J. steroid Biochem. Vol. 27, No. 4–6, pp. 905–914, 1987
Printed in Great Britain. All rights reserved
0022-4731/87 $3.00 + 0.00
Copyright © 1987 Pergamon Journals Ltd

Proceedings of the VII International Congress on Hormonal Steroids (Madrid, Spain, 1986)

AROMATASE INHIBITORS: THEIR BIOCHEMISTRY AND CLINICAL POTENTIAL

DAVID HENDERSON*
Research Laboratories of Schering AG, Berlin/Bergkamen, 1000 Berlin 65, Federal Republic of Germany

Summary—It has been proposed that one of the endocrinological factors in the pathogenesis of benign prostatic hyperplasia is estrogen stimulation of stromal growth. Current clinical experience with anti-estrogenic compounds indicates that, in the case of mammary carcinoma, aromatase inhibitors provide a viable alternative to estrogen receptor antagonists for treatment of the disease. It is proposed that inhibitors of estrogen biosynthesis could likewise provide a non-invasive therapy for benign prostate disease. Some aspects of the activity of known aromatase inhibitors as substrates for enzymes of steroid metabolism and their potential relevance to the pharmacology of the compounds are discussed.

INTRODUCTION

In the course of synthesis of steroid hormones from their common precursor cholesterol, the number of carbon atoms in the skeleton is reduced successively from 27 to 21, 19 and finally to 18 by a series of specific cytochrome P-450 catalysed enzymatic reactions [1]. The last step in the series, removal of the C-19 methyl group from the androgen precursors androstenedione† or testosterone to produce the C_{18} estrogens estrone or estradiol is carried out by cytochrome P-450_{arom}, in concert with NADPH-dependent cytochrome reductase (Fig. 1, Refs [1–4]). This enzymatic activity is commonly referred to as aromatase and is found primarily in the gonads, placenta, subcutaneous fat tissue and the brain [2, 5–8]. The fact that this synthetic step is the last in the steroid hormone synthetic cascade makes it an attractive point for pharmacological inhibition of estrogen biosynthesis: reduction of circulating estrogens will not directly influence the production of other steroids since the estrogens are unique in not being precursors for other hormones.

Aromatase inhibitors have consequently attracted attention due to their potential application in the clinical treatment of several conditions associated with the action of estrogens. For example, endometriosis, endometrial and mammary carcinomas in women are obvious targets, while in the male gynecomastia, idiopathetic oligospermia and benign prostatic hyperplasia come into question [9–12].

CLINICAL USE OF AROMATASE INHIBITORS

Mammary carcinoma

Hormone dependence of mammary carcinomas has been recognised for about 90 yr—since Beatson demonstrated the palliative effects of ovariectomy in 1896 [13]. Additional endocrine therapy in the form of adrenalectomy and hypophysectomy has lent further support to this concept [14, 15]. Recently, treatment of post-menopausal metastatic disease has usually included use of non-steroidal anti-estrogens such as tamoxifen or nafoxidine whose primary mode of action probably results from their ability to compete with estradiol for binding to the estrogen receptor [16–18]. A logical alternative to surgical ablation or receptor blockade would seem to be the use of an enzyme inhibitor to prevent synthesis of the estrogens, that is, use of an aromatase inhibitor. Clinical experience with the use of such compounds has, however, until recently been limited by the availability of suitable drugs. The first compound to find clinical application as aromatase inhibitor was aminoglutethimide, a non-steroidal compound known to have inhibitory effects on adrenal steroidogenesis as well as on aromatase [19]. While quite effective as treatment, especially in conjunction with corticoid replacement therapy to combat the adrenal side-effects, other toxic effects are too severe to warrant use of the compound in less debilitating indications [21, 22]. The steroid analogue testolactone (**1**), a rather weak aromatase inhibitor, has been shown to be effective in treatment of McCune–Albright syndrome [22]. Although massive doses are required to substantially reduce serum estradiol

*Address for correspondence: Department of Biochemical Pharmacology, Schering AG, Postfach 65 03 11, 1000 Berlin 65, Federal Republic of Germany.

†Abbreviations and trivial names: androstenedione, 4-androstene-3,17-dione; testolactone, D-homo-17a-oxa-1,4-androstadiene-3,17-dione; 4-OHA, 4-hydroxy-4-androstene-3,17-dione; ATD, 1,4,6-androstatriene-3,17-dione; SH 489, 1-methyl-1,4-androstadiene-3,17-dione; DHT, 17β-hydroxy-5α-androstan-3-one; MDL 18962, 10-propargyl-4-estrene-3,17-dione; Aminoglutethimide; 3-(4-aminophenyl)-3-ethyl-2,6-piperidinedione.

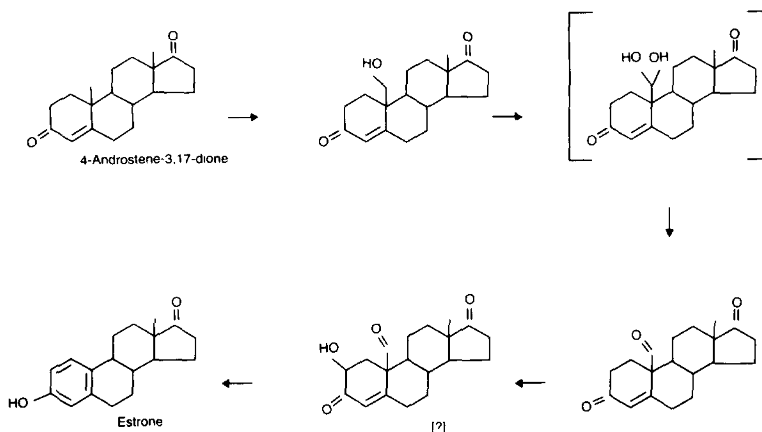


Fig. 1. Probable intermediates generated in the course of aromatization of androstenedione to estrone [82, 83].

levels, the compound has also found some use in treatment of mammary carcinoma [23].

About 2 yr ago, the first clinical data on the much more potent inhibitor 4-hydroxy-4-androstene-3,17-dione (**2**, 4-OHA) were published [24], showing that this compound is indeed effective. These studies together with the previous experience with aminoglutethimide, show that aromatase inhibitors can be used effectively to inhibit extragonadal aromatization. Aside from providing a firm practical basis for the treatment of post-menopausal breast cancer, it seems realistic to suppose that these or similar drugs could be useful in treatment of other estrogen-dependent diseases.

Potential application in prostatic hyperplasia

The possibility of using some form of medication in treatment of benign prostatic disease (BPH) has aroused interest for a number of years. A hormonal basis for BPH was postulated two centuries ago, based on the observation that men castrated early in life do not develop the condition [see 30]. This obvious androgen dependence of the disease is not, however, reflected in good clinical response to anti-androgen therapy [25]. This stands in sharp contrast to prostatic carcinoma, where surgical or medical castration are standard treatment. The answer to this apparent contradiction lies, presumably, in the etiology of the hyperplasia.

In somewhat simplified terms, the human prostate can be considered to consist of two main tissue compartments: the glandular elements, of epithelial origin, and the surrounding stromal tissue. These two compartments contain quite distinct complements of hormone receptors and also of steroid-metabolising enzymes, as will be discussed later. The histological picture of the hyperplastic prostate in the human is, however, quite heterogeneous. The hyperplastic areas tend to be well-defined nodules whose histology varies to some extent depending on the region of the organ in which they develop. Constriction of the urethra and the resulting clinical symptoms are most frequently produced by growth of nodules in the periurethral region. These nodules generally show a preponderance of stromal elements. Hyperplastic lesions more distant from the urethra tend to show greater involvement of glandular elements [26]. On the basis of histological analysis, McNeal suggested that BPH results from a reactivated embryonic induction of epithelial growth stimulated by the stromal tissue. This theory of BPH as a primarily stromal disorder was first propounded by Reischauer in 1925 [27] and found considerable support in anatomical studies in the first half of the century [28–30]. Direct experimental support of this hypothesis has been provided in the last few years by the elegant reconstruction experiments carried out primarily by Cunha and his colleagues who have been able to demonstrate that embryonic mesenchyme, which ultimately develops into the prostatic stroma, controls the differentiation and growth of adjacent epithelium and that this growth is under hormonal control [31–33].

Two principal lines of evidence indicate a probable involvement of estrogen as a growth stimulant of the prostatic stroma. First, biochemical analysis of the tissue has shown that nuclear binding of estrogens is higher in stroma than in epithelium while the opposite is true of nuclear androgen receptor. This finding has been reported for the human hyperplastic prostate as well as for normal tissue in common laboratory animals (rat, dog and guinea-pig) [34–38]. The stroma, however, would seem to be the principal site of conversion of testosterone into dihydrotestosterone, postulated to be the principle androgen responsible for growth of the glandular epithelium [34, 39–41]. This distribution of the receptors and enzymes appears to support the postulated role of stroma as a regulator of epithelial growth.

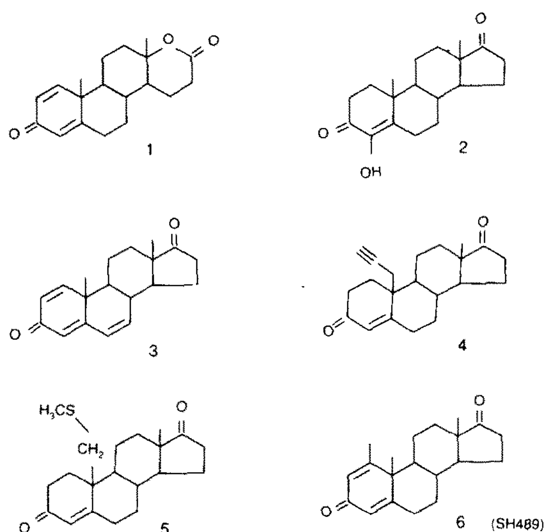
BPH is, of course, primarily a disorder of old age and it is therefore possible that clues to the hormonal control of hyperplasia might be gained by measurement of hormone levels as a function of age. With increasing age, the levels of plasma testosterone tend to fall while estrogens rise somewhat. Furthermore, the estrogen-dependent increase in levels of SHBG reduces still further the effective concentration of testosterone so that a considerable shift in the estrogen/androgen balance is seen among men between 60 and 70 yr of age as compared to young men. This shift in relative levels of the two hormones may be in part responsible for reactivation of stromal inductive activity [42–45].

To what extent can this hypothesis be supported by experimental data? A major problem in experimental analysis of prostatic hyperplasia is the extreme variation among species with respect to the anatomy and histology of their prostates, so that the question of their relevance as models for the human condition arises. Furthermore, there are very few species which naturally develop hyperplasia in old age. One notable exception is the dog, which has been the object of a number of studies, although the dog prostate shows important anatomical differences compared to man and canine hyperplasia differs considerably from the human condition in being a diffuse and not a nodular growth and being primarily an epithelial rather than stromal disorder [46–49]. Estrogens play an important role, however, in development of canine hyperplasia and the extent and nature or the hyperplasia can be influenced by the ratio of androgen to estrogen in circulation [49]. It has recently been reported that an anatomically more suitable model may be found in the baboon [50] or cynomolgus monkey (*Macaca fascicularis*, [51]). Experiments in the last two species and the dog have shown that prostate growth can be induced by treatment with the aromatisable steroids 4-androstene-3,17-dione [51] or testosterone enanthate [50]. Furthermore, the estrogenic effects of this treatment on the stromal histology can be reversed by treatment with the steroidal aromatase inhibitors 4-OHA or SH489 [51, 52].

There therefore exists a considerable body of evidence, much of it indirect but some of it direct and experimental in nature, to support the contention that:

(1) human BPH is a disease of stromal origin and
(2) estrogens play an important role in induction of prostatic growth.

A direct clinical trial of the use of an aromatase inhibitor in treatment of BPH has up to now been carried out on a limited scale using testolactone [12]. Results of this trial were encouraging enough to warrant further trials with more potent compounds, once these become available for testing. Benign prostatic hyperplasia and mammary carcinoma are therefore currently viewed as the principal indications for future clinical use of aromatase inhibitors.



Scheme 1

BIOCHEMICAL CHARACTERISTICS OF AROMATASE INHIBITORS

The last few years have seen a surge of activity in the search for new inhibitors of aromatase having sufficient specificity for the enzyme to warrant their testing clinically. The first lead for new compounds which might prove useful as inhibitors of the substrate analogue type was provided by the work of the Brodies and their colleagues, who examined the activity of a large number of steroids [53–55]. The most active compounds found in that search were androstenedione derivatives: 1,4,6-androstatriene-3,17-dione (ATD, **3**) 4-androstene-3,6,17-trione and 4-substituted compounds 4-acetoxy and 4-hydroxy-4-androstene-3,17-dione (4-acetoxy-A and 4-OHA, **2**). As already mentioned, the latter compound has in the mean time progressed to clinical trials.

All four of these compounds were shown to be potent inhibitors of aromatase *in vitro*, using both human placental microsomes and microsomes derived from the ovaries of PMSG-stimulated rats as the source of enzyme [54]. In the mean time, several other androstenedione derivatives with good inhibitory activity have been described. These include the 10-propargyl compound MDL 18962 (**4**) [56–58], 19-thio-methyl derivatives, for example (**5**) [59] and the compound 1-methyl-1,4-androstadiene-3,17-dione (SH489, **6**) which has been the object of our own interest [52, 60, 61].

These compounds show kinetics of inhibition, as analysed by Lineweaver–Burke or Dixon plots, indicative of competitive inhibition. A further common feature of the steroids **2**, **3**, **4** and **6**, however, is that they are capable of irreversibly inhibiting the enzyme. That is, on incubation of microsomes with inhibitor in the absence of substrate, but with

NADPH (required as cofactor for enzyme activity), there is a time- and concentration-dependent loss of aromatase activity [56, 58, 61–64]. The precise mechanism of inactivation of the enzyme remains a matter of discussion and it is presumably different for the 4- or 6-substituted steroids, the steroids carrying a C1–C2 double bond (testolactone, ATD, SH489) and irreversible inhibitors substituted at C10 with allenic or acetylenic groups [63–65].

The dependence of the inactivation on NADPH, protection by addition of substrate and the irreversibility of the reaction as judged by stability to prolonged washing or dialysis all point to a mechanism of the "k_{cat}" or suicide type. It is therefore presumed that the hydroxylation of these substrate analogues by cytochrome P-450_{arom} leads to formation of active intermediates which can form a covalent bond to the protein. In the case of 4-OHA, additional evidence for the covalent nature of the binding was provided by our observation that, following reaction of [^{3}H]4-OHA with partially purified human placental aromatase, the radioactive label was recovered in a protein of molecular weight 55,000 under the denaturing conditions of SDS–PAGE [66]. This molecular weight corresponds to that of the homogeneous purified cytochrom P-450_{arom} [4] and therefore also provides further support for the idea that a suicide interaction occurs between the activated substrate and the hydroxylating cytochrome. Analogous experiments with other suicide substrates have not yet been reported.

As far as the kinetics of the irreversible reaction are concerned, we have previously reported that introduction of the 1-methyl group in SH489 leads to a slower reaction than observed in unsubstituted 1,4 dienes [61]. The methyl group, however, improves substantially the reversible binding of the compound as a competitive inhibitor and prevents aromatization of the inhibitor. 4-OHA, ATD and MDL 18962 are all known to produce, in addition to reduced serum estrogen levels, a reduction of the specific activity of aromatase in target tissues *in vivo*, which is presumed to result from inactivation of the enzyme [62, 67, 68].

FURTHER BIOCHEMICAL CONSIDERATIONS

Androstenedione derivatives described as being active as aromatase inhibitors are in general not dramatically modified in structure, as is clear from the few examples shown here. This results from there being relatively few points on the molecule where modifications can be made without reducing affinity to the enzyme. In so far as they have been tested, however, such modifications have only modest effects on other enzymes of steroid metabolism (e.g. [69]). As a consequence, it must be anticipated that the steroidal inhibitors discussed here will be subject to metabolism. Apart from the obvious consequence of excretion, such substrate activity can be viewed from two standpoints: on the one hand, enzymatic modification may reduce or abolish activity of the compound as an aromatase inhibitor. Alternatively, the compound, in competing for an enzyme with its natural substrate, may produce changes in the pattern of synthesis or metabolism of the natural hormonal steroids.

It is known, for example, that the plasma half-life of 4-hydroxy-4-androstene-3,17-dione (4-OHA) is quite short. It has recently been reported that glucuronidation of the hydroxyl group is very rapid following *in vivo* administration to human or the rat and also on incubation with suspensions of rat hepatocytes [70, 71]. In addition, the compound is also subject to a number of alterations in the A-ring. While at least some of the identifiable metabolites are still capable of inhibiting aromatase, others and in particular the glucuronide are presumably inactive. Furthermore, the conjugated steroid can be expected to be rapidly excreted. These metabolic effects on the molecule therefore contribute substantially to the fact that the compound must be given in substantial doses to be effective, most particularly when applied orally [71]. The other steroidal inhibitors mentioned here differ from 4-OHA in not possessing hydroxyl groups. It is, however, likely that one or other of the ketone groups at positions 3 or 17 in these compounds, after reduction by the appropriate oxidoreductase, could provide a site for conjugation in a manner analogous to normal metabolic processing of natural steroids. Since reactions of this type might play a decisive role in the pharmacokinetics of the inhibitors, it seemed worthwhile to investigate the properties of some of the inhibitors as substrates for steroid-metabolizing enzymes. Besides providing an understanding of the metabolism of the compounds themselves, information gained in studies of this type may prove useful in future development of more effective inhibitors.

In the course of characterizing the inhibitor SH489, we have therefore compared the compound with androstenedione and also with 4-OHA for its activity as substrate and for four androgen-metabolising enzymes: 5α- and 5β-reductase and the soluble and membrane-bound forms of hepatic 17β-hydroxysteroid dehydrogenase.

5α- and 5β-reduction

The enzymes 5α- and 5β-reductase control an important step in the metabolism of both C19 and C21 steroids: only after reduction of the C4–C5 double bond is reduction of the 3-ketone possible, leading to a product which can be conjugated and excreted. The activities of both 5α- and 5β-reductases is high in the liver. 5α-Reductase is found in the nuclear membrane and in microsomes while 5β-reductase is a soluble enzyme [72]. 5α-Reductase is also found in the male reproductive organs and in tissues which are targets for androgen action. While the function of the reductases in the liver is primarily

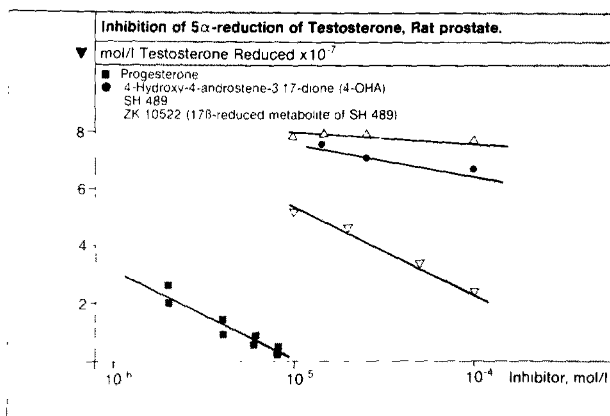


Fig. 2. Concentration dependence of inhibitory effects of progesterone (■), SH489 (△), 4-OHA (●) and 17-hydroxy-1-methyl-1,4-androstadiene-3-one (17βOH metabolite of SH489 (▽) on 5α-reduction of [^{14}C]testosterone to DHT by rat ventral prostate membranes.

in inactivation and excretion of the steroids, 5α-reductase in androgen target tissues is responsible for conversion of testosterone into dihydrotestosterone (DHT), which is an active androgen.

Figure 2 shows the effects of several steroids on reduction of testosterone to DHT by a crude membrane fraction isolated from rat ventral prostate. Results are expressed as the amount of DHT produced as a function of added competing test compound. Since [^{14}C]testosterone substrate was present at 2 μmol/1, approximately the K_m under these conditions, a 50% reduction in the yield of DHT will be produced by a test compound at its K_m (or K_i in the case of compounds which are not substrates). In this experiment, micromolar concentrations of the natural substrate progesterone reduced dramatically the production of DHT, consistent with the K_m of 3 μmol/l determined using labelled progesterone as substrate (not shown). The aromatase inhibitors SH489 and 4-OHA proved to be essentially inactive in concentrations up to 100 μmol/l. While a moderate inhibition was found using the 17β-hydroxy metabolite of SH489, indicating the preference of the enzyme for a 17β-hydroxyl over a 17-ketone group, the K_m for this compound appears to lie in the region of 100 μmol/l.

In experiments using either nuclei or microsomes from rat liver, similar results were obtained (data not shown). Thus it is anticipated that these compounds will not be readily reduced to 5α-metabolites *in vivo* and neither aromatase inhibitor is expected to interfere significantly with the conversion of testosterone to DHT.

The soluble 5β-reductase from rat liver is a NADPH-dependent enzyme with a mol. wt. of about 39 kDa. Using purified protein prepared by a slight modification of the method of Mode and Rafter[73] we find that SH489 is a good substrate for this enzyme, showing a K_m of 0.8 μmol/1 (Fig. 3), compared to about 20 μmol/1 for androstenedione itself (not shown). SH489 also shows approximately 6-fold higher V_{max} as compared to androstenedione so that 5β-reduced metabolites can be expected *in vivo*.

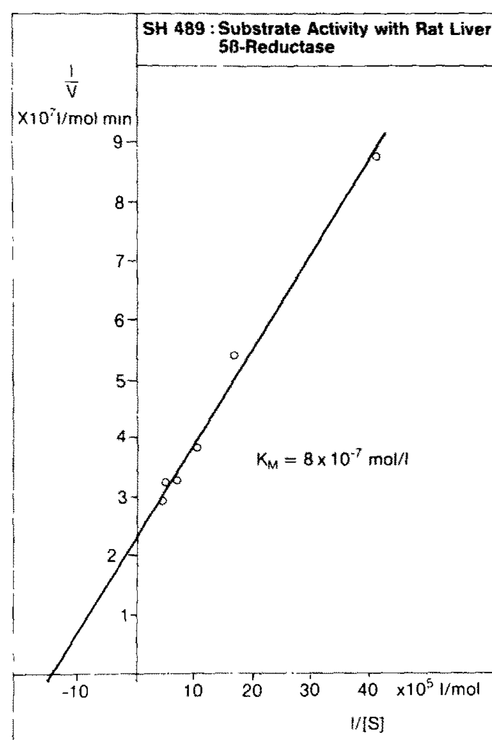


Fig. 3. K_m determination for 5β-reduction of SH489. Reaction conditions were as described in [73]. Substrate was [1-methyl ^{14}C]SH489.

17β-Hydroxysteroid dehydrogenases

The 17β-hydroxysteroid dehydrogenases are stereospecific enzymes catalysing the interconversion of a wide range of 17-keto and 17β-OH steroids. In the human, as also in the rat and guinea-pig liver, both cytoplasmic [NADP(H)-dependent] and microsomal [NAD(H)-dependent] activities are present [74]. In the present investigation, we have

Table 1. Substrate activities with hepatic 17β-hydroxysteroid dehydrogenases*

Compound	K_m(μmol/1), microsomal		K_m(μmol/1), soluble	
Androstenedione	4.9	(2)	40 ± 8	(3)
SH489	20 ± 1	(4)	37	(2)
4-hydroxy-androstenedione	15 ± 8	(3)	40	(2)

*Apparent K_m values measured for conversion of the substrates to their corresponding 17β-hydroxy derivatives by microsomes or purified soluble dehydrogenase isolated from guinea-pig liver. The numbers in parentheses are the number of determinations. Average values or mean values and SD are given.

compared the activities of the microsomal enzymes from the last two species with the soluble enzyme purified to homogeneity from guinea-pig liver. Purification was by ammonium sulphate fractionation, affinity chromatography on Blue Sepharose and ion-exchange chromatography on Mono-Q resin using a Pharmacia FPLC apparatus.

Table 1 summarises the results obtained with the guinea-pig enzymes, showing that the K_m values obtained with the soluble enzyme were the same for all three diones while the aromatase inhibitors showed increased K_m with the microsomal enzyme. Differences in V_{max} were not significant (not shown). Thus, although the aromatase inhibitors may show slightly less conversion to their corresponding 17β-hydroxy metabolites than androstenedione under analogous conditions, conversion by this metabolic route could also be expected.

A further aspect of the activity of the microsomal enzyme which deserves more detailed investigation is that of product activation. It was described in 1973 that androstenedione accelerates the conversion of testosterone into androstenedione in homogenates of human testis. Testosterone shows a similar activity for the reverse reaction [75, 76]. We have investigated the microsomal enzymes of rat liver and testis and find a similar phenomenon. Furthermore, this activity is not restricted to androstenedione alone but is demonstrable in the case of both SH489 and 4-OHA, indicating that it is probably a general property of 17-keto substrates for the enzyme. As an example, Fig. 4 shows the results obtained with rat testis microsomes displayed as a Hanes' plot. The inset shows the dependence of V_{max} on the concentration of dione present in the reaction mixture: the order of effectiveness for these three compounds was androstenedione > 4-OHA > SH489. All three compounds gave approximately the same maximal stimulation of the enzyme at the highest concentration tested (20 μmol/1).

The principle routes for interconversion of 5α-reduced androgen metabolites are shown in Fig. 5. An analogous scheme can be drawn up for 5β-reduced compounds. As displayed here, conversion between the upper and lower rows is mediated by 17-dehydrogenases. The product activation just described is predicted to lead to a relative increase in the 17-keto compounds androstenedione, androstandione and the androsterones at the expense of testosterone, DHT and androstanediols. Interes-

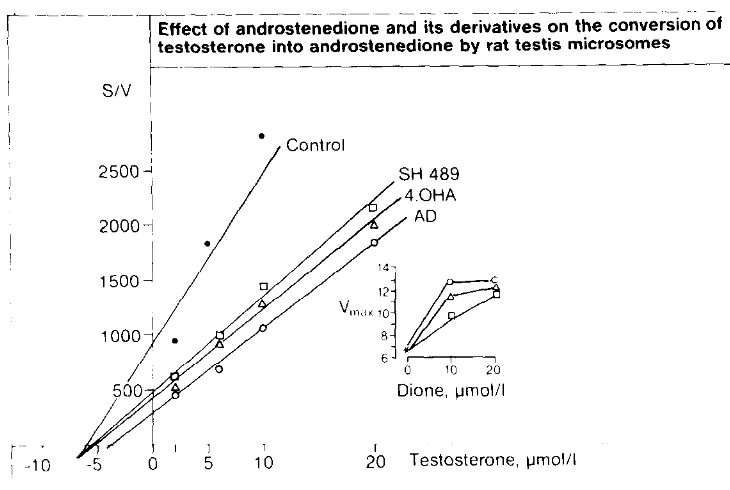


Fig. 4. Effects of androstenedione and the aromatase inhibitors 4-OHA and SH489 on conversion of [^{14}C]testosterone to androstenedione by microsomes from rat testis. The curves shown are for reactions in the presence of 20μmol/1 of the test compounds. The inset shows the concentration dependence of the effects on V_{max}(V_{max} in units of nmol/mg protein per min).

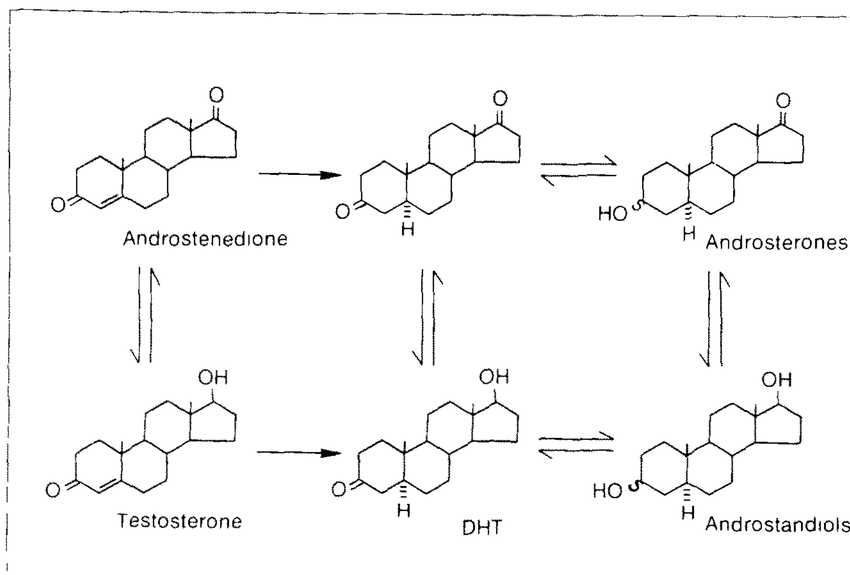


Fig. 5. Interconversion of androgens and their 5α-reduced metabolites (see text for details).

tingly, precisely this shift in metabolites has been observed in Leydig cell suspensions incubated with labelled testosterone or DHT and either SH489, 4-OHA or androstenedione [77]. It is, furthermore, likely that a similar product activation takes place *in vivo*. Thus, for example, 4-OHA and 4-acetoxy-4-androstene-3,17-dione were found to produce substantially greater reduction in serum estradiol as compared to estrone in rats [78,79], an observation which was confirmed in the course of our own studies (Nishino and El Etreby, personal communication). More recently, a similar disparity has been observed in breast cancer patients treated with 4-OHA [80]. It is conceivable, therefore, that activation of oxidative metabolism of the steroids by 17-dehydrogenase(s) induced by the aromatase inhibitor contributes to the clinical effectiveness by converting some residual estradiol to estrone. In the case of male subjects, these effects could lead to a reduction in both androgens and estrogens available to the prostate. As discussed elsewhere by Motta *et al.*[78], this may be beneficial in treatment of prostatic hyperplasia since effects on the growth of both the estrogen-dependent stroma and androgen-dependent glandular epithelia might result.

CONCLUSIONS

Results of the animal studies and clinical trials alluded to in the first part of this brief review strengthen our belief and hope that use of aromatase inhibitors will provide a useful therapy for estrogen-dependent conditions. In contrast to mammary carcinoma, however, where substantial information is already available from the clinic [20, 21, 23, 24, 80] the application of aromatase inhibitors for treatment of benign prostatic hyperplasia is more speculative. Nevertheless, the profound effects of these compounds demonstrable in animals together with the overall biochemical and endocrinological picture of the pathophysiology of BPH provide a reasonable basis for future research in this direction. As far as the inhibitors themselves are concerned, there is still much to be learned of their pharmacokinetics and metabolism. A more detailed understanding of these aspects should allow optimization of treatment schedules and might also provide clues for future development of more effective drugs or formulations.

Acknowledgements—I am indebted to my colleagues at Schering, Prof. M. F. El Etreby, Prof. F. Neumann, Drs U.-F. Habenicht, Y. Nishino, E. Schillinger and H. Schröder for their contributions to the hypotheses and results presented here. I also thank Lianne Dummann and Gisela Repenthin for their tireless assistance in the laboratory.

REFERENCES

1. Hall P. F.: Role of cytochromes P-450 in the biosynthesis of steroid hormones. *Vitamins Horm.* **42** (1985) 315–368.
2. Thompson E. A. and Siiteri P. K.: The involvement of human placental microsomal cytochrome P450 in aromatization. *J. biol. Chem.* **249** (1974) 5373–5278.
3. Bellino F.: Estrogen synthetase. Demonstration that the high molecular weight form of NADPH-cytochrome c reductase from human placental microsomes is required for androgen aromatization. *J. steroid Biochem.* **17** (1982) 261–270.
4. Mendelson C. R., Wright E. E., Evans G. T., Porter J. C. and Simpson E. R.: Preparation and characterization of polyclonal and monoclonal antibodies against human aromatase cytochrome P-450 (P-450_{arom}) and their use in its purification. *Archs Biochem. Biophys.* **243** (1985) 480–491.
5. Longcope C., Pratt J. H., Schneider S. H. and

Fineberg S. E.: Aromatization of androgens by muscle and adipose tissue *in vivo*. *J. clin. Endocr. Metab.* **46** (1978) 146–152.
6. Naftolin F. and Ryan K. J.: The metabolism of androgens in central neuroendocrine tissues. *J. steroid Biochem.* **6** (1975) 993–997.
7. Matsumine H., Hirato K., Yanaihara T., Tamada T. and Yoshida M.: Aromatization by skeletal muscle. *J. clin. Endocr. Metab.* **63** (1986) 717–720.
8. Baird D., Horton R., Longcope C. and Tait J. F.: Steroid prehormones. *Perspect. Biol. Med.* **II** (1968) 384–421.
9. Johnston J. O. and Metcalf B. W.: Aromatase: a target enzyme in breast cancer. In *Novel Approaches to Cancer Therapy* (Edited by S. Prasad). Academic Press, New York (1984) pp. 307–328.
10. Brodie A. M. H.: Aromatase inhibition and its pharmacologic implications. *Biochem. Pharmac.* **34** (1985) 3213–3219.
11. Henderson D., Habenicht U.-F., Nishino Y., Kerb U. and El Etreby M. F.: Aromatase inhibitors and benign prostatic hyperplasia. *J. steroid Biochem.* **25** (1986) 867–876.
12. Tunn U. W., Kaivers P. and Schweikert H. U.: Conservative treatment of human benign prostatic hyperplasia. In *Regulation of Androgen Action* (Edited by N. Bruchovsky, A. Chapdelaine and F. Neumann). Congressdruck R. Brückner, Berlin (1985) pp. 87–90.
13. Beatson G. T.: On the treatment of inoperable cases of carcinoma of the mamma: suggestion for a new method of treatment, with illustrative cases. *Lancet* **2** (1896) 104–107.
14. Huggins C. and Bergenstal D. M.: Inhibition of human mammary and prostatic cancers by adrenalectomy. *Cancer Res.* **12** (1952) 131–141.
15. Pearson O. H. and Ray B. S.: Hypophysectomy in the treatment of metastatic mammary cancer. *Am. J. Surg.* **99** (1960) 544–552.
16. Cole M. P., Jones C. T. A. and Todd I. D. H.: A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI 46 474. *Br. J. Cancer* **25** (1971) 270–275.
17. Heuson J. C., Englesman E., Blonk-Van der Wijst J., Maas H., Drochmans A., Michel J., Nowakowski H. and Gorins A.: Comparative trial of nafoxidine and ethinyloestradiol in advanced breast cancer: an EORTC study. *Br. med. J.* **2** (1975) 711–713.
18. Wakeling A. E.: Pharmacology of antioestrogens. In *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action* (Edited by B. J. A. Furr and A. E. Wakeling). Baillière-Tindall, London (1987) pp. 1–19.
19. Dexter R. N., Fishman L. M., Ney R. L. and Liddle G. W.: Inhibition of adrenal corticoid synthesis by aminoglutethimide: studies on the mechanism of action. *J. clin. Endocr. Metab.* **27** (1967) 473–480.
20. Santen R. J., Santner S., Davis B., Veldhuis J., Samojlik E. and Ruby E.: Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. *J. clin. Endocr. Metab.* **47** (1978) 1257–1265.
21. Santen R. J. and Misbin R. I.: Aminoglutethimide: review of pharmacology and clinical use. *Pharmacotherapy* **1** (1981) 95–120.
22. Foster C. M., Pescovitz O. H., Comite F., Feuillan P., Shawker T., Loriaux D. L. and Cutler G. B., Jr: Testolactone treatment of precocious puberty in McCune–Albright syndrome. *Acta endocr., Copenh.* **109** (1985) 254–257.
23. Barone R. M., Shamonki I. M., Siiteri P. K. and Judd H. L.: Inhibition of peripheral aromatization of androstenedione to estrone in postmenopausal women with breast cancer using Δ^1-testololactone. *J. clin. Endocr. Metab.* **49** (1979) 672–676.
24. Coombes R. C., Goss P., Dowsett M., Gazet J. C. and Brodie A. M. H.: 4-Hydroxyandrostenedione in treatment of postmenopausal patients with advanced breast cancer. *Lancet* **2** (1984) 1237–1239.
25. Tunn U. W., Senge Th., Neumann F. and Schweikert H. U.: Der Einfluß von Cyproteronacetat auf die mit Steroidhormonen induzierte experimentelle Prostatahyperplasie des Hundes und die menschliche BPH. In *Antihormone: Bedeutung in der Urologie* (Edited by J. E. Altwein, G. Bartsch and G. H. Jacobi). W. Zuckschwerdt Verlag, Munich (1981) pp. 41–50.
26. McNeal J. E.: Origin and evolution of benign prostatic enlargement. *Invest. Urol.* **15** (1978) 340–345.
27. Reischauer F.: Die Entstehung der sogenannten Prostatahypertrophie. *Virchows Arch. path. Anat.* **256** (1925) 357–389.
28. Deming C. L. and Neumann C.: Early phases of prostatic hyperplasia. *Surg. gynec. Obstet.* **68** (1939) 155–160.
29. Le Duc I. E.: The anatomy of the prostate and the pathology of early benign hypertrophy. *J. Urol.* **42** (1939) 1217–1241.
30. Franks L. M.: Benign nodular hyperplasia of prostate: a review. *Ann. R. Coll. Surg.* **14** (1954) 92–106.
31. Chung L. W. K. and Cunha G. R.: Stromal–epithelial interactions II. Regulation of prostatic growth by embryonic urogenital sinus mesenchyme. *Prostate* **4** (1983) 503–511.
32. Cunha G. R., Chung L. W. K., Shannon J. M., Taguchi O. and Fujii H.: Hormone induced morphogenesis and growth: role of mesenchymal–epithelial interactions. *Rec. Prog. Horm. Res.* **39** (1983) 559–595.
33. Cunha G. R., Bigsby R. M., Cooke P. S. and Sugimura Y.: Stromal–epithelial interactions in adult organs. *Cell. Diff.* **17** (1985) 137–148.
34. Krieg M., Klotzl G., Kaufmann J. and Voigt K. D.: Stroma of human benign prostatic hyperplasia: preferential tissue for androgen metabolism and oestrogen binding. *Acta endocr., Copenh.* **96** (1981) 422–432.
35. Kozak I., Bartsch W., Krieg M. and Voigt K. D.: Nuclei of stroma: site of highest estrogen concentration in human benign prostatic hyperplasia. *Prostate* **3** (1982) 433–438.
36. Tilley W. D., Horsfall D. J., McGee M. A., Henderson D. W. and Marshall V. R.: Distribution of oestrogen and androgen receptors between the stroma and epithelium of the guinea-pig prostate. *J. steroid Biochem.* **22** (1985) 713–719.
37. Chaisiri N. and Pierrepoint C. G.: Examination of the distribution of oestrogen receptor between the stromal and epithelial compartments of the canine prostate. *Prostate* **1** (1980) 357–366.
38. Jung-Testas I., Groyer M.-T., Brunner-Lorand J., Hechter O., Baulieu E.-E. and Robel P.: Androgen and estrogen receptors in rat ventral prostate epithelium and stroma. *Endocrinology* **109** (1981) 1287–1289.
39. Cowan, R. A., Cowan S. K., Grant J. K. and Elder H. Y.: Biochemical investigations of separated epithelium and stroma from benign hyperplastic prostatic tissue. *J. Endocr.* **74** (1977) 111–120.
40. Wilkin R. P., Bruchovsky N., Shnitka T. K. Rennie P. S. and Comeau T. L.: Stromal 5α-reductase activity is elevated in benign prostatic hyperplasia. *Acta endocr., Copenh.* **94** (1980) 284–288.
41. Bruchovsky N. and Wilson J. D.: The conversion of testosterone to 5α-androstan-17β-ol-3-one by rat prostate *in vivo* and *in vitro*. *J. biol. Chem.* **243** (1968) 2012–2021.
42. Vermeulen A., Stoica T. and Verdonck L.: The ap-

parent free testosterone concentration; an index of androgenicity. *J. clin. Endocr. Metab.* **33** (1971) 759–767.
43. Rubens R., Dhont M. and Vermeulen A.: Further studies on Leydig cell function in old age. *J. clin. Endocr. Metab.* **39** (1974) 40–45.
44. Krieg M., Bartsch W., Herzer S., Becker H. and Voigt K. D.: Quantification of androgen binding, androgen tissue levels and sex hormone binding globulin in prostate, muscle and plasma of patients with benign prostatic hyperplasia. *Acta endocr., Copenh.* **86** (1977) 200–215.
45. Rannikko S. and Adlercreutz H.: Plasma estradiol, free testosterone, sex hormone-binding globulin binding capacity and prolactin in benign prostatic hyperplasia and prostatic cancer. *Prostate* **4** (1983) 223–229.
46. Tunn U., Senge Th., Schenck, B. and Neumann F.: Biochemical and histological studies on prostates in castrated dogs after treatment with androstanediol, oestradiol and cyproterone acetate. *Acta endocr., Copenh.* **91** (1979) 373–384.
47. Tunn U. W., Schüring B., Senge Th., Neumann F., Schweikert H. U. and Rohr H. P.: Morphometric analysis of prostates in castrated dogs after treatment with androstanediol, estradiol and cyproterone acetate. *Invest. Urol.* **18** (1981) 289–292.
48. Funke P.-J., Tunn U. W., Senge Th. and Neumann F.: Histological and histochemical findings in prostates of castrated dogs after treatment with estradiol, tamoxifen, androstanediol and cyproterone acetate. In *Antihormone—Bedeutung in der Urologie* (Edited by J. E. Altwein, G. Bartsch and G. H. Jacobi) W. Zuckschwerdt Verlag, Munich (1981) pp. 51–55.
49. Walsh P. C. and Wilson J. D.: The induction of prostatic hypertrophy in the dog with androstanediol. *J. clin. Invest.* **57** (1976) 1093–1097.
50. Karr J. P., Chai L. S., Kim U., Murphy G. P., Resko J. A., Sandberg A. A. and Schneider S.: Induction of benign prostatic hypertrophy in baboons. *Urology* **23** (1984) 276–289.
51. Habenicht U. F., Schwarz K., Schweikert H. U., Neumann F. and El Etreby M. F.: Development of a model for the induction of estrogen-related prostatic hyperplasia in the dog and its response to the aromatase inhibitor 4-hydroxy-androstene-3,17-dione. *Prostate* **8** (1986) 81–94.
52. Habenicht U. F., El Etreby M. F., Tunn U. W., Schweikert H. U. and Neumann F.: Animal models for investigating the role of estrogens in the pathogenesis of benign prostatic hyperplasia. In *Ipertrofia Prostatica Benigna* (Edited by F. di Silverio, F. Neumann and M. Tannenbaum). Excerpta Medica, Elsevier, Amsterdam (1986) pp. 71–77.
53. Schwarzel W. C., Kruggel W. G. and Brodie H. J.: Studies on the mechanism of estrogen biosynthesis. VIII. The development of inhibitors of the enzyme system in human placenta. *Endocrinology* **92** (1973) 866–880.
54. Brodie A. M. H., Schwarzel W. C. and Brodie H. J.: Studies on the mechanism of estrogen biosynthesis in the rat ovary—I. *J. steroid Biochem.* **7** (1976) 787–793.
55. Marsh D. A., Brodie H. J., Garrett W., Tsai-Morris C.-H. and Brodie A. M. H.: Aromatase inhibitors. Synthesis and biological activity of androstenedione derivatives. *J. med. Chem.* **28** (1985) 788–795.
56. Metcalf B. W., Wright C. L., Burkhart, J. P. and Johnston J. O.: Substrate-induced inactivation of aromatase by allenic and acetylenic steroids. *J. Am. chem. Soc.* **103** (1981) 3221–3222.
57. Marcotte P. A. and Robinson C. H.: Synthesis and evaluation of 10β-substituted estr-4-ene-3,17-diones as inhibitors of human placental microsomal aromatase. *Steroids* **39** (1982) 325–344.
58. Covey D. F., Hood W. F. and Parikh V. D.: 10ß-Propynyl substituted steroids. Mechanism-based enzyme-activated irreversible inhibitors of estrogen biosynthesis. *J. biol. Chem.* **256** (1981) 1076–1079.
59. Loozen H. J. J. and van Luit P. J. N.: European patent application 0 149 499 (1985).
60. Nishino Y., Kerb U., Henderson D. A., Schillinger E., Beier S., Wiechert R., Neumann F. and El Etreby M. F.: *In vivo* and *in vitro* evaluation of a new selective aromatase inhibitor. *Proc. Int. Congr. Endocr.* '85 Int. Cong. Series no. 683. Elsevier, Amsterdam (1986) pp. 443–446.
61. Henderson D., Norbisrath G. and Kerb U.: 1-Methyl-1,4-androstadiene-3,17-dione (SH489): characterization of an irreversible inhibitor of estrogen biosynthesis. *J. steroid Biochem.* **24** (1986) 303–306.
62. Brodie A. M. H., Garrett W. M., Hendrickson J. R., Tsai-Morris C., Marcotte P. A. and Robinson C. H.: Inactivation of aromatase *in vitro* by 4-hydroxy-4-androstene-3,17-dione and 4-acetoxy-4-androstene-3,17-dione and sustained effects *in vivo*. *Steroids* **38** (1981) 693–702.
63. Covey D. F. and Hood W. F.: Aromatase enzyme catalysis is involved in the potent inhibition of estrogen biosynthesis caused by 4-acetoxy and 4-hydroxy-4-androstene-3,17-dione. *Molec. Pharmac.* **21** (1982) 173–180.
64. Covey D. F. and Hood W. F.: Enzyme-generated intermediates derived from 4-androstene-3,6,17-trione and 1,4,6-androstatriene-3,17-dione cause a time-dependent decrease in human placental aromatase activity. *Endocrinology* **108** (1981) 1597–1599.
65. Covey D. F. and Hood W. F.: A new hypothesis based on suicide substrate inhibitor studies for the mechanism of aromatase. *Cancer Res.* Suppl. **42** (1982) 3327s–3333s.
66. Henderson D., Dummann L. and Norbisrath G.: Reconstitution and affinity labelling of human placental aromatase. In *Proc. 5th Ovarian Workshop* (Edited by D. O. Toft and R. J. Ryan). Ovarian Workshops, Champaign, IL (1985) pp. 357–361.
67. Johnston J. O., Wright C. L. and Metcalf B. W.: Time-dependent inhibition of aromatase in trophoblastic tumor cells in tissue culture. *J. steroid Biochem.* **20** (1984) 1221–1226.
68. Ellinwood W. E., Hess D. L., Roselli C. E., Spies H. G. and Resko J. A.: Inhibition of aromatization stimulates luteinizing hormone and testosterone secretion in adult male rhesus monkeys. *J. clin. Endocr. Metab.* **59** (1984) 1088–1096.
69. Johnston J. O., Wright C. L. and Metcalf B. W.: Biochemical and endocrine properties of a mechanism-based inhibitor of aromatase. *Endocrinology* **115** (1984) 776–785.
70. Foster A. B., Jarman M., Mann J. and Parr I. B.: Metabolism of 4-hydroxyandrost-4-ene-3,17-dione by rat hepatocytes. *J. steroid Biochem.* **24** (1986) 607–617.
71. Goss P. E., Jarman M., Wilkinson J. R. and Coombes R. C.: Metabolism of the aromatase inhibitor 4-hydroxyandrostenedione *in vivo*. Identification of the glucuronide as a major urinary metabolite in patients and biliary metabolite in the rat. *J. steroid Biochem.* **24** (1986) 619–622.
72. Cark A. F.: Steroid Δ^4-reductases: their physiological role and significance. In *Steroid Biochemistry* (Edited by R. Hobkirk). CRC Press, Boca Raton, Vol. 1, (1979) pp. 1–27.
73. Mode A. and Rafter I.: The sexually differentiated Δ^4-3-ketosteroid 5β-reductase of rat liver. Puri-

fication, characterization and quantitation. *J. biol. Chem.* **260** (1985) 7137–7141.

74. Williamson D. G.: The biochemistry of the 17-hydroxysteroid dehydrogenases. In *Steroid Biochemistry* (Edited by R. Hobkirk). CRC Press, Boca Raton, Vol. 1, (1979) pp. 83–110.
75. Oshima H., Ochiai K., Niizato N. and Tamaki A.: On testicular 17β-hydroxysteroid oxidoreductase product activation of testosterone formation from androstenedione *in vitro*. *Biochim. biophys. Acta* **306** (1973) 227–236.
76. Oshima H., Yoshida K. and Troen P.: A further study of 17β-hydroxysteroid oxidoreductase in the human testis: mechanism of *in vitro* activation. *Endocr. japon.* **27** (1980) 107–115.
77. Schröder H., Ziegler M. and El Etreby M. F.: Effects of aromatase inhibiting 4-androstene derivatives on androgen metabolism in a cell suspension of the rat testis. *J. steroid Biochem.* **25** (Suppl.) (1986) 68s.
78. Brodie, A. M. H., Schwarzel W. C., Schaikh A. A. and Brodie H. J.: The effect of an aromatase inhibitor 4-hydroxy-4-androstene-3,17-dione, on estrogen-dependent processes in reproduction and breast cancer. *Endocrinology* **100** (1977) 1684–1695.
79. Brodie A. M. H., Marsh D. A. and Brodie H. J.: Aromatase inhibitors—IV. Regression of hormone-dependent mammary tumors in the rat with 4-acetoxy-4-androstene-3,17-dione. *J. steroid Biochem.* **10** (1979) 423–429.
80. Goss P. E., Powles T. J., Dowsett M., Hutchinson G., Brodie A. M. H., Gazet J.-C. and Coombes R. C.: Treatment of advanced postmenopausal breast cancer with an aromatase inhibitor, 4-hydroxyandrostenedione: Phase II report. *Cancer Res.* **46** (1986) 4823–4826.
81. Zoppi S., Cocconi M., Natali A., Constantini A., Sorio M., Martini L. and Motta M.: *In vitro* effects of an aromatase inhibitor on 5α-reductase activity in human hypertrophic prostatic tissue. *J. clin. Endocr. Metab.* **63** (1986) 269–271.
82. Kelly W. G., Judd D. and Stolee A.: Aromatization of Δ^4-androstene-3,17-dione,19-hydroxy-Δ^4-androstene-3,17-dione and 19-oxo-Δ^4-androstene-3,17-dione at a common catalytic site in human placental microsomes. *Biochemistry* **16** (1977) 140–145.
83. Fishman J. and Goto J.: Mechanism of estrogen biosynthesis. Participation of multiple enzyme sites in placental aromatase hydroxylations. *J. biol. Chem.* **256** (1981) 4466–4471.