pituitary FSH were significantly lower in all treated groups in comparison with controls and the lowest concentration was seen in the EDS-treated group (Fig.2A).

Testicular histology. With the exception of the EDS-treated group qualitatively normal spermatogenesis was observed in all groups. In quantitative terms (Table 2) some loss of germ cells following administration of CAS was observed. Strongest effects were seen after administration of EDS whilst FL did not affect germ cell numbers. Counts for A spermatogonia, preleptotene and pachytene spermatocytes, and spermatids were consistently lower following administration of CAS. This effect, although not always significant, was most pronounced

Table 2. Germ cell numbers (stage VII) and diameter of seminiferous tubules after 2 weeks in vehicle (CONT), Casodex (CAS), Flutamide (FL) and ethane dimethanesulphonate (EDS) treated rats

	CONT	CAS1	CAS2	CAS4	FL1	EDS
A spermatogia	0.41 ± 0.03	0.24 ± 0.01*	0.33 ± 0.03	0.23 ± 0.03*	0.30 ± 0.06	0.32 ± 0.04
Preleptotene spermatocytes	11.98 ± 0.32	10.10 ± 0.15*	10.78 ± 0.27	9.80 ± 0.25*	12.91 ± 0.64	15.31 ± 0.11*
Pachytene spermatocytes	9.01 ± 0.34	8.02 ± 0.25*	8.05 ± 0.30*	7.29 ± 0.14*	9.89 ± 0.24	8.00 ± 0.34*
Step VII spermatids	28.59 ± 0.81	25.52 ± 0.81	26.41 ± 1.71	24.02 ± 0.72*	28.68 ± 0.85	18.93 ± 1.13*
Elongated spermatids	29.78 ± 0.81	26.12 ± 1.14	24.94 ± 0.83	24.40 ± 1.45*	28.77 ± 2.13	12.87 ± 2.98*
Diameter of seminiferous tubules	394.0 ± 11.61	393.6 ± 7.33	416.6 ± 10.23	418.6 ± 15.83	409.5 ± 13.98	339.0 ± 16.41*

Values are mean ± SEM ($n = 8$/group). CAS1: 10 mg/kg/day; CAS2: 20 mg/kg/day; CAS4: 40 mg/kg/day; FL1: 10 mg/kg/day; EDS: 100 mg/kg once. *Significantly different from control ($P < 0.05$).

with CAS4. The diameter of seminiferous tubules differed from CONT in the EDS group only (Table 2).

Study 2

This study was performed in order to determine whether CAS can reach the testis in sufficient amounts for blocking androgen action. To avoid interference of the high intratesticular androgen levels the animals received ANT treatment to reduce gonadotropin secretion and thereby testicular androgen production.

Organ weights. Following ANT administration the weights of testes, epididymides and seminal vesicles plus coagulating glands were markedly reduced (Table 3). Pituitary weight remained unaffected. With ANT testicular weight was reduced to 0.82 ± 0.03 g compared to 1.99 ± 0.05 g in the vehicle-treated group. The addition of CAS2 or FL2 reduced further testis weight to 0.65 ± 0.02 g and 0.58 ± 0.02 g, respectively, compared to ANT alone. The weights of the epididymides and seminal vesicles were comparably suppressed in the groups receiving ANT alone or along with FL2 or CAS2. Body weight remained unaltered.

Hormone concentrations. Serum concentrations of LH and FSH, after 7 and 15 days, were suppressed to undetectable levels or levels close to the detection limit of the assay (Fig. 3). Pituitary gonadotropin concentrations were reduced to a similar degree in all ANT-administered groups (Table 4). Serum testosterone concentrations were totally inhibited by ANT in the presence and absence of antiandrogens (Fig. 3C) and testicular androgen concentrations were 6–8% of control levels.

Spermatogenesis. The number of 2C cells remained within the control range following ANT treatment but was significantly lower in ANT + CAS2 or ANT + FL2 groups (Fig. 4). After administration of ANT alone the number of 4C, 1C and 1CC cells were reduced to 54, 30 and 8 respectively, compared to the control group. The addition of CAS2 or FL2 further lowered the number of 4C and 1C cells from 58.73 ± 6.59 million/testis in the ANT alone group to 20.11 ± 4.49 and 19.14 ± 5.20 million/testis, respectively. Elongated spermatids (1CC) were absent in groups receiving the combination of ANT and CAS or FL.

DISCUSSION

The aim of the present investigation was to evaluate the endocrine profile (study 1) of the antiandrogen CAS and its effects on spermatogenesis (study 1 and 2) in adult rats. The rationale for this study was based upon the report by Furr and coworkers [12,13] that CAS is a peripherally selective antiandrogen without affecting gonadotropin secretion. Therefore, CAS was employed to evaluate whether selective blockade of androgen action within the

Table 3. Weight of organs (study 2) after 2 weeks of treatment with GnRH antagonist (ANT) alone or in combination with Casodex (CAS2) or Flutamide (FL2)

	Testes (g)	Epididymides (g)	Seminal vesicles (g)	Pituitaries (mg)
CONT	1.995 ± 0.054	1.138 ± 0.021	0.562 ± 0.031	13.175 ± 0.568
ANT	0.817 ± 0.028*	0.361 ± 0.024*	0.176 ± 0.007*	11.912 ± 0.331
ANT + CAS2	0.651 ± 0.021*	0.339 ± 0.016*	0.163 ± 0.004*	13.062 ± 0.442
ANT + FL2	0.582 ± 0.024*	0.316 ± 0.011*	0.168 ± 0.011*	11.60 ± 0.649

Values are mean ± SEM ($n = 8$/group). ANT (200 μg/day); CAS2 (20 mg/kg/day) and FL2 (20 mg/kg/day). *Significantly different from control ($P < 0.05$).

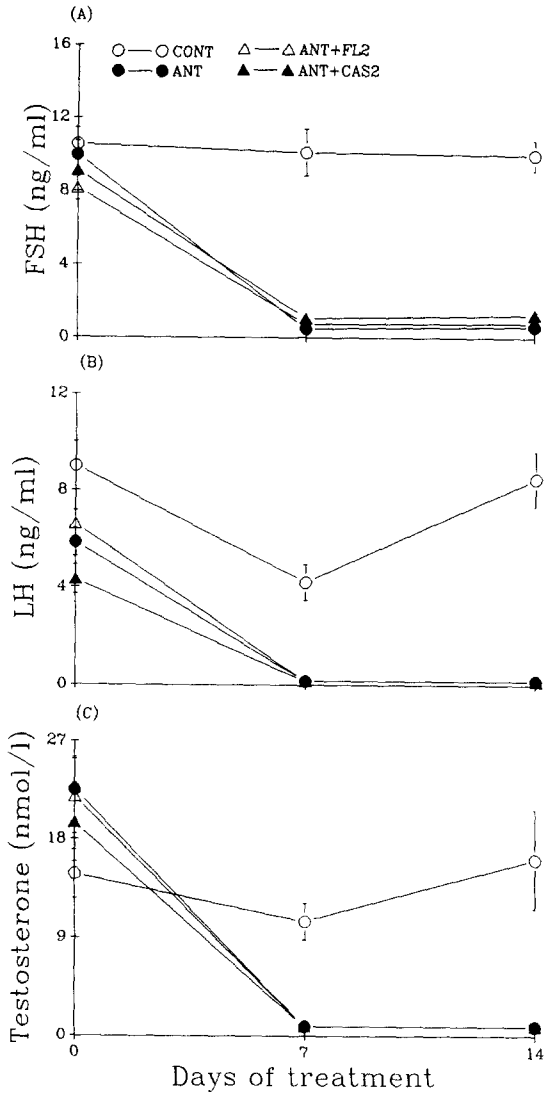

Fig. 3. Serum concentration of FSH (A) LH (B) and testosterone (C) during 2 weeks of treatment with vehicle (CONT); GnRH antagonist 200 μg/day (ANT); ANT + Casodex 20 mg/kg/day (CAS2) and ANT + Flutamide 20 mg/kg/day (FL2). The values are means ± SEM, $n = 8$.

testis by CAS could be used to define the importance of androgens for spermatogenesis in the presence of unaltered FSH levels.

Casodex at daily doses up to 20 mg/kg did not significantly influence serum concentrations of LH, FSH and testosterone although a trend towards raised hormone levels was seen by day 15. At a dose of 40 mg/kg serum gonadotropin levels were significantly elevated by day 15 only, without an accompanying rise of testosterone. In contrast FL induced a marked hypersecretion of all hormones as early as 5 days after initiation of treatment. These data are consistent with previous reports [10, 11, 24]. Daily amounts of 25 mg/kg for 14 days did not affect serum levels

Table 4. Concentration of pituitary LH and FSH after 2 weeks of treatment with GnRH antagonist (ANT) alone or in combination with Casodex (CAS2) or Flutamide (FL2)

	LH (ng/mg)	FSH (ng/mg)
CONT	1693 ± 209	520 ± 46
ANT	1067 ± 81*	171 ± 16*
ANT + CAS2	897 ± 44*	212 ± 9*
ANT + FL2	1116 ± 90*	217 ± 20*

Values are mean ± SEM ($n = 8$/group). ANT (200 μg/day); CAS2 (20 mg/kg/day) and FL2 (20 mg/kg/day). *Significantly different from control ($P < 0.05$).

of LH or testosterone [12, 13]. Snyder *et al.* [25] reported an elevation of serum testosterone levels following 14 days of CAS administration but 5-fold less than flutamide. The differences in the endocrine profiles of CAS and FL indicate different mechanisms of action of these antiandrogens. Most probably FL exerts a central effect by inhibiting the negative feedback action of testosterone on gonadotropin release. Since FL failed to counteract the action of androgens on LH secretion in cultured pituitary cells [24] this antiandrogen might act at the hypothalamic level. Indeed, FL has been reported to increase in gonadotropin-releasing hormone content in the median eminence [26]. On the other hand, CAS has been claimed to be a peripherally selective antiandrogen [12, 13] and for this the explanation was presented on the basis of its exclusion from central tissue and hence an inability to inhibit androgen feedback to hypothalamic regulatory centres [27]. The data obtained in the present investigation are compatible with this view.

Following CAS administration at all doses a comparable reduction in weights of seminal vesicles and epididymides was observed demonstrating the biological effectiveness of CAS to block androgen action in peripheral androgen-target organs. With respect to the effects on seminal vesicle weight CAS appeared to be more effective than FL as suggested previously [12, 13, 25]. The observation that the reduction in weights of seminal vesicles was similarly pronounced with CAS and EDS is important because it shows that Casodex action is equivalent to initial removal of peripheral androgens [28–30]. Epididymal weights were equally influenced by CAS and FL. Lowest epididymal weights were seen with EDS. This effect, however, might be due to direct effects of EDS on the epididymis [31, 32].

Administration of CAS led to a slight but significant reduction of germ cell numbers

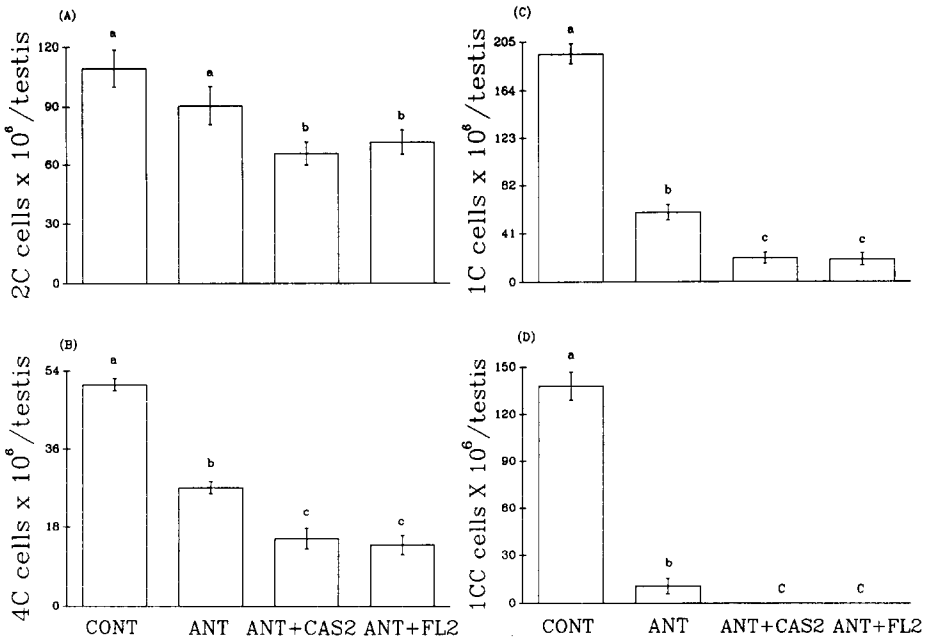

Fig. 4. Flow cytometric analysis of germ cells/testis (A) 2C cells, (B) 4C cells, (C) 1C cells and (D) 1CC cells after 2 weeks of treatment with vehicle (CONT); GnRH antagonist 200 μg/day (ANT); ANT + Casodex 20 mg/kg/day (CAS2) and ANT + Flutamide 20 mg/kg/day (FL2). The values with different superscript are significantly different from the others ($P < 0.05$). The values are means ± SEM, $n = 8$.

during stage VII of spermatogenesis. At this stage spermatogenesis is very sensitive to androgen deprivation [18]. Since antiandrogens act via inhibition of androgen uptake and/or nuclear binding [33] and form biologically inactive complexes with the nuclear androgen receptor [34] the observed effects of CAS on germ cell development were most likely due to interference with testicular androgen action. Sertoli cells and peritubular cells are the prime androgen target cells in the male gonad [35–37]. Different from CAS, FL which drastically elevated intratesticular androgen levels, had no advertent effect on gametogenesis. This observation is entirely consistent with previous reports that the compensatory rise in LH secretion and testosterone production counteracts the effects of FL in androgen-dependent tissues [10]. In contrast to steroidal antiandrogens such as cyproterone acetate, non-steroidal compounds do not directly affect spermatogenesis [39]. Viguier-Martinez *et al.* [11] observed a slight reduction of preleptotene spermatocyte numbers after 14 days of treatment with 10 mg/kg of FL. However, in this study only the uncorrected cell counts were considered which might explain the observed differences.

In the present study, administration of EDS induced a clearly more pronounced loss of germ cells compared to CAS. It is likely that EDS acts on Sertoli cells [6] and germ cells [7] directly. Alternatively, an experimental period of two weeks might have been too short to disclose marked effects of blocked androgen effects in the presence of normal serum FSH levels as FSH is undoubtedly involved in the maintenance of the spermatogenic process [40]. Nonetheless, it is equally possible that the administered amounts of CAS were insufficient to entirely block testicular androgen action because the intratesticular concentrations for androgens far exceed those present in serum [3]. In order to test whether CAS can principally enter the testis and interfere with androgen action, study 2 was undertaken. A GnRH antagonist was added to the treatment regimen to reduce gonadotropin release and subsequently testicular androgen production. In a previous experiment we observed that FL could remarkably enhance ANT-induced testicular involution to a similar extent as seen with ANT plus EDS [38]. In terms of total number of germ cells, quantitated by flow cytometry, CAS was as effective as FL. These findings support the view that CAS, at the dosages used, reached the testis and compromised the androgen effects.

In the course of the present studies unexpected effects of CAS and FL on pituitary gonadotropin concentrations were observed. Following FL treatment hypophyseal LH and

FSH concentrations were reduced. Furthermore, CAS selectively lowered pituitary FSH levels. The decrease of pituitary gonadotropin content may be a consequence of increased release, thereby depleting the pituitary stores. Such explanation, however, does not hold for CAS since serum gonadotropin levels were only marginally affected. At present, no obvious reason for these observations can be provided.

In summary, the present study demonstrates that (a) CAS is a peripherally selective antiandrogen and (b) CAS might provide a feasible approach to study androgen dependence of spermatogenesis in the presence of normal FSH levels. To ultimately clarify the latter point longer administration periods of CAS are required.

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