History of LHRH agonist and combination therapy in prostate cancer

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Abstract

An LHRH agonist was first administered to a prostate cancer patient 16 years ago in 1980 while combination therapy with an LHRH agonist and a pure antiandrogen was first administered 14 years ago in 1982. We take this opportunity to review briefly the events which, in our opinion, led to such fundamental changes in the endocrine therapy of prostate cancer. Following the observations of Huggins and his colleagues in 1941, orchiectomy and treatment with high doses of estrogens remained the standard therapy of prostate cancer for 50 years. Discovery of the structure of LHRH in 1971 by Schally and his colleagues stimulated the synthesis of highly potent analogs of LHRH with the objective of treating infertility. However, difficulties were met in finding the proper schedule of administration as well as the dose of LHRH agonists which could maintain stimulatory effects upon repeated administration. In fact, contrary to the expectations of a stimulatory effect, we found in 1977 that treatment of adult male rats with an LHRH agonist for a few days caused some inhibition of ventral prostate and seminal vesicle weight, although the inhibitory effects achieved were small compared with those of castration. It was then believed that the high serum LH levels induced by LHRH agonist treatment caused desensitization of the steroidogenic response in the testes.

Even more unexpected was the finding that of all the species studied, man was the most sensitive to the inhibitory action of LHRH agonists on testicular androgen biosynthesis and that medical castration could be easily achieved with LHRH agonists in adult men. In fact, a single intranasal administration of an LHRH agonist to healthy men in 1979 caused the expected acute rise in serum levels of testosterone and its precursors. This increase, however, was followed by a loss of diurnal cyclicity and lowered serum androgen levels which lasted for 3 to 4 days, thus suggesting that man is exquisitely sensitive to the inhibitory action of LHRH agonists. When, in 1980, the first prostate cancer patient received an LHRH agonist at the Laval University Medical Center, it was found that treatment with a high dose of the peptide caused a dramatic reduction in serum testosterone and dihydrotestosterone (DHT) after 2 weeks of administration. Contrary to the usual pattern in medical discoveries, the castration effect of LHRH agonists was first observed...
in men and not in experimental animals where castration is difficult or sometimes impossible to achieve with daily administration of LHRH agonists. A limitation to the use of LHRH agonists to achieve castration in prostate cancer patients was the temporary rise in serum testosterone and DHT at the start of treatment: such an elevation of serum androgens could cause an exacerbation of the symptoms or flare of prostate cancer. It was then decided to combine a pure antiandrogen with the LHRH agonist. In 1978, we found that the inhibitory effects observed in the rat with the combination of an LHRH agonist associated with a pure antiandrogen were more than additive, especially on seminal vesicle weight. These promising results suggested the use of a pure antiandrogen, not only to avoid the risk of disease flare during the first days of treatment with an LHRH agonist but also to offer the possibility of potentiating the inhibitory effects of the LHRH agonist on androgen-sensitive parameters.

Combination therapy with an LHRH agonist and a pure antiandrogen was first administered to a prostate cancer patient in March 1982, also at the Laval University Medical Center. The first patients with advanced prostate cancer treated with combination therapy in a non-randomized study showed a rapid fall in serum prostatic acid phosphatase and a marked improvement of the signs and symptoms of prostate cancer which were highly suggestive of the advantage of combination therapy compared with previous treatments. In fact, the first 58 stage D2 prostate cancer patients showed a positive objective response to combination therapy while a 94% rate of positive response was achieved in the total group of 260 patients entered in this first study. The highly promising results obtained in this initial study provided the stimulus for large-scale randomized and placebo-controlled clinical trials which confirmed the unique benefits of combination therapy on both duration of response and, most importantly, on survival. In fact, combination therapy became the first treatment shown to prolong life in prostate cancer and it has been the gold standard for the endocrine therapy of prostate cancer since 1989.

Since localized disease provides the only opportunity for cure of prostate cancer, the same combination therapy was next administered to patients at earlier stages of the disease. Randomized studies performed in patients with localized disease have recently demonstrated that combination therapy administered for 3 months before radical prostatectomy increases the proportion of patients having organ-confined disease by about 50% while the same approach associated with radiotherapy has been shown to delay the time to progression. Although the impact on survival of this adjuvant and adjuvant use of combination therapy, in association with radical prostatectomy or radiotherapy, remains to be assessed by long-term follow-up of the patients, it certainly raises the hope of a further significant improvement in the therapy of prostate cancer.

Since screening for prostate cancer with serum prostatic specific antigen is gaining wide acceptance, the diagnosis of prostate cancer is made at earlier stages of the disease where the reversibility of medical castration with LHRH agonists is the only acceptable approach. It has thus become clear that LHRH agonists should almost completely replace orchiectomy in the near future while there is no more valid reason to use treatment with estrogens because of their serious and life-threatening cardiovascular side-effects.

Knowledge of the structure of the genes responsible for the formation of DHT in the human prostate provides the scientific basis for the observation that about 50% of total androgens responsible for the growth of prostate cancer are synthesized in the prostatic tissue itself. Fortunately, prostate cancer is exquisitively sensitive to androgen deprivation, thus providing a powerful tool to control this cancer. The data summarized above indicate that the use of a pure antiandrogen in association with chemical castration represents the most acceptable approach to block androgen action maximally and thus cause maximal induction of cell cycle arrest and apoptosis in prostate cancer. Since the available antiandrogens still leave some free DHT in the prostatic tissue, research efforts should be
directed to improving androgen blockade further with the development of more potent antiandrogens as well as efficient inhibitors of androgen formation. Simultaneously, efforts should be made to detect and treat prostate cancer at an earlier stage, when endocrine therapy alone or combined with radical prostatectomy or radiotherapy is clearly most efficient.

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Introduction

In 1941, Huggins and colleagues (Huggins & Hodges 1941, Huggins et al. 1941a,b) observed some dramatic responses in metastatic prostate cancer patients following castration or treatment with estrogens. During the 50 years that followed the introduction of the concept of androgen dependency of prostate cancer, orchietomy and high doses of estrogens have been the gold standard for the treatment of advanced prostate cancer (Fig. 1). Although this treatment is limited to blockade of the androgens of testicular origin, reports from many groups have shown that such treatment achieves a positive response in 60 to 70% of patients, although for a limited period of time (Nesbit & Baum 1950, Staubitz et al. 1954, VACURG 1967, Mettlin et al. 1982, Murphy et al. 1983). As indicated by such a high proportion of positive responses observed after only partial blockade of androgens, prostate cancer is the most sensitive of all hormone-sensitive cancers to endocrine therapy. However, since it is relatively easy to obtain a positive response to hormone therapy in prostate cancer, it is particularly important to make sure that the chosen treatment provides the best possible response and not simply a statistically significant but suboptimal response.

The serious and frequently lethal cardio- and cerebrovascular complications of estrogens (VACURG 1967, Robinson & Thomas 1971, Peeling 1989), on one hand, and the psychological (Lunglmayr et al. 1988, Cassileth et al. 1989) as well as physical limitations of orchietomy, on the other hand, have generally delayed endocrine treatment until late stages of the disease when pain and debility had developed. Typically, at such a late stage, the large and disseminated tumors show poor and short-lived responses, thus limiting the success of endocrine therapy. In fact, in analogy with all other types of cancers, androgen blockade loses its effectiveness with increasing size of the tumors (Chen et al. 1996). It should be added that although well recognized to reduce pain and improve quality of life in a large

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**Figure 1** Landmarks in the development of the endocrine therapy of prostate cancer.
agonists could be easily achieved in men (Labrie et al. 1980, 1982, Faure et al. 1982, Tolis et al. 1982) has eliminated all the above-indicated limitations including the serious and even life-threatening side-effects of previous therapies (VACURG 1967, Robinson & Thomas 1971, Peeling 1989). It is quite remarkable that medical castration with LHRH agonists was first achieved in men and not in experimental animals (Labrie et al. 1980, 1982, Faure et al. 1982, Tolis et al. 1982). In fact, in experimental animals, only partial inhibition of total testicular androgen biosynthesis could be achieved with daily administration of LHRH agonists (Auclair et al. 1977a,b, Labrie et al. 1978, Rivier & Vale 1979). The availability of a safe and highly efficient method of medical castration with LHRH agonists free of the side-effects of estrogens and surgical castration has generated renewed interest in the treatment of prostate cancer and has stimulated an unprecedented number of clinical studies which rapidly led to the worldwide commercialization of a series of LHRH agonists having equivalent characteristics, mechanisms of action, and efficacy (Fig. 2).

Following the studies initiated in 1978 in experimental animals, a pure antiandrogen was first added to an LHRH agonist to treat prostate cancer in men in 1982 (Labrie et al. 1982). An important objective of adding a pure antiandrogen to an LHRH agonist was to neutralize the initial rise in serum androgens induced during the first days of treatment with...
LHRH agonists (Bélanger et al. 1980b, Labrie et al. 1980). Moreover, the addition of the pure antiandrogen made it possible to take advantage of the synergistic inhibitory effects on prostate and seminal vesicle weight previously observed in the rat (Fig. 3). The encouraging results obtained in the first patients who received combination therapy renewed the interest in research on the endocrinology of prostate cancer and stimulated large-scale clinical studies that led to the acceptance of combination therapy as the new gold standard for the treatment of prostate cancer in 1989. In fact, combination therapy is the first and only treatment shown to prolong life in advanced prostate cancer (Crawford et al. 1989, Denis et al. 1993).

While the clinical studies were progressing, the powerful and highly efficient technology of molecular biology allowed the isolation and characterization of the genes encoding the enzymes responsible for the formation of the androgens testosterone and dihydrotestosterone (DHT) in the human prostate, thus providing an explanation for the important role played by the androgens of adrenal origin (Labrie et al. 1985a, 1995, Labrie 1991). These data provide the scientific rationale for the necessity to use combination therapy (Fig. 4) (instead of monotherapy) in order to block androgens of both testicular and adrenal origins simultaneously at the start of treatment of prostate cancer (Labrie et al. 1982).

We shall briefly review those observations made in experimental animals and men which, in our opinion, have most significantly contributed to our improved understanding of the endocrine control of prostate cancer growth as well as its treatment since Huggins’ introduction of the important concept of androgen sensitivity of the disease in 1941.

**Medical castration with LHRH agonists**

Although the castration effect of LHRH agonists was first discovered in man, and not in experimental animals, it is of interest to review the research performed in the field of LHRH agonists in the laboratory in the 1970s.

**Interactions of LHRH with sex steroids at the pituitary level**

The discovery by Schally and his colleagues (Matsuo et al. 1971) of the structure of LHRH, the hypothalamic hormone that controls the secretion of LH and follicle-stimulating hormone (FSH) by the anterior pituitary gland, was a major breakthrough in this field. In fact, the availability of synthetic LHRH has allowed detailed studies of the mechanism of action of LHRH on LH and FSH secretion in the anterior pituitary gland (Borgeat et al. 1974) while providing

**Figure 4** Schematic representation of the two sources of androgens that control the development, growth and function of the human prostate. LHRH secreted in a pulsatile fashion by the hypothalamus stimulates the secretion of LH by the anterior pituitary gland. Transported in the general circulation, LH reaches the Leydig cells of the testicles where it stimulates the secretion of androgens, especially testosterone, which, in turn, is transported by the blood to the prostate where it is converted into the more potent androgen DHT by 5α-reductase. In order to maintain circulating androgens at a constant level, testosterone exerts an inhibitory action at the hypothalamic and pituitary levels on the secretion of LHRH and LH respectively. As a result, LHRH and LH secretion increase when circulating androgens decrease while an inhibition of LHRH and LH secretion occurs when the concentration of circulating androgens increases. This regulatory feedback mechanism is responsible for maintaining circulating androgens at a physiological level. On the other hand, the pituitary hormone ACTH stimulates secretion by the adrenals of DHEA and its sulfated form DHEA-S which also reach the prostate by the bloodstream and are converted locally into testosterone and DHT by the appropriate steroidogenic enzymes. In adult men, it is estimated that approximately equal amounts of androgens are from testicular and adrenal origins (Labrie 1991, Labrie et al. 1995).
essential information on the interactions of LHRH with androgens, estrogens, and progesterins at the pituitary level (Drouin & Labrie 1976, Drouin et al. 1976, Ferland et al. 1976). Such studies were fundamental for a proper understanding of the delicate feedback mechanism controlling LHRH secretion by the hypothalamus as well as the action of LHRH on LH and FSH secretion in the anterior pituitary gland with their resultant impact on gonadal functions and fertility (Fig. 4).

Synthesis of LHRH agonists
In addition to providing the possibility of performing extensive biological studies with LHRH, knowledge of the structure of this neurohormone offered chemists the opportunity of designing and synthesizing peptides more potent than LHRH itself. The objective of that research program was to obtain potent LHRH agonists for the treatment of infertility (Schally et al. 1976, Schwarztein 1976). It was then rapidly discovered that positions 6 and 10 of the LHRH molecule were particularly sensitive to structural modifications (Fugino et al. 1972, Monahan et al. 1973, Coy et al. 1974, 1975, 1976, Rivier et al. 1975): changes at these two positions, especially when made simultaneously in the same molecule, led to a marked increase in biological activity. The success of the chemistry of LHRH analogs has been particularly impressive. In fact, within 5 years after the discovery of the structure of LHRH, super-agonists of LHRH having 100 to 200 times the in vivo biological activity of LHRH were already available (Coy et al. 1975, 1976, Rivier et al. 1975). The original objective determined in 1971 to obtain highly active LHRH agonists which would require small quantities of compound for administration in the human had been achieved within 5 years. In fact, in 1975, no further significant improvement of the molecules of LHRH agonists in terms of potency or otherwise was then expected or needed, or even seen. In fact, all of the LHRH agonists which later became commercially available possess equivalent potency, efficacy, and mechanism of action (Fig. 2).

As mentioned above, following the discovery of the structure of LHRH in 1971 (Matsuo et al. 1971), most research efforts in endocrinology and reproduction were concentrated on the use of LHRH and its agonists to stimulate gonadal functions and treat infertility (Schally et al. 1976, Schwarztein 1976, Jaramillo et al. 1977, Krabbe & Shakkebaek 1977, Wiegelmann et al. 1977, Jacobi & Wenderoth 1982). All research groups working in this field, including ours, were then attempting to find the dose and vehicle as well as the dosing schedule and route of administration which would permit the use of LHRH agonists to stimulate fertility in experimental animals and man. In parallel, experiments were performed to improve our understanding of the mechanisms involved in the action of LHRH and its agonists at the pituitary as well as the testicular levels.

Paradoxical partial inhibition of testicular functions by LHRH agonists in the rat
In the course of our studies on LHRH agonists in the male rat, we then made the unexpected observation that treatment of adult animals for a few days led to variable degrees of inhibition of serum testosterone levels accompanied by a relatively smaller but usually significant inhibition of ventral prostate, seminal vesicle, and testis weight (Auclair et al. 1977a,b, Labrie et al. 1978, Rivier et al. 1978, 1979, Rivier & Vale 1979). This unexpected finding did not fit the well-established dogma in medicine where the synthesis of an analog more potent than the natural hormone is always found to induce a higher biological response. In fact, such superactive molecules are synthesized in order to permit the use of a lower dose and thus minimize the risk of side-effects associated with high doses of compounds. Because of its paradoxical nature and its lack of conformity with standard biology, the understanding of the partial inhibitory effects exerted on testicular functions by LHRH agonists in the rat remained an unsolved problem for many years. The interpretation of this unique observation was further complicated by the fact that the inhibition of serum testosterone levels induced by LHRH agonist treatment was always of greater amplitude than the inconsistent and weaker effects observed on prostate and seminal vesicle weight.

LH-induced decrease of testicular functions
The recently available endocrinological information in the rat led to the suggestion that the prolonged LH secretion induced by LHRH agonists was responsible for their inhibitory effects on testicular functions (Auclair et al. 1977a,b, Labrie et al. 1978). In fact, comparable inhibitory effects on testicular LH receptors as well as on testis weight were observed following the single administration of an
LH-like molecule, namely human chorionic gonadotropin (hCG) or an LHRH agonist (Auclair et al. 1977b). Such data suggested that the inhibitory effects of LHRH agonist treatment on the gonads were secondary to the increased serum levels of LH (Auclair et al. 1977a,b, Labrie et al. 1978, 1980). In strong support for this proposed mechanism, a marked loss of testicular LH receptors (Hsueh et al. 1976) and of the steroidogenic response to gonadotropins (Hsueh et al. 1977) had recently been observed in the rat after systemic administration of ovine LH or hCG, thus leading to the suggestion: ‘it seemed possible that changes in endogenous LH secretion induced by administration of LHRH or its agonists could lead to significant loss of LH receptors’ (Labrie et al. 1978). Consequently, it was suggested: ‘... therapeutic approaches using LHRH and its agonists will have to take into account the unexpected inhibitory effect of rises of plasma gonadotropin concentrations on the sensitivity of the testicular response to pituitary hormones’. Similarly, Sharpe & Fraser (1980) suggested that the increased LH release was responsible for the decreased formation of steroids by the testis: ‘These changes are identical to those observed after administration of high doses of LH and hCG, suggesting that the actions of the LHRH agonists are mediated by the release of pituitary LH.’

Many additional data were in agreement with the suggestion of an inhibitory role of elevated serum LH levels on testicular functions. For example, serum gonadotropin levels were elevated 870% above control following treatment of male rats with an LHRH agonist (Rivier et al. 1980). In addition, up to a 300% elevation in basal serum LH was observed up to 9 days of treatment with an LHRH agonist (Rivier et al. 1979). The lack of evidence of an antagonistic action of LHRH agonists in the intact male rat is particularly well illustrated in Fig. 5 which shows that treatment for 12 weeks with an LHRH agonist caused up to a 300% increase in serum LH (Fig. 5A) while ventral prostate and seminal vesicle weight decreased by only 35% and 25% respectively (Fig. 5B) (Cusan et al. 1979).

The observations made in the rat suggested that the important site of inhibitory action of LHRH agonists was at the testicular and not at the pituitary level. Although the acute LH response to daily administration of LHRH agonists in male rats was known to decrease during chronic treatment with LHRH agonists (Sandow et al. 1978, Rivier & Vale 1979), the concentration of serum LH under basal

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**Figure 5** Effect of treatment with 100 ng LHRH-A on (A) plasma LH levels in adult male rats as well as on (B) seminal vesicle and ventral prostate weight. Animals received single injections twice a week during the periods indicated up to 12 weeks.
conditions never decreased below normal intact values, thus excluding the possibility of using daily administration of LHRH agonists to inhibit gonadotropin secretion. As an example, Rivier & Vale (1979) found that basal plasma LH levels remained within normal limits during 6 out of 7 days of treatment with an LHRH agonist while being elevated on 1 day. It was later observed that after 5 months of daily treatment with an LHRH agonist, no significant effect was observed on either basal plasma LH or FSH concentrations (Lefebvre et al. 1982). Moreover, pituitary LH content remained unchanged after such treatment while adenohypophysial FSH content increased.

When castrated rats were used, treatment with the LHRH agonist Buserelin reduced plasma LH levels from days 14 to 28 of treatment with no significant effect earlier (Sandow et al. 1978). Such a late effect could not explain the rapid inhibition of serum testosterone as well as the decrease in ventral prostate and seminal vesicle weight observed during the first days of treatment with LHRH agonists (Auclair et al. 1977a,b, Labrie et al. 1978, Rivier et al. 1978). In addition, the absence of sex steroids in castrated animals makes difficult the extrapolation of such data to the situation in intact animals where androgens and estrogens play a crucial role in the control of LH secretion at the pituitary level (Drouin & Labrie 1976, Drouin et al. 1976, Ferland et al. 1976).

Relatively small decrease in prostate and seminal vesicle weight

As mentioned above, the inhibitory effects of LHRH agonists observed on prostate and seminal weight in experimental animals (Auclair et al. 1977a,b, Labrie et al. 1978, Rivier et al. 1978) have never been more than partial and always of small magnitude compared with the dramatic inhibitory effects achieved by castration. For example, following 12 weeks of daily treatment with an LHRH agonist, ventral prostate and seminal vesicle weight were only reduced by 25 and 30% respectively, while castration is known to cause a 90-95% inhibition of the values of the same parameters (Fig. 5; Labrie et al. 1978). Frequently, no significant inhibitory effect could be observed on ventral prostate and/or seminal vesicle weight (Fig. 3; Johnson 1977, Lefebvre et al. 1982). The absence of effect or the relatively small inhibitory effects observed compared with castration on prostate and seminal vesicle weight in the rat offered no hope of using LHRH agonists to replace orchietomy or high-dose estrogen in the treatment of prostate cancer.

LHRH agonist-induced increase in androgenic 5α-reduced steroids compensates for the decrease in testosterone secretion in the rat

We thus decided to study in detail the metabolites of testosterone after treatment of adult rats with an LHRH agonist. It was then discovered that the major discrepancy observed between the important inhibition of serum testosterone levels compared with the small decrease in prostate and seminal vesicle weight in rats treated with an LHRH agonist was due to the concomitant LHRH agonist-induced increase in the formation of the testis of androstane-3α,17β-diol (3α-diol), a steroid easily converted to DHT, an androgen approximately three times more potent than testosterone (Bélanger et al. 1979, 1980a, Labrie et al. 1980). Basal and ovine LH (oLH)-stimulated levels of testicular progesterone, testosterone, and 5α-reduced metabolites in the testes of intact rats are shown in Fig. 6A. Although the main testicular androgen was testosterone, the production of 5α-reduced metabolites (DHT and 3α-diol) contributed significantly to total androgen content in intact animals. However, the testicular response to oLH stimulation in control animals was characterized by a major increase in testosterone concentration.

Although basal and oLH-stimulated testicular progesterone levels remained unchanged after LHRH agonist treatment, both basal and oLH-stimulated testosterone levels were dramatically reduced (Fig. 6B). It is of considerable interest that the basal testicular levels of 3α-diol increased markedly after treatment with the LHRH agonist from 26±4 to 76±12 ng/g testis, whereas DHT levels remained unchanged. This effect of the LHRH agonist on 3α-diol levels became even more apparent when testicular steroidogenesis was measured 2 h after the administration of oLH, the levels of this metabolite markedly increasing from 76±12 to 670±20 ng/g testis. The major increase in the levels of 3α-diol in animals treated with the LHRH analog compensated for the almost complete inhibition of the testosterone response. This increase in 3α-diol levels was so elevated that it led to a similar total androgen response in both groups. The effects of treatment with the LHRH agonist on the basal and
LH-stimulated levels of testosterone and 3α-diol in the plasma (Fig. 6A) were similar to the effects of this treatment on the concentration of the same steroids in the testis (Labrie et al. 1980; Fig. 6B). The present data strongly suggest an increase of testicular 5α-reductase and 3α-hydroxysteroid dehydrogenase activities after treatment with an LHRH agonist in the rat.

We had previously observed that tritiated progesterone is not metabolized when incubated with desensitized Leydig cells obtained from animals treated with an LHRH agonist. It was also well known that seminiferous tubules contain an appreciable amount of 5α-reductase activity, along with other steroid-converting enzymes, and that steroids from interstitial tissue diffuse freely into the seminiferous tubules. This information led us to suggest that the increased formation of 3α-diol could represent a direct consequence of the rapid transformation of the steroid precursors (such as progesterone) by the enzymes of the seminiferous tubules. However, we did not exclude the possibility that 3α-diol could be formed from testosterone.

As summarized above, during chronic treatment of male rats with LHRH agonists, the partial decrease in serum testosterone is fully (Johnson 1977) (Fig. 3) or partially (Auclair et al. 1977a,b, Labrie et al. 1978, 1980, Rivier et al. 1978, 1979, Sandow et al. 1978, Tcholakian et al. 1978) compensated by the above-indicated increase in 3α-diol and DHT formation by the rat testis. Another example of the lack of correlation between serum testosterone levels and changes in secondary sex organ weight was the absence of effect on seminal vesicle weight observed in male rats treated for 12 days with an LHRH agonist while serum and testicular testosterone levels were reduced by 61% and 94% respectively (Bélanger et al. 1979). Serum and testicular levels of progesterone, however, were increased by 360% and 225% above control respectively, thus providing substrate for 5α-reductase and increased formation of DHT and 3α-diol. Such an increase in 5α-reduced steroids could fully compensate for the inhibition of testosterone formation, thus resulting in a null effect of LHRH agonist treatment on seminal vesicle weight. The LHRH agonist-induced increase in the secretion of 5α-reduced steroids having androgenic activity was a serious limitation to the use of LHRH agonists for inhibitory purposes and the inhibitory effects observed could not be compared with the 100% inhibition of serum androgens achieved by castration.

**Direct action of LHRH agonists at the testicular and prostatic levels**

Another possible explanation for the inhibitory effect of LHRH agonists in the male rat is the direct inhibitory action of LHRH agonists at the testicular level (Tcholakian et al. 1978, Bourne et al. 1980, Sharpe & Fraser 1980, Marchetti & Labrie 1984) as well as the possible direct action of LHRH agonists on the prostate (Dondi et al. 1994, Limonta et al. 1996). Moreover, LHRH receptors have been found in Dunning R-3327H prostate tumors (Hierowski et al. 1980).
1983). Such data led to the suggestion by SchALLY et al. (1984) that, in addition to their action at the pituitary level, LHRH agonists might also act directly on prostatic tumors as well as exert a possible action on the gonads.

LHRH receptors are in fact present in the rat testis and a direct inhibitory action of LHRH or its agonists on Leydig cell function has been observed in this species, but not in the mouse (BOURNE et al. 1980, Sharpe & Fraser 1980). It should be mentioned that attempts to characterize LHRH receptors in the human testis have been unsuccessful (CLAYTON 1982). LHRH receptors have been described in both androgen-sensitive (LNCaP) and androgen-insensitive (DU-145) human prostatic carcinoma cells (LIMONTA 1992, DONDI et al. 1994). In fact, high

**Figure 7** Effect of single intranasal administration of 500 μg [D-Ser(TBU)]₆, des-Gly-NH₂₁₀-LHRH ethylamide on serum (A) pregnenolone, (B) 17-OH-pregnenolone, (C) progesterone, (D) 17-OH-progesterone, (E) 5-diol, (F) testosterone, (G) DHT, and (H) 17β-estradiol in normal young adult men. Steroid levels were measured on 2 pretreatment, 1 treatment and 6 post-treatment days. Data are expressed as the mean± S.E.M. of values obtained from six subjects (BÉLANGER et al. 1980b).
concentrations of an LHRH agonist have been shown to exert inhibitory effects on the growth of both LNCaP and DU-145 cells (Limonta 1992). These last two mechanisms, however, have not been shown to play a significant role in the inhibitory action of LHRH agonists in men.

**LHRH agonists for male contraception: early studies**

In healthy men treated with the low 5 μg daily subcutaneous dose of the LHRH agonist Buserelin for 17 weeks in an attempt to achieve contraception, the mean levels of serum testosterone decreased by 35% during that period while basal serum LH decreased only by 43% (Bergquist et al. 1979a). In fact, the limited 35% decrease in serum testosterone achieved in men should be compared with the 95% fall in serum testosterone observed after orchiectomy or treatment with high-dose estrogens (Labrie et al. 1985a). As stated by Bergquist et al. (1979b): 'the reduction was not very pronounced and spermatogenesis and potency were unaffected during the prolonged treatment'. There was thus no indication, with the data available in the literature before 1980, that LHRH agonists could achieve medical castration in men.

The situation in 1979 concerning the potential applications of LHRH agonists was summarized as follows: 'The intranasal administration of selected analogues of LHRH has great potential in the treatment of conditions associated with deficient gonadotropin secretion, provided the pituitary over-stimulation, which may eventually lead to a decrease in LH and FSH output by the pituitary gland, is avoided. It seems likely that alternate day administration of one of the long acting analogues will be all that is required, but care will have to be taken that chronic over-stimulation of the pituitary receptor sites for LHRH does not lead to a decrease in LH and FSH output by the anterior pituitary' (Wass et al. 1979).

**Exquisite sensitivity of men to inhibition of testicular functions by LHRH agonists**

Marked sensitivity differences exist between animal species to the inhibitory effects of LHRH agonists on testicular functions. Thus, male mice and monkeys (Wickings et al. 1981, Resko et al. 1982, Nieschlag et al. 1984, van Steenbrugge et al. 1984, our own observations) are relatively insensitive to LHRH agonists while rats, as mentioned above, are moderately sensitive.

The possibility that man would be more sensitive to the inhibitory effects of LHRH agonists on testicular steroidogenesis than any other species previously studied first arose when an experiment performed in early 1979 showed that a single intranasal administration of 500 μg Buserelin to healthy 30- to 40-year-old men caused long-lasting inhibitory effects on the serum levels of many steroids of testicular origin (Bélanger et al. 1980b), especially the steroids which act as precursors of androgens, namely androst-5-ene-3β,17β-diol (5-diol), 17-OH-pregnenolone, 17-OH-progesterone, and testosterone itself (Fig. 7). Moreover, contrary to the stimulation of serum DHT concentration observed in rats following administration of LHRH agonists, serum DHT levels had, on the contrary, a tendency towards lower values up to the last time-interval studied, namely 7 days after administration of the LHRH agonist (Fig. 7G). We then conceived the notion that the inhibition of serum testosterone in men would not be compensated for by an increase in serum DHT, as observed in the rat (Bélanger et al. 1980a,b). Most impressive was the observation of a loss of diurnal cyclicity of the blood levels of all testicular steroids up to 3 to 6 days after a single intranasal administration of the LHRH agonist (Fig. 7). In fact, single administration of the LHRH agonist Buserelin caused a long-lasting increase in serum 17-OH-pregnenolone (Fig. 7B), 17-OH-progesterone (Fig. 7D), 5-diol (Fig. 7E), testosterone (Fig. 7F), and estradiol (Fig. 7H) which lasted for at least 24 h. The prolonged elevation in serum steroid levels was followed by a surprising and unexpected loss of the cyclical diurnal variation in the concentration of these steroids which lasted for 3 to 6 days. In addition, the serum levels of 17-OH-progesterone and testosterone were found to be inhibited by 50% and 40% respectively, on days 2 to 4 after administration of the peptide (Fig. 7).

**First prostate cancer patient treated with an LHRH agonist**

**First prostate cancer patient**

The above-summarized data suggesting hypersensitivity of the hypothalmo-pituitary-testicular axis of men to the inhibitory effect of LHRH agonists led us
to study the effect of administration of the same high dose of the same LHRH agonist (Buserelin) to a patient suffering from stage B prostate cancer. Thus, in the first prostate cancer patient treated with an LHRH agonist, the 500 μg dose of the LHRH agonist Buserelin administered intranasally caused 70% and 85% inhibitions of the serum levels of testosterone and DHT respectively, as early as 2 weeks after the start of therapy (Fig. 8; Labrie et al. 1980). This marked inhibition of the serum concentration of both testosterone and DHT followed an initial period of stimulation that lasted approximately 1 week. Most importantly, it can be seen that the serum DHT concentration was decreased even further than that of serum testosterone, thus clearly indicating that treatment of adult men with an LHRH agonist was not accompanied, contrary to the rat (Labrie et al. 1980), by a simultaneous increase in the concentration of DHT which would compensate for the inhibition of serum testosterone. Medical castration induced by an LHRH agonist had thus become a clear possibility in men.

Optimal dose and route of administration of LHRH agonists

Following our observation that administration of the LHRH agonist Buserelin led to an almost complete inhibition of serum testosterone and DHT levels within 2 weeks using the intranasal route, a less than optimal route of administration (Labrie et al. 1980), a detailed study of the effect of various doses of the same LHRH agonist was performed after administration by the intranasal and subcutaneous routes (Faure et al. 1982). The effect of chronic treatment with the LHRH agonist administered by nasal spray (200 or 500 μg twice daily) or subcutaneously (50 μg daily) for periods up to 8 months was thus studied on serum sex steroid and LH levels in patients with stage A or B prostate cancer who had been treated surgically by prostatectomy and had no sign of active disease. Basal serum testosterone concentration decreased to 71.1±18.3% (not significant) and 28.6±9.3% (P<0.01) of control in patients receiving the 200 μg and 500 μg doses by nasal spray respectively (Fig. 9). In patients treated subcutaneously with the 50 μg dose, a more rapid inhibition of serum testosterone levels to 19.6±6.4% of control (P<0.01) was observed (Faure et al. 1982). A more complete and rapid inhibition of serum testosterone levels to 5-8% of control (castrated levels) was achieved with the daily 200 μg and 500 μg doses subcutaneously (Fig. 9).

The importance of such results obtained with LHRH agonists was well recognized by Jacobi & Wendaroth (1982): 'What medical developments have urologists witnessed since orchietomy and estrogen treatment were introduced by Huggins 40 years ago? Gestagens, antiandrogens, adrenal inhibitors, antiprolactins, antiestrogens, cytotoxic agents? In principle, the gain in terms of efficacy and the loss as a result of toxicity have never been balanced to a degree which could establish one of the afore-
mentioned drugs as the generally accepted standard treatment to replace estrogens. LHRH analogues may prove to be the first non-toxic medical castration measure applicable for general use in the future.

**Mechanisms of medical castration by LHRH agonists in men: loss of LH bioactivity**

Our findings of low serum 17-OH-progesterone and testosterone levels in the presence of normal progesterone and pregnenolone concentrations during the first days of treatment of prostate cancer patients with LHRH agonists (Labrie et al. 1980, Faure et al. 1982) suggested that treatment of men with LHRH agonists inhibits the steroidogenic pathway at the level of 17-hydroxylase and 17,20-desmolase activities during the first 2 to 3 weeks of treatment with LHRH agonists (Labrie et al. 1985a). These sites of enzymatic blockage are identical to those described after similar treatment in experimental animals (Bélanger et al. 1979, 1980a, Labrie et al. 1980). This early period after the start of treatment with LHRH agonists, however, is not characterized by castration levels of serum androgens which are only attained 2 to 3 weeks after the start of treatment.

It was only in 1983 that it was discovered that the biological activity of LH was progressively lost during long-term treatment of prostate cancer patients with LHRH agonists (Kelly et al. 1983, St-Arnaud et al. 1986), thus explaining the castration effect of LHRH agonists in men. In fact, in the presence of a greater than 95% inhibition of serum testosterone and DHT levels, serum LH measured by RIA can remain normal or be only slightly decreased (Faure et al. 1982). Because we had previously found a discrepancy between serum LH measured by RIA and by bioassay in rhesus monkeys treated with a high dose of an LHRH agonist (Resko et al. 1982), we performed a similar study in men. We then observed that although the values of serum LH measured by RIA and bioassay (mouse Leydig cell assay) varied in a parallel manner during the first 2 weeks of treatment, a progressive and marked loss of bioactivity was measured at later time-intervals. Thus, after 3 months of treatment, LH bioactivity was reduced to about 5% of control, whereas the radioimmunoassayable LH was reduced by only 40-50% (Fig. 10) (Kelly et al. 1983). These data indicate that the loss of LH bioactivity, rather than testicular desensitization, is the major factor responsible for the complete inhibition of testicular steroidogenesis which occurs after 2 to 3 weeks of treatment with LHRH agonists in men. In men, LHRH agonists thus achieve a medical hypophysectomy selective for gonadotrophs.

**Medical castration with an LHRH agonist versus surgical castration or orchiectomy**

As indicated above, the major difference between medical castration achieved with an LHRH agonist...
and surgical castration or orchiectomy is the elevation of the blood levels of testosterone and DHT which lasts for 7 to 10 days at the start of treatment with an LHRH agonist (Fig. 11). The transient increase in serum testosterone lasting for a few days was seen in the first patient treated with an LHRH agonist (Labrie et al. 1980). This transient elevation of serum testosterone is followed by a gradual decrease to castration levels which are reached at 2 to 3 weeks. On the other hand, it is well known that serum androgens decrease to castration levels within a few hours after orchiectomy (Fig. 11). Such a difference is of major importance for the treatment of a highly androgen-sensitive disease such as prostate cancer. In fact, exposure to testosterone assessed by the area under the curve of the serum levels of testosterone is approximately 20-fold higher during the first 2 weeks of treatment with an LHRH agonist, than after orchiectomy. With this knowledge, we decided that patients with advanced disease should not be treated with an LHRH agonist alone but always in combination with a pure antiandrogen. Although the LHRH agonist-induced secretion of androgens observed during the first days of treatment with an LHRH agonist alone carries the unacceptable risk of disease flare, the addition of a sufficient dose of a pure antiandrogen completely eliminates this risk.

The suspected risk of flare of prostate cancer symptoms during the first days of treatment of advanced prostate cancer with LHRH agonists given alone was confirmed later in several clinical studies. In a recent review of nine series including a total of 765 patients with advanced prostate cancer who suffered disease flare, 15 men died during the acute phase of exacerbation of the disease (Thompson et al. 1990). In these series of patients, an average of 10.9% of patients suffered from disease flare. These symptoms not only included worsening of skeletal pain but also urological obstruction, flank pain, lymphedema, urological symptoms, and spinal cord compression (Waxman et al. 1985, Thompson et al. 1990).

**Combination of an LHRH agonist and a pure antiandrogen (combination therapy)**

As indicated above, the use of LHRH agonists to replace orchiectomy and high-dose estrogen for the treatment of prostate cancer had become a possibility in 1979-80. However, the elevation of serum androgens which lasted for at least 1 day after a single administration of an LHRH agonist (Fig. 7) and up to 7 to 10 days after consecutive daily administration of the peptide (Fig. 8) was unacceptable because of the risk of stimulation of growth of prostate cancer or disease flare.

It was thus deemed essential to neutralize the potentially harmful effects of increased serum androgen levels observed at the start of treatment with LHRH agonists by the addition of a pure antiandrogen (Labrie et al. 1982). We could then observe, in our first patients who received a pure antiandrogen (Flutamide or Anandron) in association with an LHRH agonist (Labrie et al. 1982, 1983, 1984a,b, 1985a, 1987c), that serum prostatic acid phosphatase (PAP) decreased rapidly at a time when serum androgens were maximally increased (Labrie et al. 1982, 1983, 1984a,b, 1987c). These early data clearly indicated that the addition of a pure antiandrogen completely eliminated the risk of disease flare associated with the use of the otherwise exceptionally well-tolerated LHRH agonists in prostate cancer patients.
Different categories of antiandrogens

The chemical structures of four commercially available antiandrogens are indicated in Fig. 12. The first available antiandrogen was cyproterone acetate (Androcur), the progestational derivative developed at Schering AG (Neumann & Hamada 1964). In addition to being only about 50% as potent as Flutamide as an antiandrogen, this progestin derivative possesses significant intrinsic androgenic and estrogenic activities (Fig. 13). Although to a somewhat lower degree than diethylstilbestrol (DES), cyproterone acetate causes estrogenic-like complications such as thrombosis, cardiovascular side-effects, gynecomastia, and adverse effects on serum lipoproteins (Tveter et al. 1978, Neumann & Jacobi 1982, Paisley et al. 1986). Cyproterone acetate also affects carbohydrate metabolism (Seed et al. 1984, Harris & Cantwell 1985). The induction of liver tumors by cyproterone acetate in man is another matter of potential concern (Barradell & Faulds 1994, Rabe et al. 1994).

As early as 1974, the androgenic activity of cyproterone acetate and megestrol acetate was described under the term synandrogenic (Mowszowicz et al. 1974). Virilization effects were seen in all the female fetuses examined when pregnant guinea-pigs were given cyproterone acetate from the 15th to the 40th day postcoitum (Graf et al. 1974), thus providing early evidence for the androgenic activity of cyproterone acetate. Similar virilization effects were reported later in female rabbit fetuses (Elger 1966). The effects of Flutamide and of the steroidal derivatives chlormadinone acetate, megestrol acetate, cyproterone acetate and medroxyprogesterone acetate were compared in vivo in female nude mice bearing androgen-sensitive Shionogi tumors.

Figure 12 Chemical structures of four commercially available antiandrogens.

Figure 13 Schematic representation of the differences in the specificities of action of pure antiandrogens of the class of Flutamide and the progestin derivatives cyproterone acetate and megestrol acetate. AR, androgen receptor; ER, estrogen receptor; GR, glucocorticoid receptor; PR, progesterone receptor.
As an example, it can be seen in Fig. 14 that all of the steroidal compounds stimulated tumor growth while Flutamide, in agreement with its pure antiandrogenic properties, had no stimulatory effect.

![Figure 14](image)

**Figure 14** Effect of 21 days of treatment with 250 µg (twice daily) Flutamide (FLU), chlormadinone acetate (CMA), megestrol acetate (MEG), cyproterone acetate (CPA) and medroxyprogesterone acetate (MPA) on the size (cm²) of the Shionogi mammary carcinoma in intact female mice. Control animals were injected with the vehicle alone. Results are presented as means± S.E.M. (Plante et al. 1988). **P<0.01 vs control.

Due to its lower antiandrogenic potency (Neri et al. 1967, Sufrin & Coffey 1976) and the much lower dose used compared with Flutamide as well as its intrinsic androgenic properties which stimulate androgen-sensitive parameters, including cancer growth (Elger 1966, Neri et al. 1967, Graf et al. 1974, Poyet & Labrie 1985, El Etreby et al. 1987, Labrie et al. 1987a, 1990, Luthy et al. 1988, Plante et al. 1988) (Fig. 14), cyproterone acetate added to castration has never been shown in any controlled study to prolong disease-free survival or overall survival in prostate cancer when compared with castration alone (Pescatore et al. 1980, Robinson 1988, Di Silverio et al. 1990, Sciarra et al. 1990).

The first pure antiandrogen was discovered in 1967 by Neri and his colleagues at Schering-Plough (Neri et al. 1967). Flutamide was thus the first compound to block the interaction of DHT and testosterone with the androgen receptor without exerting any androgenic, progestational, glucocorticoid, or estrogenic activity (Fig. 13). Nilutamide (Raynaud et al. 1979, Mogulewsky et al. 1987) and biculatum (Furr et al. 1987), two analogs of Flutamide, then became available.

![Figure 15](image)

**Figure 15** Schematic representation of the mechanism of action of combination therapy using a pure antiandrogen associated with chemical castration achieved by treatment with an LHRH agonist. After 2 to 3 weeks of treatment with an LHRH agonist, castration levels of serum androgens are obtained due to the loss of biological activity of LH, thus resulting in a complete arrest of testosterone secretion by the Leydig cells of the testis. On the other hand, the pure antiandrogen competes at the level of the androgen receptors in the prostate with the androgens made locally in the prostate from the inactive precursors of adrenal origin, namely DHEA, DHEA-S and androstenedione.

Our early knowledge of the androgenic activities of cyproterone acetate and megestrol acetate has completely eliminated this class of compounds from our strategy aimed at achieving an optimal blockade of androgens. In fact, prostate cancer is so exquisitely sensitive to androgens that using a compound having intrinsic androgenic activity did not appear logical or recommendable. Consequently, there seemed to be no rationale to support the use of a mixed androgen-antiandrogen when pure antiandrogens were available and such compounds were the only ones which
could theoretically achieve a complete blockade of androgens. Combination therapy thus dictated the use of a pure antiandrogen (Fig. 15).

Combination therapy in experimental animals

Although a progressive increase in serum gonadotropin and androgen secretion was well known to occur in intact rats treated with a pure antiandrogen (Neumann et al. 1977, Raynaud et al. 1979), the pure antiandrogenic characteristics of a compound such as Flutamide were of sufficient interest to suggest an experiment combining this compound with an LHRH agonist in experimental animals. The possibility of interference by the pure antiandrogen with the inhibitory action of the LHRH agonist on testicular testosterone secretion was more than compensated, in our opinion, by the attractive characteristics of such a molecule. In fact, such a pure antiandrogen should exert a more efficient blockade of androgen action than could be achieved by the mixed agonist-antagonists of androgen action of the cyproterone acetate class.

The first study combining an LHRH agonist with a pure antiandrogen was performed in 1978 in our laboratory (Fig. 3). Subcutaneous treatment of adult male rats every second day for 2 weeks with 0.1 μg [D-Ala^6,des-Gly-NH_2^10]-LHRH ethylamide alone had no significant effect on ventral prostate weight while Flutamide, at the subcutaneous twice-daily dose of 2 mg caused a 29% (P<0.05) decrease in ventral prostate weight. No significant change was observed on seminal vesicle weight following treatment with either the LHRH agonist or the pure antiandrogen alone. Interestingly, however, combination of the two drugs caused dramatic 62% (P<0.01) and 59% (P<0.01) decreases in ventral prostate and seminal vesicle weight respectively. Not only were the inhibitory effects of the two drugs additive but, more interestingly, they were synergistic: while additive effects would have reduced ventral prostate weight from 115.4±6.3 mg in control intact rats to only 68.6 mg, the value observed in animals treated with the combination LHRH agonist plus Flutamide was reduced much further to 44.2±6.9 mg, thus representing a 51% increase in efficacy compared with the simple additive effects of each compound. Even more pronounced was the synergistic effect observed on seminal vesicle weight where addition of the individual effects of each drug would have only reduced seminal vesicle weight from 60.1±2.9 mg to 51 mg while the value measured after combination therapy was as low as 24.7±2.9 mg. This inhibitory effect of the combination therapy represents a 335% increase in efficacy over the additive effects of each drug used alone.

Synergistic effects of 33% and 75% were seen on ventral prostate and seminal vesicle weight in subsequent experiments (Séguin et al. 1981). In fact, in no instance where daily administration of an LHRH agonist in the male rat was combined with a pure antiandrogen, have the effects observed been less than the addition of the effect of each individual compound. Such highly promising data clearly indicated the efficient complementarity of the mechanisms involved and the absence of interference by the pure antiandrogen of the inhibitory actions of the LHRH agonist. On the contrary, synergistic inhibitory effects were seen.

Dramatic synergistic effects were also observed under long-term conditions of treatment. In fact, simultaneous treatment for 5 months with an LHRH agonist and a pure antiandrogen markedly potentiated the activity of each compound, thus leading to a dramatic inhibition of both ventral prostate and seminal vesicle weight while each compound alone had little or no inhibitory effect (Lefebvre et al. 1982). In that study, combined daily administration for 5 months of a potent LHRH agonist and a pure antiandrogen at daily doses of 250 ng and 5 mg respectively caused a marked 91% inhibition of ventral prostate weight, whereas treatment with either drug alone had no significant effect on prostate weight. Although seminal vesicle weight was decreased by 26% and 34% of control respectively after LHRH agonist and Anadron treatment alone (P<0.05), a combination of the two drugs caused a much greater 85% inhibition of seminal vesicle weight (P<0.01).

The mechanisms responsible for the synergistic action of the LHRH agonist and the pure antiandrogen are still unclear. It should be mentioned that in the experiment described above (Fig. 3), Flutamide decreased the stimulation of plasma testosterone levels induced by treatment with hCG, thus suggesting an inhibitory action of Flutamide at some step(s) in the steroidogenic pathway leading to androgen formation.

Such benefits of combination therapy, first demonstrated in 1978, led us to believe that combination
of a pure antiandrogen with an LHRH agonist would not only neutralize the transient increase in serum testosterone which always accompanies the first days of treatment with an LHRH agonist (with its associated risk of disease flare) but would also exert synergistic inhibitory effects in prostate cancer (Labrie et al. 1984a, 1987c, Thompson et al. 1990).

First patients treated with combination therapy

With the knowledge of the above-summarized preclinical and clinical data, including those showing the optimal dose of LHRH agonist needed to achieve medical castration, it was decided to investigate the potential benefits of combination therapy adding a pure antiandrogen to an LHRH agonist in prostate cancer patients suffering from advanced or metastatic disease (Labrie et al. 1982, 1983, 1984a, 1985a).

Treatment of the first patient with combination therapy with a pure antiandrogen and an LHRH agonist was started on 25 March 1982 at the Laval University Medical Center (CHUL) in Quebec City. This patient lived 1136 days or 3.1 years from the start of therapy. An important observation made in this first series of patients who received combination therapy was the absence of interference by the antiandrogen of the LHRH agonist-induced blockade of androgen formation. In fact, a previous limitation to the use of pure antiandrogens for long periods of time had been the progressive increase in gonadotropin and androgen secretion caused by neutralization by the antiandrogen of the inhibitory feedback action of androgens at the hypothalamo-adenohypophyseal level (Neumann et al. 1977, Raynaud et al. 1979). Our first data rapidly showed that daily administration of the LHRH agonist prevented this escape phenomenon induced by the antiandrogen alone. Moreover, serum androgen concentrations were inhibited to castration levels within 2 to 4 weeks (Labrie et al. 1982, 1983).

The first preliminary report described the results obtained in nine stage D2 and one stage C patients

Figure 16 Changes of serum PAP (dotted line) and testosterone (solid line) levels during the first month of treatment in four previously untreated advanced prostatic cancer patients (A-D) receiving the combined administration of the LHRH agonist Buserelin and the pure antiandrogen Anadron. Note the rapid and marked decrease of serum PAP in the presence of elevated serum testosterone levels, thus indicating the efficiency of the antiandrogen to neutralize the transient rise in serum androgens (Labrie et al. 1983).
treated with combination therapy for 10 to 29 weeks (Labrie et al. 1982). Eight patients had bone pain at the start of treatment, including two bedridden, while prostatism was present in eight patients. Marked objective and subjective improvement was rapidly observed in all stage D2 patients treated with the combination therapy while, in the stage C patient, subjective improvement of urinary symptoms could be documented (Labrie et al. 1982). In conclusion, it was stated: ‘Due to the ease of its application and the lack of secondary effects other than those related to hypoandrogenicity, the treatment of cancer of the prostate with the combined use of an LHRH agonist and an antiandrogen appears promising. Certainly at this point it seems warranted to begin a randomized prospective clinical trial in which the combined therapy is compared with treatment with an LHRH agonist alone and to treatment with estrogen and/or orchiectomy.’ Although the follow-up was short, the ‘rapidity and degree of subjective and objective improvement appeared to be appreciably more favorable than previously observed with either castration or estrogen therapy’. The preliminary data obtained in these first ten patients were most encouraging and led to rapid enrolment of other patients suffering from advanced disease.

The subsequent report included 27 previously untreated stage D2 and 10 stage C patients who received combination therapy with an LHRH agonist and a pure antiandrogen (Labrie et al. 1983). Thirty-six patients previously castrated or treated with estrogens also received combination therapy. A particularly striking observation was the marked and rapid fall in serum PAP measured soon after the start of combination therapy. As judged by a return of serum PAP to normal in 26 of 27 patients who had elevated serum PAP levels before treatment and/or an improvement in the 9 patients who had evaluation of their response by bone scintigraphy, a positive response was then observed in 29 of 30 (97%) previously untreated stage C or D2 patients who could be evaluated by serum PAP and/or bone scan up to 12 months of treatment (Labrie et al. 1983). The objective responses observed were parallel to a rapid and marked improvement of the clinical signs and symptoms related to prostate cancer (prostatism, bone pain, and general well being) in previously untreated patients.

In the previously untreated patients receiving the combination therapy, a 60% fall in serum PAP was observed as early as 5 days after starting treatment, at a time when the serum androgen concentration was 100% to 200% above control (Fig. 16). It could then be concluded with confidence that combined treatment with a pure antiandrogen had sufficient androgen-blocking potency to completely prevent flare-up of the disease, a complication already suspected and later reported in a significant proportion of patients treated with an LHRH agonist alone (Waxman et al. 1985, Thompson et al. 1990). In marked contrast, the same combination therapy applied to patients previously treated with high doses of DES (13 patients) achieved a positive objective response in only 55% of cases. In 23 previously castrated patients showing relapse, an objective response was seen in only 25% of cases after neutralization of adrenal androgens by the antiandrogen.

It could also be concluded from that study that the proportion of androgen-sensitive tumors decreases from more than 95% in untreated patients to 20% to 50% after orchiectomy or treatment with high doses of estrogens. Since the previous rate of positive response following monotherapy ranged between 60% and 80% (VACURG 1967, Mettlin et al. 1982, Murphy et al. 1983), the above-summarized data, indicating a 95% response rate, suggested that partial hormonal blockade limited to the neutralization of testicular androgens left 15% to 35% of hypersensitive tumors growing while such growth could be inhibited at the start of therapy by simple addition of a pure antiandrogen. No clinical or biochemical side-effect could be detected except those related to reduced serum androgen levels. Due to the ease of its application and the lack of secondary effects other than those related to hypoandrogenicity, these preliminary data suggested that complete (instead of partial) androgen withdrawal should be performed as early as possible after diagnosis, at least in advanced prostatic cancer, to reduce the development of androgen-insensitive cancer cell clones (Labrie et al. 1983).

Following the inclusion of a few additional patients, the next report described the results obtained in 44 previously untreated stage D2 patients, 35 of whom received an LHRH agonist and a pure antiandrogen while 9 had orchiectomy and received a pure antiandrogen. With a short follow-up ranging from 4 to 18 months (average=9.9 months), all 44 patients could be documented as having a positive objective response as assessed by a return of
elevated serum PAP to normal and/or improved bone scintigraphy while, at that time, 2 patients had shown relapse of their disease after experiencing a positive response (Labrie et al. 1984b). By contrast, in the group of 53 patients previously castrated and/or treated with high doses of estrogens, a positive response was observed in only 45% of patients using the same objective criteria of response.

The most widely cited description of the results obtained in this first series of patients treated with combination therapy was published in 1985 (Labrie et al. 1985a). With a follow-up ranging up to 23 months, the results were presented for 47 and 37 previously untreated patients at stages D2 and C respectively. For stage D2, comparison was made with 53 patients previously castrated or treated with estrogens. Of the 47 previously untreated patients, 38 achieved complete androgen blockade by combined treatment with an LHRH agonist and the pure antiandrogen 5,5-dimethyl-3-[4-nitro-3-(trifluoromethyl)phenyl]-24-imidazolidinedione (RU-23908, Anandron) or 4'-nitro-3'-trifluoromethylisobutylamidine (Flutamide), whereas 9 patients were orchiectomized and received a pure antiandrogen. The LHRH agonist was injected daily subcutaneously, whereas the antiandrogen was given orally. The criteria for objective response developed by the National Prostate Cancer Project (NPCP) were used for stage D2 patients (Murphy & Slack 1980) while the criteria for positive response in stage C disease were as follows. (1) Serum PAP, when elevated, has returned to normal. (2) Regression of prostatic volume due to cancer by more than 30% at echography and rectal examination. (3) Signs of prostatism due to cancer (including flowmetry) improved by more than 50%. (4) Signs of hydronephrosis due to cancer, if present, improved by more than 50%.

As illustrated in Fig. 17, the concentration of serum PAP decreased rapidly to 45% of pretreatment values as early as 5 days after the start of the combined antihormonal therapy using an LHRH agonist and a pure antiandrogen in previously untreated patients. The serum PAP values then continued to decrease and normal values were reached within 2 months in all except five patients in whom normal serum PAP levels were reached at 4 months. A similar pattern was seen in the 9 previously untreated patients who were surgically castrated (instead of medical castration with the LHRH agonist) and who received the same treatment with the antiandrogen (data not shown).

Contrary to this extremely rapid and constant decrease in serum PAP levels observed when the combination therapy was applied to previously
untreated patients, only a limited success was obtained when the same combined androgen blockade was administered to patients who had received previous monotherapy by orchietomy or treatment with DES. Unfortunately, in those patients who had received previous hormonal therapy, the concentration of serum PAP continued to increase in about 50% of cases even when neutralization of the remaining androgens had been achieved with the same dose of antiandrogen (Labrie et al. 1985a). In patients previously treated with DES, combined treatment with the LHRH agonist and the antiandrogen was given, whereas only the antiandrogen was administered to patients previously orchietomized. It should be mentioned, however, that not all tumors had become androgen-insensitive in the previously treated patients, as indicated by the fall in serum PAP levels observed in about 30% of cases upon starting the combined treatment (Labrie et al. 1985a).

In stage C patients, on the other hand, a positive response was observed in all cases. The improvement in signs of prostatism and hydronephrosis was rapid, a significant effect usually being seen within 2 weeks.

It is of interest to mention that the first 58 consecutive previously untreated stage D2 patients showed a positive objective response to combination therapy as evaluated by the objective criteria of the NPCP (Murphy & Slack 1980). Our study was then extended to 260 previously untreated stage D2 patients and the best responses achieved are indicated in Table 1. These data should be compared with a 87-88% positive response to similar combination therapy in the NCI study (Crawford et al. 1989). Following orchietomy or treatment with a high dose of DES, a positive response rate of 81% was found (Murphy et al. 1983). A value of approximately 80% has also been observed after treatment with an LHRH agonist alone (Crawford et al. 1989).

As can be seen in Fig. 18, the median time to progression (Kaplan-Meier estimate) in the cohort of 260 previously untreated stage D2 patients at the Laval University Medical Center was 2.3 years (118 weeks). The duration of survival, on the other hand, was calculated at 3.58 years (186 weeks) (Fig. 19). The median survival in our cohort of patients of 3.58 years should be compared with 2.96 years and 2.85 years in the studies of Crawford et al. (1989) and Denis et al. (1993) (Fig. 20) respectively, where similar treatments with an LHRH agonist and Flutamide were given. It should be noticed that when all causes of death are considered, the survival advantages in the NCI and EORTC studies were 32 and 31 weeks respectively, or a 26% prolongation of life. The 32 and 38 weeks longer duration of life in our cohort of patients could be related to the greater proportion of patients having 'minimal' metastatic disease. In fact, 101 of 261 (38.8%) evaluable patients had one to five bone metastases while 43 (16.5%) had six to ten bone metastases, 47 (18.1%) had 11 to 40 bone metastases, and 62 (23.8%) had disseminated disease (of the remaining seven 2.7%
had soft tissue metastasis. The median survival times estimated according to Kaplan-Meier were 6.81 years, 3.44 years, 2.37 years and 1.75 years, in the corresponding groups. Such data clearly demonstrate the importance of early treatment and the much longer survival achieved in patients having minimal disease, a finding also observed in both the NCI and EORTC studies.

**Heterogeneity of sensitivity to androgen deprivation: difficulty of inhibiting growth of hypersensitive tumors**

**Heterogeneity of androgen sensitivity**

Before 1982, the effect of combined androgen blockade had never been tested as an initial form of therapy (Labrie et al. 1982, 1983). A logical conclusion from our first clinical results obtained with combination therapy was that prostate cancer is more sensitive to androgens than previously expected. Contrary to the general belief that the lack of response to orchietomy or treatment with estrogens in 20-40% of prostate cancer patients was due to the presence of androgen-resistant tumors present before the start of treatment, a large proportion of these tumors are in fact androgen-hypersensitive since their growth can be inhibited by further blockade with the addition of a pure antiandrogen after castration. That most carcinomas of the prostate are androgen-sensitive, even at a late stage, is also supported by the observation that treatment with exogenous androgens stimulated cancer growth in almost all cases of prostate cancer in progression after castration (Fowler & Whitmore 1981).

![Figure 19](image1.png) Survival (solid line) with 95% confidence interval (dotted lines) in previously untreated stage D2 prostate cancer patients who received combination therapy (Laval University Prostate Cancer Program). *, number of patients at risk; †, number of deaths during period.

![Figure 20](image2.png) Median survival in previously untreated stage D2 prostate cancer patients according to Denis et al. (1993), Crawford et al. (1989), and the Laval University Prostate Cancer program (data updated in March 1996).

Clinical experience in the management of prostate cancer clearly suggests a marked heterogeneity of the response to hormonal therapy. The mostly subjective response obtained in 30 to 50% of patients in progression after monotherapy by the addition of adrenalectomy, hypophysectomy or Flutamide suggests that tumors left growing under standard hormonal therapy can be blocked by further androgen blockade. Such a response to further androgen blockade in patients already castrated can only be explained by the presence in these patients of tumors which were still growing in the low androgenic environment provided by the adrenal androgens that remain after medical or surgical castration.

In addition to the second short-lived and mostly subjective response observed in 30 to 50% of
castrated patients receiving combination therapy as second-line therapy after failure of castration, additional proof for the presence of androgen-hypersensitive prostatic carcinoma is provided by the findings that combined treatment of advanced prostate cancer with an LHRH agonist (or orchietomy) in association with the pure antiandrogen Flutamide causes a positive response in 85-95% of patients (Labrie et al. 1985a, 1986, Crawford et al. 1989). Since only 60 to 80% of patients respond to castration (medical or surgical) (Nesbit & Baum 1950, Jordan et al. 1977, Mettlin et al. 1982, Murphy et al. 1983), the increase in responders from 60-80 to 85-95% following the addition of a pure antiandrogen (combination therapy) indicates the presence of androgen-hypersensitive tumors in at least 20% of patients with advanced prostate cancer. These patients were previously thought to have 20 to 40% of 'androgen-resistant' tumors at the start of treatment while, on the contrary, they have androgen-hypersensitive tumors. While 20 to 40% of patients not responding to castration benefit from a positive response to combination therapy, it is also reasonable to expect that the 60 to 80% of patients who show a positive response to castration should obtain additional benefits from combination therapy through an improvement of their positive response which should translate into an increased duration of response and longer survival.

The approximately 5-15% of patients who do not reach an objective response to combination therapy might be having some truly androgen-resistant tumors or, alternatively, these tumors could well be even more androgen-sensitive and are thus able to grow under the influence of the androgens remaining in their prostatic tumors in the presence of therapeutic doses of the antiandrogen and castration. Further blockade of adrenal androgen secretion and/or action will be needed to differentiate between these two possibilities.

While the expression of all androgen-sensitive genes in a normal androgen-sensitive tissue, for example the prostate, generally decreases in parallel with the lowering of androgen levels (Pelletier et al. 1988), the situation is very different in cancer tissue. In fact, in prostate cancer, the growth of some tumors, in the same patient, can be inhibited by partial blockade of androgens achieved by castration while other tumors require the more complete androgen blockade achieved by combination therapy. The marked heterogeneity of the sensitivity to androgens of prostate tumors is an essential characteristic of prostate cancer which needs to be taken into account for the design of an optimal therapy of the disease. In fact, while the tumors requiring the highest concentrations of DHT will regress following partial androgen blockade, it is essential to block androgens more completely in order to inhibit the growth of hypersensitive tumors (Fig. 21). It is

Figure 21 Effect of increasing concentrations of DHT on the maximal androgenic response (DNA content) in the three clones obtained from a single Shionogi mouse mammary tumor (Tumor 1). In order to facilitate visualization of the differences in androgen sensitivity, all data are expressed as a percentage of the maximal response to DHT. Note that there were also marked differences in basal growth in the absence of DHT as well as in the maximal response to DHT between the different clones (Labrie & Veilleux 1986).
Model of androgen hypersensitivity and clinical significance of low serum androgens

The situation in human prostate cancer thus appears to be analogous to the data obtained in androgen-sensitive Shionogi tumors where a proportion of clones obtained from a single tumor show supersensitivity to DHT with half-maximal growth in culture at a concentration of DHT as low as 0.008 ng/ml (0.024 nM) (Fig. 21). Since the castration levels of serum DHT range between 0.04 and 0.08 ng/ml (Labrie et al. 1985a), it is clear that such hypersensitive tumors can continue to grow at a maximal rate following castration. For these androgen-hypersensitive tumors, castration levels of DHT are sufficient to maintain a maximal growth rate. This is the type of tumor which continues to grow after castration but can be blocked by adding a pure antiandrogen. These are the androgen-hypersensitive tumors.

The available clinical data clearly indicate that the situation in prostate cancer is analogous to that presented above where a proportion of clones show high sensitivity to DHT with half-maximal growth at concentrations of DHT as low as 0.01 nM. Since castration levels of serum DHT range from 0.04 to 0.08 nM (Labrie et al. 1985a,b, Labrie & Veilleux 1986), it is clear that such hypersensitive tumors can continue to grow at a maximal rate following castration. Instead of being androgen-resistant, these tumors are, on the contrary, androgen-hypersensitive and are treatment-resistant. Blockade of their growth necessitates more efficient androgen blockade.

The influence of progressive blockade of androgens on the growth of tumors having heterogeneous sensitivity to androgens is illustrated in Fig. 22. More complete blockade of androgens achieved by the addition of Flutamide results in lower concentrations of free DHT in the prostatic tissue, thus causing regression of a larger proportion of tumors. Moreover, for the tumors already affected by castration, the addition of Flutamide should cause a greater degree of growth inhibition for a longer time-interval.

Importance of low androgen levels

An interpretation of paramount importance in this area is the one concerning the biological role of the apparently low levels of serum testosterone and DHT which remain after surgical or medical castration in men. Castration levels of serum testosterone were previously considered the ultimate objective as well as the biochemical evidence of the success of endocrine therapy. As clearly demonstrated (Bartsch et al. 1983, Labrie & Veilleux 1986), this endocrine parameter, however, is highly misleading, since a 90-95% reduction in serum levels of testosterone cannot
be taken as evidence for a similar degree of reduction of androgen action in target tissues.

As mentioned earlier, serum testosterone levels in intact adult men usually range between 4 and 8 ng/ml while values of 0.2 to 0.4 ng testosterone/ml (5% of control) are observed after surgical or medical castration. As an example, Fig. 23 shows respective values of plasma testosterone of 4.99±0.35 and 0.31±0.02 ng/ml before and after 1 month of treatment of 12 men suffering from prostate cancer with the LHRH agonist [D-Trp^6]-LHRH ethylamide. As illustrated in Fig. 23, the most significant finding is that the apparently low castration levels of serum testosterone at 0.2 to 0.4 ng/ml stimulate growth of the androgen-sensitive mouse mammary carcinoma cells at 36-62% of the maximal growth rate which can be achieved at testosterone levels corresponding to those found in intact men (4 to 8 ng/ml) (Labrie & Veilleux 1986).

The concentration of serum DHT in intact adult men usually ranges between 0.4 and 0.8 ng/ml while castration levels of DHT are at 0.04 to 0.08 ng/ml (Labrie et al. 1985b). The same group of patients shown in Fig. 23 had pretreatment and treatment values of serum DHT at 0.55±0.07 and 0.05±0.03 ng/ml respectively. Again, the impressive finding was that a reduction of serum DHT to 10% of control values decreases androgen-sensitive cell growth only from 74-84% to 27-41% of maximal androgen-sensitive cell growth (data not shown) (Labrie & Veilleux 1986) (Fig. 23).

Contrary to the widely accepted dogma in the endocrine therapy of prostate cancer, such data clearly demonstrate that castration levels of testosterone and DHT retain an important stimulatory effect on androgen-sensitive growth in both normal and cancer tissue. In fact, the growth of a typical androgen-sensitive carcinoma cell line, namely the Shionogi mouse mammary carcinoma (SC-115), is stimulated at 27 to 62% of maximal growth by castration levels of testosterone or DHT in the incubation medium. These data are in direct contradiction of the belief that the apparently low (5 to 10% of control) serum concentration of serum testosterone and DHT

Figure 23 Effect on the growth of Shionogi mouse mammary carcinoma cells of concentrations of testosterone corresponding to the serum values found in the serum of intact men (4-8 ng/ml) compared with growth achieved at testosterone levels corresponding to those obtained after surgical or medical castration (0.2-0.4 ng/ml). Note that a 95% reduction in testosterone concentration, in the culture medium, causes only a 38 to 64% reduction in cell growth. The left-hand panel shows, as an example, the serum testosterone levels measured before and after 1 month of treatment of adult men suffering from prostate cancer with the LHRH agonist [D-Trp^6]-LHRH ethylamide (Labrie et al. 1985b).
remaining after surgical or medical castration should have little, if any, influence on the growth of prostate cancer (Gittes 1991).

The above-summarized data obtained in an androgen-sensitive cancer cell line are also in agreement with the elegant study of Barstch et al. (1983) performed in the normal rat prostate. These authors have shown, using Silastic implants of testosterone, that the maintenance in castrated rats of serum testosterone at the concentration found in the serum of castrated men leads to an approximately tenfold higher concentration of DHT in the prostatic tissue, thus causing a stimulation of prostatic weight as high as 30 to 40% of the value found in intact animals.

The physiological importance of relatively low levels of testosterone and DHT in the function of normal tissue has also been demonstrated in rat anterior pituitary cells in culture (Labrie et al. 1985a, 1986). Androgens are in fact well known to exert specific inhibitory effects on LHRH-induced LH release in adenohypophyseal cells in culture. Using this precise and well-established in vitro system, we have observed that reduction of the concentration of testosterone in the incubation medium to the values found in the serum of castrated men reduced the androgenic activity, as reflected by the inhibition of LH release by only 50 to 70%.

The data summarized above (Bartsch et al. 1983) as well as our previous observations (Marchetti et al. 1988, Labrie et al. 1985a, 1986, Labrie & Veilleux 1986), clearly demonstrate that low concentrations of androgens are highly active and that small variations of these low concentrations of androgens can cause major changes in the responses observed in various androgen-sensitive systems, namely cancer cell growth (Labrie & Veilleux 1986), growth of the normal rat prostate (Bartsch et al. 1983, Marchetti et al. 1988) as well as LHRH-induced LH release in normal rat gonadotrophs in culture (Labrie et al. 1985a, 1986). In these three different systems, one common finding is that low castration levels of testosterone at approximately 5% of the concentration found in the blood of castrated men can maintain a high level of biological activity at 30 to 60% of the level found under maximal stimulatory conditions. Concentrations of androgens below the adult

![Diagram](image_url)
physiological range thus have major biological importance.

**Role of androgens of adrenal origin**

Although the evidence for a significant role of adrenal androgens in prostate cancer was not recognized before the 1980s, it seems appropriate to briefly summarize the recent data which demonstrate their essential role in the present and future endocrine therapies of prostate cancer.

**Poor response to endocrine therapy in patients previously castrated or treated with estrogens**

Following the first bilateral adrenalectomy of a prostate cancer patient performed by Huggins & Scott (1945), bilateral adrenalectomy and hypophysectomy have been performed in patients previously castrated or previously treated with estrogens with a low rate of mostly subjective and short-lived responses (Harrison et al. 1953, Morales et al. 1955, MacFarlane et al. 1960, Bhanalaph et al. 1974, Ferguson 1975, Sanford et al. 1977). Similarly, treatment with Flutamide of patients already castrated or treated with estrogens yielded a 33 to 39% positive response rate (Stoliar & Albert 1974, Sogani et al. 1975). The low rate of clinical response to second-line endocrine therapy and the short duration of these mostly subjective responses were not suggestive of a significant role of adrenal androgens in prostate cancer.

**Lack of early evidence for a significant role of adrenal androgens**

Only a small level of transformation of the adrenal steroids dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) into active androgens in the prostate had been reported (Harper et al. 1974). Moreover, the direct secretion of androgens by the adrenals was known to be minimal (Baird et al. 1969). It was in fact believed that standard approaches such as castration and high doses of estrogens left only 5% of circulating androgens of adrenal origin (Bhanalaph et al. 1974, Cowley et al. 1976, Sanford et al. 1977, Horton 1978). In a recent assessment of the role of adrenal androgens, Griffiths et al. (1995) wrote that ‘of the testosterone in human male plasma, 5-10% would be considered of adrenal origin’. It was also mentioned that the role of adrenal C19 steroids in maintaining the growth of hormone-dependent cancer cells in patients who have been castrated, either surgically or medically is doubtful (Griffiths et al. 1989). As another example, Scott (1983) states in a review: ‘I sincerely believe that we have gone as far as we can go in the hormonal treatment of advanced prostate cancer and that it is unlikely that further search will reveal a better treatment than castration-estrogen therapy.’ Even more recently, it was mentioned: ‘In conclusion, the concept that the adrenal androgens have a major stimulatory effect and are thus responsible for the relapse of prostatic cancer after testicular androgen withdrawal does not appear supportable based on the scientific facts presently available’ (Schulze & Isaacs 1986).

Geller et al. (1978) measured the intraprostatic concentration of DHT in untreated prostate cancer patients as well as in patients castrated and/or treated with estrogens. In untreated prostate cancer, the average DHT concentration was 4.1 ng/g tissue. Based upon the concentration of DHT in non-androgen-sensitive target tissues, a value of 2.0 ng/g tissue was designated as a biochemical index indicating differentiation or hormone-dependence. Based upon the observation that intraprostatic DHT values below 2.0 ng/g tissue resemble values of non-androgen target tissues, Geller (1985) proposed a cut-off value of 2.4 ng DHT/g prostatic tissue. In fact, since three of 28 (10%) surgically castrated patients had prostatic DHT above 2.4 ng/g tissue, it was then concluded that ‘certainly, in the castrated male, adrenal androgens cannot sustain the growth and development of the prostate’. It was then suggested that ‘DHT of adrenal origin may account for up to one sixth (16.7%) of the total prostate DHT’ (Geller 1985) while, based upon a similar interpretation of prostatic DHT values, it was indicated in 1991 by the same author that ‘the adrenal contribution to prostatic DHT is small’ (Geller 1991). Similarly, in a report where 2 of 20 (10%) surgically castrated patients had DHT levels about 2.4 ng/g tissue, it was said that if plasma and tissue DHT levels are at castrate levels (tissue levels less than 2.4 ng/g tissue), then further hormonal therapy is not indicated (Geller et al. 1984).
Serum levels of androgen metabolites of adrenal origin

The most direct and straightforward evidence of a role for the adrenal androgens in prostatic cancer is the observation that the active androgen DHT remains at relatively high concentrations in prostatic cancer tissue following removal of testicular androgens by orchiectomy or treatment with estrogens. As illustrated in Fig. 24A, a high concentration of the active androgen, DHT, remains in prostatic cancer tissue following castration. Although orchiectomy, estrogens, or LHRH agonists (through blockade of release of bioactive LH) cause a 90-95% reduction in serum testosterone concentrations (Warner et al. 1983, Labrie et al. 1980, 1985a, Waxman et al. 1983) (Fig. 24A), a much smaller effect is observed on the most meaningful parameter of androgenic action, namely the intraprostatic concentration of the potent androgen DHT that is reduced by only 50 to 60% (Geller et al. 1978, Labrie et al. 1985a, Bélanger et al. 1986) (Fig. 24A).

Measurements of testosterone and DHT concentrations in serum have little or no value except as an index of testicular activity. In fact, the intraprostatic DHT concentration is the only significant parameter which indicates the level of the active androgen at its site of action in prostatic cancer tissue. Based on the intraprostatic levels of DHT measured after castration, estrogen treatment or treatment with LHRH agonists alone, the testes and adrenals are of approximately equal importance in stimulating prostate cancer growth.

As a measure of the global importance of adrenal androgens in adult men, the serum levels of the main metabolites of androgens, namely 3α-diol, androstene, and their glucuronidated derivatives, are only reduced by 50-70% following castration (Moghissi et al. 1984, Bélanger et al. 1986), thus indicating that the adrenals contribute 30-50% of the total androgen pool in adult men. As illustrated in Fig. 24B, the plasma concentration of the two main metabolites of androgens, namely androstanol-3α,17β-diol glucuronide (3α-diol-G) and androsterone glucuronide (ADT-G) remain at 28% and 37% respectively of control after castration in adult men (Bélanger et al. 1986), thus reflecting the high level of adrenal precursors converted into DHT in castrated prostate cancer patients. Such data demonstrate the major contribution of the adrenals to the pool of androgens in men, despite their advanced age. It is in fact well known that the secretion of DHEA and DHEA-S, the precursors of DHT in the prostate, by the human adrenals is decreased by 60 to 70% at 70 years of age (Labrie et al. 1995).

Our interpretation, contrary to that of Geller, is that 1.8 or 2.4 ng DHT/g tissue should not be considered ‘insignificant’ concentrations of intraprostatic DHT (Geller et al. 1978, 1984). We do indeed believe that the presence of significant levels of DHT in non-androgenic tissues, such as, for example, muscle (Geller et al. 1978, 1984), is not relevant to the role of DHT in the prostate. In fact, the concentration of DHT in tissues other than the prostate has no relevance to prostate cancer. It seemed more appropriate to consider that the prostatic cells respond to the concentrations of DHT present in these cells independently of the presence or absence of androgens in non-androgen-sensitive tissues. Since a concentration of 2.4 ng DHT/g tissue or 7.9 nM is 10- to 25-fold above the K_i value of interaction of DHT with the androgen receptor, it seems clear that such a concentration of DHT in the prostate cancer tissue is more than sufficient to exert a major stimulatory effect on cancer growth.

The important role of adrenal androgens is demonstrated in previously untreated prostate cancer

The major difference in the response rate, type of response and duration of response to combination therapy first suggested by Labrie et al. (1982) and the results obtained by previous blockade of the androgens of adrenal origin by adrenalectomy, hypophysectomy, Flutamide, aminoglupetidamide or glucocorticoids (Huggins & Scott 1945, Harrison et al. 1953, Morales et al. 1955, MacFarlane et al. 1960, Bhanalaph et al. 1974, Resnick & Grayhack 1975) is that before 1982 the blockade of adrenal androgens achieved by various means was always performed in patients showing relapse of their disease after previous castration or treatment with estrogens. In fact, while the blockade of androgens of adrenal origin in patients previously castrated or treated with estrogens results in a short-lived and mostly subjective response in only 20 to 50% of cases (Morales et al. 1955, MacFarlane et al. 1960, Stoliar & Albert 1974, Ferguson 1975, Sogani et al. 1975, Sanford et al. 1977, Markland et al. 1978, Labrie et al. 1983, 1984a, 1985a), the objective response rate to combination therapy in previously untreated patients
reaches 85-95%. Moreover, as mentioned above, this combination therapy became the first and only treatment of advanced prostate cancer demonstrated to prolong life in well-controlled and randomized clinical trials (Crawford et al. 1989, Denis et al. 1993, Janknegt et al. 1993).

Prior to our study published in 1982 (Labrie et al. 1982), ‘the effect of combination therapy had never been assessed as initial form of hormonal therapy’ (Labrie et al. 1983). This observation was well recognized by Sciarrà et al. (1990) who wrote: ‘In 1982, the concept of total androgen blockade was introduced to the endocrine therapy of prostate cancer’ while referring to the paper by Labrie et al. (1982).

**Intracrinology: adrenal androgens account for 40-50%, not 5-10%, of total prostatic androgens in 65-year-old men**

Following the demonstration of the role of testicular androgens in prostate cancer in 1941 (Huggins et al. 1941b), the most important discovery in the endocrinology of prostate cancer is likely to be the recognition that humans and some other primates are unique among animal species in having adrenals that secrete large amounts of the inactive precursor steroids DHEA, DHEA-S and androstenedione, which are converted into potent androgens in peripheral tissues, including the prostate (Fig. 25). In fact, plasma DHEA-S levels in adult men are 100 to 500 times higher than those of testosterone (Labrie et al. 1985a), thus providing high levels of the substrate required for conversion into androgens in the prostate as well as other peripheral tissues.

As mentioned above, the local synthesis of active steroids in peripheral target tissues has been called intracrinology (Labrie et al. 1988, Labrie 1991). The active androgens made locally in the prostate exert their action by interacting with the androgen receptors in the same cells where their synthesis takes place without being released in the extracellular environment. Contrary to the previous erroneous belief that the testes are responsible for 95% of total androgen production in men (as suggested by simple measurement of circulating serum testosterone), it is now well demonstrated that the prostatic tissue efficiently transforms the inactive steroid precursors DHEA-S, DHEA, and androstenedione into the active androgen DHT. In fact, the prostate synthesizes its own androgens.

The amounts of androgens that are synthesized from DHEA-S and DHEA in each cell and tissue depend upon the relative levels of expression of 3β-HSD isoenzymes as well as the other steroidogenic

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**Figure 25** Intracrine activity of the human prostate or biosynthetic steps involved in the formation of the active androgen DHT from testicular testosterone as well as from the adrenal precursors DHEA, DHEA-S and androstenedione (Δ4-dione) in human prostatic tissue. 17β-HSD=17β-hydroxysteroid dehydrogenase; 3β-HSD=3β-hydroxysteroid dehydrogenase/Δ5,Δ4-isomerase. The widths of the arrows indicate the relative importance of the sources of DHT in the human prostate: 60% originating from the testes and 40% from the adrenals in 65-year-old men. The testis secretes testosterone (T) which is transformed into the more potent androgen DHT by 5α-reductase in the prostate. Instead of secreting testosterone or DHT directly, the adrenal secretes very large amounts of DHEA, DHEA-S and Δ4-dione which are transported in the blood to the prostate and other peripheral tissues. These inactive precursors are then transformed locally into the active androgens testosterone and DHT. The enzymatic complexes DHEA sulfatase, 3β-HSD, 17β-HSD and 5α-reductase are all present in the prostatic cells, thus providing 40% of total DHT in this tissue.
enzymes including steroid sulfatase, 17β-HSD, and 5α-reductase activities (Labrie et al. 1985a, 1988, Labrie 1991). As a measure of the importance of adrenal precursor sex steroids in adult men, serum levels of the main metabolites of androgens, namely 3α-diol, ADT, and their glucuronidated derivatives, 3α-diol-G and ADT-G, are reduced by only 50-70% following surgical or medical castration (Fig. 24; Bélanger et al. 1986), thus suggesting that the conversion of adrenal precursor sex steroids accounts for 30 to 50% of total androgens in adult men. In agreement with the above-mentioned clinical findings, we have observed that plasma concentrations of DHEA and androstenedione, maintained at levels comparable with those found in adult men, exert potent stimulatory effects on androgen-dependent growth and gene expression in the rat ventral prostate (Labrie et al. 1988, 1989).

It is clear that measurements of serum concentrations of active androgens are poor markers of intracellular androgenic activity which can only be evaluated by the circulating levels of the metabolites of androgens, namely ADT-G, 3α-diol-G, androstane-3β,17β-diol-G (3β-diol-G), and ADT-sulfate (ADT-S). For example, percutaneous administration of DHEA to men which led to a twofold increase in serum DHEA had no significant effect on the serum levels of testosterone or DHT, while the concentrations of ADT-G, 3α-diol-G, 3β-diol-G and ADT-S were significantly increased (F Labrie, unpublished data).

At least partially responsible for the delayed progress in intracrinology is the fact that commonly used laboratory animals do not secrete significant amounts of adrenal DHEA and DHEA-S (Bélanger et al. 1989), thus focusing all attention on the testes and ovaries as the exclusive sources of androgens and estrogens respectively for target tissue growth and function. Since local formation of androgens and estrogens plays such a major role in both normal and neoplastic hormone-sensitive tissues in the human, a major proportion of the research program of our group has recently been devoted to this challenging and therapeutically promising area (for a review see Labrie 1993, Labrie et al. 1994). It is important to mention that 41% of all cancers, namely, breast, prostate, ovarian, and uterine cancers (Parker et al. 1996) are hormone-sensitive and thus prime candidates for approaches based on the control of intracrine activity.

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