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**Steroid Inhibitors**

# AROMATASE INHIBITORS AND HORMONE-DEPENDENT CANCERS

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**Summary**—Aromatase (estrogen synthetase) occurs in a variety of tissues. Using immunocytochemistry, we have recently located this enzyme in cellular compartments of several types of human tissue. Furthermore, we found the mRNA was located in the same structures where tested. As both gonadal and peripherally formed estrogen contribute to growth of hormone sensitive cancers, we have developed aromatase inhibitors to block synthesis of this hormone. We have determined that 4-hydroxyandrostenedione (4-OHA) selectively inhibits aromatase activity in ovarian and peripheral tissues and reduces plasma estrogen levels in rat and non-human primate species. 4-OHA was also found to inhibit gonadotropin levels and reduce estrogen and progesterone receptor levels in treated animals. The mechanism of these effects appear to be associated with the weak androgenic activity of the compound. These effects together with aromatase inhibition may result in a synergistic response reducing estrogen production and action. In postmenopausal women, estrogens are mainly of peripheral origin. When postmenopausal breast cancer patients were administered either daily oral or parenteral weekly treatment with 4-OHA at doses that did not affect their gonadotropin levels, plasma estrogen concentrations were significantly reduced. Complete or partial response to treatment occurred in 34% of 100 patients with advanced breast cancer, while the disease was stabilized in 12%. These results indicate that 4-OHA is of benefit in postmenopausal patients with advanced disease who have relapsed from prior hormonal therapies, and that steroidal inhibitors may be of value in premenopausal patients.

## INTRODUCTION

As estrogens have an important role in the growth of breast and other hormone sensitive cancers, we have focused our research over the past several years, on the development of compounds which would reduce production of this steroid by inhibiting the aromatase enzyme.

Aromatase is an enzyme complex consisting of a cytochrome *P*-450 ($P$-$450_{Arom}$) hemoprotein and a flavoprotein, NADPH-cytochrome *P*-450 reductase [1]. The $P$-$450_{Arom}$ binds the C-19 androgen substrates e.g. androstenedione and testosterone and catalyzes their conversion to estrogens. Aromatization of androgens appears to involve three hydroxylation steps [2], eventual loss of the angular methyl group at C-19 and the elimination of the 1$\beta$ and 2$\beta$ hydrogens resulting in aromatization of the A-ring of the androgen molecule to form estrogen. Two of these hydroxylations appear to occur at the C-19 position [3, 4], while it is presently uncertain whether the third hydroxylation takes place at this position [5] or at a separate site, such as the C-2$\beta$ position [6]. The cytochrome $P$-$450_{Arom}$ has recently been purified and the DNA cloned from human placenta by several groups [7–10]. The role of the NADPH-cytochrome *P*-450 reductase is to donate electrons to the cytochrome *P*-450. The reductase is common to most cell types and the same enzyme appears to perform this function for most cytochrome *P*-450 reactions [11].

Studies of the gene sequence of cytochrome $P$-$450_{Arom}$ indicates that its highly conserved, consistent with the fact that aromatase occurs in a wide variety of tissues in both males and females of many species. The overall homology with other cytochrome *P*-450 genes is only about 30%. Aromatase is therefore considered to be in a separate gene family within the overall superfamily of cytochrome *P*-450 genes [12].

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We have recently been able to investigate the location of the enzyme in sections of several human tissues by immunocytochemistry using a monoclonal antibody of Mendelson *et al.* to purified $P\text{-}450_{Arom}$ [13] in the peroxidase-antiperoxidase [PAP] staining technique or the biotin–avidin technique [14]. Our studies reveal that in the human ovary, aromatase is located in the thecal compartment of the developing follicle, but as the follicle progresses towards ovulation, there is increased expression of the enzyme in granulosa cells [15]. Utilizing the same technique we have identified over-expression of aromatase located in the Sertoli cells of a testicular tumor of a boy with gynecomastia [16]. In the human placenta, which is the major source of estrogen during pregnancy, we have also investigated the site of transcription. From the results of *in situ* hybridization histochemistry on tissue sections, the enzyme appears to be synthesized in the cytoplasm of syncytiotrophoblasts in the outer layer of the chorionic villi. These are the same cells in which we detected the presence of the enzyme [17]. In other tissue, aromatase has been identified by conventional biochemical techniques in several locations in the brain including the hypothalamus [18] amygdala and hippocampus [19], adipose tissue of both men and women [20, 21] and the testis [22]. There are a number of reports indicating the presence of aromatase in breast tumors [23–26].

Although it is currently believed that a single gene encodes only one species of aromatase in the human [27], regulation of the enzyme appears to be tissue-specific. In the gonads, aromatase is regulated by gonadotropins. Information concerning the regulation of aromatase in other tissues is sparse, except for adipose tissue in which glucocorticoids and growth factors have been shown to be involved [28]. Production of estrone by adipose tissue is also positively correlated with obesity and ageing [21]. Thus, estrogens from many sources, both gonadal and extragonadal, can contribute to promoting the growth of estrogen sensitive cancers.

We reasoned therefore that inhibitors of aromatase by interacting with the enzyme in all tissues, could provide both selective and effective reduction of estrogen production. Such inhibitors would be potentially useful treatment for diseases in which estrogens have a role, e.g. breast and endometrial cancer. Breast cancer is most prevalent among postmenopausal women; about 75% of patients have hormone-responsive tumors, whereas about 60% of premenopausal patients have hormone-responsive tumors [29]. Methods of controlling estrogen production or action result in objectively quantifiable breast tumor regression which can often be long lasting [29]. Until recently, all inhibitors of estrogen action were also weak or partial agonists. Thus, tamoxifen may not be the optimal antiestrogen. It appears to be insufficiently effective in consistently blocking the action of the high levels of estrogen which occur in premenopausal women [30]. Thus, aromatase inhibitors have the potential for being more effective than tamoxifen. Since we began our studies on aromatase inhibitors, it has become apparent that tamoxifen provides superior response rates and less toxicity, than cytotoxic agents in postmenopausal patients with estrogen receptor-positive tumors [30]. Nevertheless, as with other anticancer agents, patients ultimately relapse from tamoxifen treatment. Aromatase inhibitors, therefore, offer an alternative approach to patients who have failed to respond to, or who have relapsed from tamoxifen treatment. In addition, a goal of our work was to find agents that have low toxicity and could therefore be used for long periods and in more advantageous strategies, such as in adjuvant therapy.

In the early seventies, we began a program to synthesize and identify compounds which are potent aromatase inhibitors [32]. We envisaged that selective inhibition of aromatase was a feasible goal, because aromatization of the androgen molecule is a unique feature of estrogen biosynthesis. In addition, since estrogens are the last steroids to be synthesized in the biosynthetic sequence, their inhibition would not interfere with the production of other essential hormones, such as cortisol.

The most potent inhibitor *in vitro* and *in vivo* that we have described is 4-hydroxyandrostene-3,17-dione (4-OHA) [33, 34]. This is the first compound designed as an aromatase inhibitor to be evaluated for clinical use in breast cancer patients. Studies have been initiated in postmenopausal patients with advanced metastatic disease who have relapsed from the other treatments [35, 36]. The efficacy of 4-OHA compared to tamoxifen in breast cancer patients still remains to be determined.

## *IN VITRO* STUDIES WITH AROMATASE INHIBITORS

In our initial studies, the most active aromatase inhibitors were found to be C-19, 17-

keto androstenedione derivatives. Although these compounds exhibited properties typical of competitive inhibitors, we also observed that several of the more potent ones caused inactivation of aromatase. This phenomenon was demonstrated with 4-OHA by preincubating the compound for various lengths of time with microsomes from human placental tissue or rat ovaries in the presence of NADPH [37]. Following removal of the compound, a time-dependent loss of enzyme activity was observed. The reaction followed pseudo-first-order kinetics and appears to be irreversible. Thus, 4-OHA appears to be acting as a mechanism-based inhibitor.

A number of other steroidal aromatase inhibitors have also been reported to cause inactivation and appear to be acting by a similar mechanism e.g. 10(2-propynlestr-4-ene-3,17-dione [38] and analogs of 7α-(4′-amino)-phenylthio-androstene-3,17-dione [39].

In addition to these steroid inhibitors, several non-steroidal aromatase inhibitors have been described. However, none have been reported to act by the above mechanism. Several of these inhibitors are imidazoles or structurally related to aminoglutethimide (AG). AG is an inhibitor of steroid hydroxylase reactions and lacks specificity for aromatase [40]. Cortisol replacement therapy is therefore required when this drug is administered. Because of its potency for aromatase and its previous clinical use as an anticonvulsant, AG became the first compound used to test the concept of aromatase inhibition as a means of treatment for postmenopausal breast cancer [41]. Several new agents are reported to be very potent inhibitors of aromatase, such as 4-(5,6,7,8-tetrahydrimidazol [1,5-α] pyridin-5-yl)benzonitrile monohydro-chloride (CGS16949A) [42] and 6[(4-chlorophenyl) (1H-1,2,4-triazol-1-yl)methyl]-1-methyl-1-H-benzotriazole (R76713) [43]. Their actions on other enzymes, such as adrenal corticosteroids, are therefore likely to be minor [42].

## THE EFFECTS OF 4-OHA ON CYCLING ANIMALS

The dimethylbenz(a)anthracene (DMBA)-induced, hormone-dependent, mammary tumors of the rat [34] are comparable to premenopausal breast cancer as growth of these tumors is dependent upon ovarian steroids. 4-OHA (50 mg/kg/day, s.c.) was found to be highly effective in causing regression of DMBA-induced tumors.

Although 4-OHA did not have significant actions on ovarian steroidogenic enzymes other than aromatase *in vitro*, we considered it important to evaluate the effect of the compound on plasma hormone levels and on steroid receptor levels in the rat model. At the end of 4 weeks of treatment with 4-OHA, ovarian aromatase activity and estrogen secretion as well as peripheral estradiol levels, were markedly reduced in 4-OHA-treated rats compared to vehicle injected control animals [34]. However, despite the low levels of peripheral estradiol concentrations, LH and FSH concentrations were found to be significantly reduced. Treatment with 4-OHA also was found to prevent the reflex rise in gonadotropins that usually occurs following ovariectomy. Furthermore, the high levels (> 1000 ng/ml) of gonadotropins in long-term ovariectomized animals could be reduced by 4-OHA treatment administered for 2 weeks. These effects of 4-OHA could be completely reversed by coadministration of the antiandrogen, flutamide, suggesting that 4-OHA acts as an androgen to inhibit LH and FSH [44].

The effects of a non-steroidal aromatase inhibitor, aminoglutethimide were also compared in similar experiments [45]. AG reduced ovarian estradiol secretion initially but long-term treatment of intact rats resulted in estradiol secretion and ovarian aromatase activity which were higher than those measured 3 h after treatment. In the same animals the mean ovarian weight was significantly increased and gonadotropin levels were 1.8-fold higher than values for intact control rats (Table 1). These observations suggest that the increase in LH and FSH levels resulting initially from reduced estrogen secretion, may subsequently act to stimulate the ovary and lead to an increase in estrogen synthesis. This hypothesis is substantiated by the report that the mid-cycle surge of estradiol in premenopausal breast cancer patients is not consistently suppressed by AG [46]. We also observed that in studies of the non-human primate menstrual cycle, LH, as well as estradiol levels, were not increased above very low basal values by long-term treatment (> 1 month) with 4-OHA (50 mg/kg/day, s.c.) [47]. These results suggest that potent steroidal aromatase inhibitors which also have a direct action on the hypothalamus–pituitary axis, may be effective in reducing ovarian estrogen production in premenopausal patients.

Further studies performed in the rat model indicate that 4-OHA inhibits estrogen receptor

Table 1. Effect of 4-OHA and AG treatment on rats with DMBA-induced mammary tumor

| Treatment | Ovarian wt (mg)[a] | Aromatase (% of activity/ mg of protein) | Estradiol (pg/ml) | LH (ng/ml)[a] |
|---|---|---|---|---|
| Control | 79.6 ± 5.5[b,c] (7)[d,e] | 3.10 ± 1.03[c] [6] | 474.0 ± 100.3[c] (5) | 167.7 ± 29.8[c] (7) |
| 4-OHA | 45.8 ± 5.2[c] (8) | 0.43 ± 0.06[c] (8) | 140.0 ± 18.7[c] (8) | 14.7 ± 6.4[c] (10) |
| AG | 117.3 ± 7.3[c] (8) | 1.58 ± 0.25 (6) | 326.8 ± 153.4 (5) | 297.3 ± 50.8 (7) |

[a]Statistical analyses of differences were performed on $\log_e$-transformed data, since Cochran's test of raw values revealed heterogeneous variances.
[b]Mean ± SE.
[c]Significance ($P < 0.01$).
[d]Numbers in parentheses, number of animals.
[e]Significance ($P < 0.005$).
Rats were given injections s.c. twice daily for 4 weeks of 4-OHA or AG (50 mg/kg/day). Blood was collected from the ovarian vein 3 h after the last injection, and then ovaries were removed, stored frozen, and homogenized, and aromatase activity was determined. Heart blood was collected at sacrifice, and LH concentrations were determined in the plasma. (From Wing *et al.* [45].)

(ER) and progesterone receptors (PR) in the rat uterus and DMBA-induced mammary tumors [45]. 4-OHA does not interact directly with these receptors *in vivo* even at $10^{-6}$ M, as determined by competitive binding assays. However, concomitant treatment of ovariectomized rats with 4-OHA and antiandrogen, but not antiestrogen, counteracted the reduction in both ER and PR, suggesting that the effect of 4-OHA on receptor levels is also via an androgenic mechanism. The effect of the antiestrogen clomiphene was to enhance the level of PR in the uterus. 4-OHA has about 1% the androgenic activity of testosterone in bioassays [34]. This level of activity was confirmed in a competitive binding assay for androgen receptors [48]. Others have reported similar effects of androgens on reducing ER in human cancer cell lines [49] and progesterone receptors in rat mammary tumors [50].

In summary, at the doses administered to cycling animals, 4-OHA acts to inhibit ovarian aromatase but also directly inhibits gonadotropin production and reduces estrogen and progesterone receptors. These effects appear to be mediated by interaction with androgen receptors, but not estrogen receptors, and may result in a synergistic response by reducing estrogen production and its action.

## THE EFFECT OF 4-OHA ON PERIPHERAL AROMATASE

Peripheral aromatization is an important source of estrogens which contributes to such conditions as endometrial as well as breast cancer in postmenopausal women, and gynecomastia in men. Several studies imply that prostatic tissue contain estrogen receptors [51] and that estrogens may have a role in prostatic disease [52].

We recently carried out a study to determine the presence of aromatase in the prostate [53]. We employed both a product isolation method using HPLC, as well as the $^3H_2O$ assay in which the $^3H$-label released during aromatization from the C-1$\beta$ and C-2$\beta$ of the androstenedione substrate is measured as $^3H_2O$. We, and others, have used the $^3H_2O$ assay in placental and ovarian tissue and found that the amount of $^3H_2O$ released is equivalent to estrogen produced [15]. Although $^3H_2O$ was released during incubations of prostatic tissue from both BPH and cancer patients, no estradiol or estrone was detected. We are currently uncertain why $^3H_2O$ is released, but conclude that prostatic tissue does not contain significant amounts of aromatase. 4-OHA is effective in inhibiting peripheral aromatization as demonstrated in male non-human primates (rhesus monkeys) [54]. Thus, if estrogen has a role in prostatic diseases, aromatase inhibitors may be of value by inhibiting peripheral production of estrogens.

## STUDIES WITH 4-OHA IN POSTMENOPAUSAL BREAST CANCER PATIENTS

Based on results from the above studies which demonstrated that 4-OHA is a potent aromatase inhibitor *in vitro*, inhibits ovarian aromatase activity and estrogen production, as well as peripheral aromatization, we have now evaluated 4-OHA in 128 postmenopausal patients with advanced, metastatic breast cancer [55]. The first few patients received material prepared in our laboratories [35]. All subsequent patients received material supplied by Ciba-Geigy Pharmaceuticals (CGP 32349).

The effect of 4-OHA on suppression of plasma estradiol was determined in groups of patients administered the compound orally or

parenterally [56]. A single injection of 4-OHA of 500 mg i.m. suppressed estradiol levels to a mean of 36.3 ± 3.3% (SE) of base-line 4–7 days later in 14 patients. This suppression was maintained in 6 of 7 patients for more than 14 days. The half-life of 4-OHA following a single injection was approx. 8 days. When the plasma level of 4-OHA had fallen to less than 3 ng/ml, estradiol concentrations begun to rise. Results from patients receiving a single dose of 125 mg 4-OHA i.m. were similar to those with 500 mg i.m. except that estradiol levels began to rise earlier. When 4-OHA was administered orally (500 mg per day), further suppression occurred each day of treatment and by 7 days of treatment, the mean suppression was similar to that with 500 mg 4-OHA i.m. by days 4–7. These results suggest that a dose of 125 mg i.m. is sufficient to maintain maximal estradiol suppression for more than 7 days. Also, despite rapid glucuronidation of 4-OHA by the liver [56], 4-OHA given orally is equally effective in suppressing estradiol [56].

No effect of 4-OHA treatment was observed on serum gonadotropin, sex steroid binding globulin or dehydroepiandrosterone sulfate levels [36], suggesting that suppression of estradiol by 4-OHA at a dose of 500 mg i.m. was the result of inhibition of peripheral aromatization. Recently, inhibition of peripheral aromatization in postmenopausal patients [58] treated with 4-OHA was confirmed by measuring conversion of radiolabeled precursor [54].

Of the 128 patients treated with 4-OHA, 100 were assessable. Groups of patients received 500 mg i.m. once a week, 250 mg i.m. every 2 weeks, or 500 mg orally every day. All were either postmenopausal or had been previously ovariectomized and had received 1–4 treatments. The patients had assessable locally advanced or metastatic disease. Assessment was made at 3, 6 and 9 months and at the end of treatment using the criteria of the International Union Against Cancer. Of parients responding to treatment, 91% had ER positive tumors and of patients with disease stabilization 86% had ER positive tumors, whereas 53% of patients whose disease progressed had ER positive tumors. Eighty of the 100 patients had received prior endocrine treatment (ovariectomy, tamoxifen, AG or megace). Patients who had previously responded to ovariectomy, had an excellent (88%) chance of responding to 4-OHA. The compound was well tolerated by the patients. The only major side-effect was local reaction at the site of injection in 13% of patients who received 500 mg i.m. This was not a problem in patients receiving the lower dose. The results are shown in Table 2. Overall, 34% of patients experienced complete or partial response to treatment, the disease was stabilized in 12% but progressed in 54% of patients [55].

Table 2. Overall response of breast cancer patients to 4-OHA

| Dose | Number of patients | Number (percentage) responding | | |
|---|---|---|---|---|
| | | CR/PR[a] | NC[b] | PD[c] |
| 500 mg i.m. | 51 | 17 (33) | 7 (14) | 27 (53) |
| 250 mg i.m. | 25 | 9 (36) | 5 (4) | 11 (44) |
| 500 mg orally | 24 | 8 (33) | 0 | 16 (67) |
| Total | 100 | 34 | 12 | 54 |

[a]Complete or partial response.
[b]No change.
[c]Progressive disease.
Postmenopausal patients with advanced disease were injected with 500 mg i.m. once weekly, 250 mg i.m. every 2 weeks, or 500 mg orally each day. (Data from Coombes *et al.* [55].)

In conclusion, we have demonstrated that 4-OHA is a selective inhibitor of aromatase and causes inactivation of the enzyme. The compound inhibits both ovarian and peripheral aromatase in animal models. In addition, 4-OHA directly inhibits gonadotropins and reduces ER and PR in the uterus and mammary tumors of the rat. These effects could be reversed by antiandrogen but not by antiestrogen treatment, suggesting that these actions of 4-OHA may be mediated via the androgen receptor. At the doses administered to the non-human primate (baboons) during the menstrual cycle, 4-OHA treatment also reduced steroid and gonadotropin levels to base-line after one month of treatment. Our findings suggest that inhibition of the rise in gonadotropins is important in maintaining suppression of ovarian estradiol during the cycle. In the first clinical studies with 4-OHA, 34% of postmenopausal breast cancer patients experienced objective response to treatment. The compound administered by both parenteral and oral routes reduced plasma estrogen levels, had few side effects and was effective in patients who had relapsed from previous endocrine treatment.

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## REFERENCES

1. Thompson E. A. Jr and Siiteri P. K.: The involvement of human placental microsomal cytochrome P-450 in aromatization. *J. Biol. Chem.* **249** (1974) 5373–5378.

2. Thompson E. A. Jr and Siiteri P. K.: Utilization of oxygen and reduced nicotinamide adenine dinucleotide phosphate by human placental microsomes during aromatization of androstenedione. *J. Biol. Chem.* **249** (1974) 5364–5372.
3. Meyer A. S.: Conversion of 19-hydroxy-4-androstene-3,17-dione to estrone by endocrine tissue. *Biochim. Biophys. Acta* **17** (1955) 441–442.
4. Morato T., Hayano M., Dorfman R. I. and Axelrod L. R.: The intermediate steps in the biosynthesis of estrogens from androgens. *Biochem. Biophys. Res. Commun.* **6** (1961) 334–338.
5. Cole P. A. and Robinson C. H.: A peroxide model reaction for placental aromatase. *J. Am. Chem. Soc.* **110** (1988) 1284–1285.
6. Goto J. and Fishman J.: Participation of a non-enzymatic transformation in the biosynthesis of estrogens from androgens. *Science* **80** (1977) 195–196.
7. Corbin C. J., Graham-Lorence S., McPhaul M., Mason J. I., Mendelson C. R. and Simpson E. R.: Isolation of a full-length cDNA insert encoding human aromatase system cytochrome P-450 and its expression in non-steroidogenic cells. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 8948–8952.
8. Harada N.: Cloning of a complete cDNA encoding human aromatase: Immunochemical identification and sequence analysis. *Biochem. Biophys. Res. Commun.* **156** (1988) 725–732.
9. McPhaul M., Noble J. F., Simpson E. R., Mendelson C. R. and Wilson J. D.: The expression of a functional cDNA encoding the chicken cytochrome $P\text{-}450_{Arom}$ (aromatase) that catalyses formation of estrogen from androgen. *J. Biol. Chem.* **263** (1988) 16358–16363.
10. Chen S., Besinan M. J., Sparkes R. S., Zollman S., Klisak I., Mohandes T., Hall P. F. and Shively J. E.: Human aromatase: cDNA cloning, Southern blot analysis, and assignment of the gene to chromosome 15. *DNA* **7** (1988) 27.
11. Simmons D. L., Lalley P. A. and Kasper C. B.: Chromosomal assignments of genes coding for components of the mixed-function oxidase system in mice. *J. Biol. Chem.* **260** (1985) 515–521.
12. Nebert D. W., Adesnik M., Coon M. J., Estabrook R. W., Gonzales F. J., Guengerich F. P., Gunsalus I. C., Johnson E. F., Kemper B., Levin W., Philips I. R., Sato R. and Waterman M. R.: The P-450 gene superfamily; recommended nomenclature. *DNA* **6** (1987) 1–11.
13. Mendelson C. R., Wright E. E., Evans C. T., Porter J. C. and Simpson E. R.: Preparation and characterization of polyclonal and monoclonal antibodies against human aromatase cytochrome P-450 and their use in its purification. *Archs Biochem. Biophys.* **243** (1985) 480–491.
14. Inkster S. E. and Brodie A. M. H.: Immunocytochemical studies of aromatase in early and full term human placental tissues: comparison with biochemical assays. *Biol. Reprod.* **41** (1989) 889–898.
15. Inkster S. and Brodie A.: Immunocytochemical localization of aromatase in human ovarian follicles. *71st Ann. Mtg of the Endocrine Soc.*, Seattle (1989) Abstr. 915.
16. Coen P., Kulin H., Ballantine T., Zaino R., Frauenhoffer E., Boal D., Inkster S., Brodie A. and Santen R.: An aromatase producing sex cord tumor resulting in prepubertal gynecomastia. *New Engl. J. Med.* (In press).
17. Banks P., Brodie A., Petersen S., Zoller T. and Barraclough C.: Localization of aromatase mRNA in human term placenta by *in situ* hybridization histochemistry. *71st Ann. Mtg of the Endocrine Soc.*, Seattle (1989) Abstr. 76.
18. Naftolin F., Ryan K. J., Davis I. J., Reddy V. V., Flores F., Petro Z. and Kuhn M.: The formation of estrogen by central neuroendocrine tissues. *Recent Prog. Horm. Res.* **31** (1975) 295–319.
19. Roselli C. E., Horton L. E. and Resko J. A.: Distribution and regulation of aromatase activity in the rat hypothalamus and limbic systems. *Endocrinology* **117** (1985) 2471–2477.
20. 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.
21. Hemsell D. L., Grodin J., Breuner P. F., Siiteri P. K. and MacDonald P. C.: Plasma precursors of estrogen. II Correlation of the extent of conversion of plasma androstenedione to estrone with age. *J. Clin. Endocr. Metab.* **38** (1974) 476–479.
22. Baird D. T., Horton R., Longcope C. and Tait J. F.: Steroid dynamics under steady-state conditions. *Recent Prog. Horm. Res.* **25** (1969) 611–634.
23. Miller W. R., Forrest A. P. M. and Hamilton T.: Steroid metabolism by human breast and rat mammary carcinoma. *Steroids* **23** (1974) 379–395.
24. Varela R. M. and Dao T. L.: Estrogen synthesis and estradiol binding by human mammary tumors. *Cancer Res.* **38** (1978) 2429–2433.
25. Lipton A., Santner S. J., Santen R. J., Harvey H. A., Feil P. D., White-Hershey D., Bartholomew M. J. and Antle C. E.: Aromatase activity in primary and metastatic human breast cancer. *Cancer* **59** (1987) 779–782.
26. Perel E., Daniilescu D., Kharlip L., Blackstein M. and Killinger D. W.: Steroid modulation of aromatase activity in human cultured breast carcinoma cells. *J. Steroid Biochem.* **29** (1988) 393–399.
27. Simpson E. R., Merrill J. C., Hollub A. J., Graham-Lorence S., Mendelson C. R.: Regulation of estrogen biosynthesis by human adipose cells. *Endocr. Rev.* **10** (1989) 136–148.
28. Simpson E. R., Ackerman G. E., Smith M. E., Mendelson C. R.: Estrogen formation in stromal cells of adipose tissue of women: induction by glucocorticoids. *Proc. Natn. Acad. Sci. U.S.A.* **78** (1981) 5690–5695.
29. McGuire W. L.: An update on estrogen and progesterone receptors in prognosis for primary and advanced breast cancer. In *Hormones and Cancer* (Edited by S. Iacobelli, R. J. B. King, H. R. Lindner and M. E. Lippman). Raven Press, New York Vol. 15 (1980) pp. 337–344.
30. Manni A. and Pearson O. H.: Antiestrogen induced remissions in premenopausal women with stage IV breast cancer: affects on ovarian function. *Cancer Treat. Rep.* **64** (1980) 111–116.
31. Early Breast Cancer Trialists' Collaborative Group: Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. *New Engl. J. Med.* **319** (1988) 1681–1692.
32. Brodie A. M. H., Schwarzel W. C. and Brodie H. J.: Studies on the mechanism of estrogen biosynthesis in the rat ovary. *J. Steroid Biochem.* **7** (1976) 787–793.
33. Schwarzel W. C., Kruggel W. and Brodie H. J.: Studies on the mechanism of estrogen biosynthesis. VII. The development of inhibitors of the enzyme system in human placenta. *Endocrinology* **92** (1973) 866–880.
34. Brodie A. M. H., Schwarzel W. C., Shaikh 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.
35. Coombes R. C., Goss P., Dowsett M., Gazet J. C. and Brodie A. M. H.: 4-Hydroxyandrostenedione treatment of postmenopausal patients with advanced breast cancer. *Lancet* **2** (1984) 1237–1239.
36. Goss P. E., Coombes R. C., Powles T. J., Dowsett M. and Brodie A. M. H.: Treatment of advanced postmenopausal breast cancer with aromatase inhibitor, 4-hydroxyandrostenedione. Phase 2 report. *Cancer Res.* **46** (1986) 4823–4826.

37. Brodie A. M. H., Garrett W. M., Hendrickson J. R., Marcotte P. A. and Robinson C. H.: Inactivation of aromatase activity in placental and ovarian microsomes by 4-hydroxyandrostene-3,17-dione and 4-acetoxyandrostene-3,17-dione. *Steroids* **38** (1981) 693–702.
38. 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.
39. Brueggemeier R. W., Li P.-K., Snider C. E., Darby M. V. and Katlic N. E.: 7 α-substituted androstenediones as effective *in vitro* and *in vivo* inhibitors of aromatase. *Steroids* **50** (1987) 163–178.
40. Uzgris V. I., Whipple C. A. and Salhanick H. A.: Stereoselective inhibition of cholesterol side chain cleavage by enantiomers of aminoglutethimide. *Endocrinology* **101** (1977) 89–92.
41. Santen R. J., Santner S., Davis B., Veldhuis J., Samojlik E. and Ruby E.: Aminoglutethimide inhibits extraglandular oestrogen production in postmenopausal women with breast carcinoma. *J. Clin. Endocr. Metab.* **47** (1978) 1257–1265.
42. Steel R. E., Mellor L. B., Sawyer W. K., Wasvary J. M. and Browne L. J.: *In vitro* and *in vivo* studies demonstrating potent and selective estrogen inhibition with the nonsteroidal aromatase inhibitor CGS 16949A. *Steroids* **50** (1987) 147–161.
43. Wouters W., De Coster R., Beerens D., Doolaege R., Gruwez J. A., Van Camp K., Van Der Pas H. and van Herendael B.: Potency and selectivity of the aromatase inhibitor R76 713. A study in human ovarian, adipose stromal, testicular and adrenal cells. *J. Steroid Biochem.* **36** (1990) 57–66.
44. Wing L.-Y. C., Hammond J. O. and Brodie A. M. H.: Differential responses of sex steroid target tissue of rats treated with aromatase inhibitor 4-hydroxyandrostenedione. *Endocrinology* **122** (1988) 2418–2427.
45. Wing L.-Y., Garrett W. M. and Brodie A. M. H.: The effect of aromatase inhibitors, aminoglutethimide and 4-hydroxyandrostenedione in cyclic rats with DMBA-induced mammary tumors. *Cancer Res.* **45** (1985) 2425–2428.
46. Santen R. J., Samojlik E. and Wells S. A.: Resistance of the ovary to blockade of aromatization with aminoglutethimide. *J. Clin. Endocr. Metab.* **51** (1980) 473–477.
47. Brodie A. M. H., Hammond J. O., Ghosh M., Meyer K. and Albrecht E.: The effect of treatment with aromatase inhibitor 4-OHA on the primate menstrual cycle. *Cancer Res.* **49** (1989) 4780–4784.
48. Eils C. and Edelson S. K.: The use of human skin fibroblasts to obtain potency estimates of drugs binding to androgen receptors. *J. Clin. Endocr. Metab.* **59** (1980) 51–55.
49. Poulin R., Simard J., Labrie C., Petitclerc L., Dumont M., Lagace L. and Labrie F.: Down-regulation of estrogen receptors by androgens in the ZR-75-1 human breast cancer cell line. *Endocrinology* **125** (1989) 392–399.
50. Ip M. M., Milholland P. J., Kin V. and Rosen F.: Androgen control of cytosol progesterone receptor levels in the MT-W9B transplantable mammary tumor in the rat. *J. Natn. Cancer Inst.* **69** (1982) 673–681.
51. Ekman P., Barrack E. R., Greene G. L., Jensen E. V. and Walsh P. C.: Estrogen receptors in human prostate: evidence for multiple binding sites. *J. Clin. Endocr. Metab.* **57** (1983) 166–176.
52. Henderson D., Habenicht U.-F., Nishino Y. and El Etreby M. F.: Estrogens and benign prostatic hyperplasia: The basis for aromatase inhibitor therapy. *Steroids* **50** (1987) 219–233.
53. Brodie A. M. H., Son C., King D. A., Meyer K. M. and Inkster S. E.: Lack of evidence for aromatase in human prostatic tissues: effects of 4-hydroxyandrostenedione and other inhibitors on androgen metabolism. *Cancer Res.* **49** (1989) 6551–6555.
54. Brodie A. M. H. and Longcope C.: Inhibition of peripheral aromatization by aromatase inhibitors, 4-hydroxy- and 4-acetoxyandrostenedione. *Endocrinology* **106** (1980) 19–21.
55. Coombes R. C., Goss P. E., Dowsett M., Hutchinson G., Cunningham D., Jarman M. and Brodie A. M. H.: 4-hydroxyandrostenedione treatment for postmenopausal patients with advanced breast cancer. *Steroids* **50** (1987) 245–252.
56. Dowsett M., Goss P. E., Powles T. J., Hutchinson G., Brodie A. M. H., Jeffcoate S. L. and Coombes R. C.: Use of the aromatase inhibitor 4-hydroxyandrostenedione in postmenopausal breast cancer; optimization of therapeutic dose and route. *Cancer Res.* **47** (1987) 1957–1961.
57. 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.
58. Reed M. J., Lai L. C., Owen A. M., Singh A., Coldham N. G., Purohit A., Ghilchik M. W., Shaikh N. A. and James V. H. T.: Effect of treatment with 4-hydroxyandrostenedione on the peripheral conversion of androstenedione to oestrone and *in vitro* tumor aromatase activity in postmenopausal women with breast cancer. *Caner Res.* **50** (1990) 193–196.