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MECHANISMS OF PHYSIOLOGICAL AND PHARMACOLOGICAL SEX HORMONE ACTION ON THE MAMMALIAN LIVER*

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Summary—Androgen and oestrogen receptors have been demonstrated in mammalian liver, but since it is generally accepted that they are probably non-functional at endogenous steroid concentrations, it is not apparent how they mediate physiological influences on this organ. Nor is it certain to what extent pharmacological actions of sex hormones reflect overstimulation of physiological routes or whether alternative mechanisms become available once threshold values have been reached. In this presentation an attempt has been made to answer some of these questions using data obtained from a study of the regulation of the activities of microsomal 3α-hydroxysteroid dehydrogenase (3α-HSDH) and 5α-reductase in rat liver.

Androgens exert their primary physiological and pharmacological influences at the level of the hypothalamus. Oestrogens can elicit three different types of effect—physiological, antiandrogenic and pharmacological—of which the first two involve primary effects on the pituitary. Hepatic oestrogen receptors only become activated when oestrogen concentrations reach pharmacological levels. Progestins probably have no physiological influence on the livers of non-pregnant rats. Their pharmacological actions may either be traced back to secondary androgenic (e.g. medroxyprogesterone acetate, levonorgestrel) or oestrogenic (e.g. norethynodrel, lynestrenol) properties, involving the routes described above, or to independent effects on the central nervous system (e.g. cyproterone acetate modulation of 5α-reductase activity).

In 1987 the contraceptive "pill" will have been available for 28 yr—as many years as the "pill month" has days. It is sometimes quite a shock to look back over these years and to realize what enormous steps we have made in understanding the mechanisms of action of steroid hormones during this period. Perhaps one should put it more bluntly: at the time steroidal contraceptives were introduced we knew nothing about steroid action at a molecular level. Today the genes for several steroid hormone receptors have been cloned, their primary structure is known, and, even if there is continuing debate and controversy concerning their cellular location, we believe we have a fair idea of how they function.

If the two and a half decades which have passed since the introduction of the pill have dispelled major worries about possible long-term cancerogenicity, it has also demonstrated that the prolonged use of large doses of synthetic sex hormones is associated with quite a number of unwanted side-effects, some of which seem to be the chronic expression of normal physiological actions, others of which seem to be associated only with the pharmacological use of these steroids. A detailed study of these effects reveals that many of these pathological changes can be traced back to direct or indirect alterations in hepatic metabolism [1, 2]. Although the involvement of the liver in the pathogenesis of these changes is now universally recognized, it must be admitted that our knowledge of liver–sex hormone interactions is not very advanced. For instance, although the presence of the mammalian hepatic oestrogen receptor (ER) has been recognized since 1974, up to 1985 there was as good as no information about which particular protein or mRNA syntheses were mediated by binding of oestrogens to this receptor. In fact the only experiment actually designed to test for direct hepatic oestrogen action was performed in 1972 by Nasjletti and Masson[3], who demonstrated the synthesis of angiotensinogen following perfusion of isolated livers with diethylstilbestrol (DES). No such experiments have been performed for androgens or progestins.

It will be the purpose of this paper to examine the evidence for and against direct sex hormone action on the liver. After discussing the changes in hepatic function in response to sex hormones, I shall briefly review the evidence for the presence of sex hormone receptors and binding proteins in the liver and finally attempt to evaluate whether these "receptor systems" are actually involved in hepatic responses. Much of the evidence that will be presented has been derived from work with animal models, in particular from studies on hepatic steroid metabolism in the rat. Where possible, the relevance of these studies for the human situation will considered.

*For Prof. Dr. med. H. Schriefers: a very belated 60th birthday present.

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KNOWN ACTIONS OF SEX HORMONES ON HEPATIC FUNCTION AND METABOLISM

The influence of sex hormones on the liver can be detected in two distinct manners, namely by the

study of changes that occur (a) at defined stages of sexual differentiation (e.g. puberty, oestrous/menstrual cycle/menopause) or (b) following administration of exogenous hormone. While both methods demonstrate that alterations in circulating sex hormone levels might influence the liver, neither of them necessarily indicates which individual sex hormone is responsible. In the case of normal differentiation, the changes may be constitutive with regard to sex hormones; following pharmacological hormone administration it is not clear whether the presence of the administered steroid or the loss of endogenous hormones due to negative feedback is responsible. An unequivocal result can only be achieved if such experiments are confirmed using hormone-free organisms. Thus, virtually all experimentation has been performed on gonadectomized animals, in particular the rat.

As far as the rat is concerned, there is little doubt that a vast number of hepatic proteins show physiological sex differences in their concentrations. Perhaps the best examples are the enzymes of steroid and drug metabolism [4, 5], but certainly many other enzymes must be included [6, 7] as must hormone-binding proteins such as the prolactin receptor [8] or the atypical hepatic sex-hormone-binding protein (HASP) [9] as well as excretory proteins such as α_{2u}-globulin [7]. These differences are not constitutive, but start to develop around the onset of puberty (day 25–30) and reach the mature adult level about 6 weeks later. Gonadectomy and re-administration of sex hormones has revealed that the majority of such differences can be attributed to the presence of androgens. However, this is not invariably the case as a number of oestrogen-dependent enzyme activities are known [10]. There is also no rule about the direction of change that a particular sex hormone may cause; although most androgen-dependent protein syntheses are stimulated by the male hormone, there are examples of repression (e.g. 5α-reductase, prolactin receptor). The same rule applies for oestrogens.

Androgens can exert two distinct types of effect on hepatic protein syntheses. Some syntheses that occur in adult life are obviously dependent on a continuous supply of androgens-testectomy results in complete feminization of the parameter; others require the presence of androgens only during a short span of neonatal life in order to be able to maintain their normal adult levels [11]. Many androgen-dependent enzyme activities of hepatic steroid hormone metabolism appear to be maintained at their male level by a combination of both components, both continuous hormone supply plus neonatal androgen imprinting. The presence of androgens in the neonatal period also increases the sensitivity of these activities to androgens in adult life [12].

Although Pfaffenberger and Hornung [13] have presented evidence, based on urine analysis of glucocorticoid metabolites of normal subjects, suggesting that some sex differences may be present, there is no evidence for such hepatic sexual dimorphism in humans from direct examination of liver biopsy tissue [14, 15]. This fact must place serious doubts on the extrapolation of data obtained from rat to the human situation.

The situation for pharmacological sex hormone administration is quite the reverse. Here there is no doubt whatsoever that similar hepatic effects can be elicited in both rats and humans, and indeed many of the noted actions were first observed in humans being treated with hormonal preparations. Changes which fall into this class are those such as alterations in bile function and constitution, porphyrin metabolism, lipoprotein metabolism, vascularity, and liver cell transformation [1, 16–20]. Nor are the hepatic actions limited to the liver; changes in the rates of secretion of serum proteins and clotting factors have wide-ranging implications for coronary and vascular disease. Most of these effects have been correlated with the oestrogen content of the steroid preparations administered, but there are a number of changes which can be elicited by progestins [20, 21] and by androgens as well [22, 23].

HEPATIC SEX-HORMONE-BINDING PROTEINS

Oestrogen-binding proteins

There is little doubt that hepatic ER are physically and chemically identical to those found in other oestrogen target organs. The hepatic receptor shows the same specificity towards natural and synthetic oestrogens [24, 25] as does the receptor in uterus endometrium. In accordance with the expected results, its K_d for oestradiol lies between 0.5×10^{-10} and 2×10^{-9} mol/l [24–26], its sedimentation constant lies in the range 3–4 S and/or 8–10 S depending on buffer conditions [24, 26], and it possesses a pI between 6.4 and 6.9 [27, 28]. Rat liver cytosol binds about 100 fmol ostradiol/mg protein [24, 26] which corresponds to *ca.* 7000 binding sites/cell, a value that correlates well with *in vivo* nuclear uptake measurements [29]. This is about one-half to one-third of the normal ER complement in uterine tissue. ER are present in both sexes [27]. A hepatic ER has been detected in normal human liver tissue [30] as well as in hepatocellular carcinoma [31] and focal nodular hyperplasia [32].

The similarities between the hepatic ER and those from classical target organs seems to be limited to these physico-chemical properties. In many other respects there is a wide divergence. ER in liver can only be detected in substantial concentrations after the onset of puberty [24, 33]. Hypophysectomy causes a complete loss of hepatic ER [27, 33]. Normal concentrations can be restored in adult animals if growth hormone (GH), prolactin (PRL) and glucocorticoids are administered simultaneously [34]. The concentration of ER is also influenced by endogenous oestradiol levels; ovariectomy leads to an

increase in the number of binding sites [27], an effect that is reversed by androgen administration [35].

Translocation of the hepatic ER into cell nuclei has been demonstrated in several models, including the live animal [29, 36], isolated liver cells [37] and cell-free systems [38] and has been shown to correlate *in vivo* with the synthesis of angiotensinogen [39]. In contrast to other oestrogen-dependent organs, translocation of ER into the nucleus is not associated with the induction of progestin receptors (PR) or peroxidase. Compared to the oestradiol doses required to elicit translocation into uterine endometrial nuclei, massive dosages are necessary to achieve the same effect in the liver [29, 36, 40]. This is clearly due to the metabolic capacity of the liver since the use of synthetic oestrogens such as ethynyl oestradiol (EE_2) permits the same degree of translocation at much lower doses, in spite of the fact that hepatic ER shows the same affinity to both steroids. Moreover, low oestradiol doses become considerably more effective when inhibitors of oestradiol metabolism such as SKF-525A or testosterone are present [41–43]. The fact that only exceptionally high doses of natural oestrogens can cause translocation raises considerable doubt about the possible physiological function of hepatic ER. The capacity of the liver to metabolize oestrogens may make it redundant under normal conditions. Only administration of synthetic oestrogens (e.g. the contraceptive pill) or excessively high levels of natural oestrogens (last stages of pregnancy [44]) can respectively by-pass or saturate the hepatic metabolic capacity.

There is evidence that translocation of ER in the liver may be under hormonal regulation at a stage beyond binding of oestradiol to the receptor. Fasting or diabetes leads to a failure of ER to translocate into the cell nucleus following injection of large doses of EE_2. This effect cannot be demonstrated in the uterus in the fasting animal [45]. Since the concentrations of ER in both organs are only minimally affected by starvation, this effect marks another distinction between liver receptors and those from "classical" oestrogen target organs. These effects are due to changes in the activation of the cytoplasmic receptor.

Androgen-binding proteins

In spite of its peculiarities in its regulation, there is no doubt that the hepatic ER exists and that, under the appropriate conditions, it can mediate oestrogenic action on the liver. This is unfortunately not the case for those binding proteins which have been described for androgens and progestins. Two different proteins have been forwarded as possible candidates for the hepatic androgen receptor (AR). The first of these was originally described by Milin and Roy [46]; it is a cytoplasmic protein with a sedimentation coefficient of 3.5 S and a K_d of 2.5×10^{-7} mol/l for 5α-dihydrotestosterone (DHT). It is present in very high concentrations in male (>1 pmol/mg protein), but not female liver [9]. Its induction in males correlates well with the synthesis of α_{2u}-globulin by the liver [46]. Apart from the rather large K_d and large binding capacity, there are several reasons for rejecting this protein as a classical AR. The most important of these is its specificity towards other steroids, especially the fact that it binds natural oestrogens, in particular oestriol, much better than androgens [9]; nonsteroidal oestrogens such as DES are not bound. For this reason we have designated this protein as the hepatic atypical sex-hormone-binding protein (HASP); several other designations have been used by other authors. The synthesis of HASP is under strict hormonal control. It is neonatally androgen imprinted [47], but can be induced in females by exogenous androgen administration [9]. Both these effects are themselves dependent on the action of other hormones. Both hypophysectomy [33] and continuous administration of human GH (hGH) [48] suppresses the androgenic induction. There is no evidence that HASP can be translocated into the nucleus and it is not evident what function this protein fulfils *in vivo*. Several authors have proposed that it is a sequestering protein—either acting as a sponge to mop up occasional oestrogen surges or as a concentrating mechanism for channelling oestrogen to ER in the face of the higher metabolic capacity of the male liver. Our own experiments have suggested that HASP may be located in the microsomal fraction under *in vivo* conditions.

HASP is not the only androgen-binding protein in liver cytosol. A testosterone-binding protein with a sedimentation coefficient of 10 S was originally detected in cytosol and in liver cell nuclei by Gustafsson *et al.* [49]. This protein has been investigated extensively by Sato *et al.* [50]. It binds testosterone with a K_d between 1 and 6×10^{-7} mol/l and is present at a concentration of about 150 fmol/mg protein. This protein differs from HASP on a number of counts, the most important being its specificity which resembles that of AR from classical target organs, with the exception that it binds 4-androstenedione well. Although this protein binds the synthetic androgen, methyltrienolone, it is not known whether it is the same protein as that described in rabbit and rat liver cytosol described by Sheets and other authors [51–53] as the concentration of this latter protein in rat liver is very low. This protein which was detected using methyltrienolone as ligand, has a K_d of 9×10^{-10} mol/l, is present in rabbit liver cytosol at 80 fmol/mg protein and with a specificity in accordance with classical AR. Presumably this protein is a hepatic AR. It can be translocated *in vivo* following injection of 100 μg methyltrienolone. This is a large dose of an exceptionally metabolically stable steroid. It should be emphasized that *in vivo* translocation of natural androgens has never been demonstrated. Hepatic AR have been demonstrated

in human liver biopsy material obtained during removal of hepatic carcinoma [54, 55].

Progestin-binding proteins

Cytoplasmic PR has never been demonstrated in normal liver tissue from any mammalian species. As mentioned above, in contrast to other oestrogen-dependent tissues, PR synthesis is not induced following administration of oestrogens. However, hepatic microsomal progesterone-binding proteins (PBP) have been described by a number of authors [56, 57]. Since progestins are known to affect hepatic function and these proteins are the only recognized binders with sufficiently low dissociation constants to be possibly considered as candidates for hepatic PR, a few words must be addressed to this topic. These PBP are present in both sexes with the male liver exhibiting higher concentrations on a mg/protein basis. The K_d for progesterone lies between 2 and 6×10^{-8} mol/l; mean concentrations are between 1 and 4 pmol/mg protein. The specificity of these sites for steroids has not been thoroughly studied though all authors seem to agree that 17α-hydroxyprogesterone and testosterone show moderate affinity for these sites.

Because the mechanisms of progestin action on the liver is one of the topics we have been studying quite extensively during the last few years, we decided to undertake a thorough examination of the PBP. Our results differ markedly from those previously reported. Whilst we also found specific protesterone binding with a K_d of about 2×10^{-8} mol/l and a capacity of about 2 pmol/mg microsomal protein in female rats, we found that a vast number of steroids-in particular most of those containing a 3-oxo-4-ene group or a 17α-acetylene substitution-competed. However, the extended incubation period (>24 h at 15°C) required to reach equilibrium as well as a weak sigmoidal trend in the specific binding vs microsome concentration plot, made us suspect that the ligand was being metabolized during the incubation. These suspicions were well founded. Although the metabolism of the labelled progesterone in the incubation medium was relatively limited considering the conditions, analysis of the protein-bound steroids, revealed that progesterone itself was only bound in a nonspecific manner, whereas the specific binding could be attributed to a sole metabolite, namely 3α-hydroxy-5α-pregnan-20-one (HPO). We have recently synthesized large quantities of this steroid in both the labelled and unlabelled form, and have repeated our binding studies. HPO binds to microsomes specifically with a K_d of 1×10^{-8} mol/l at a capacity of 8 pmol/mg protein in female rat liver, i.e. similar data to those found with progesterone. However, in contrast to the situation for progesterone, HPO binds to the microsomes very rapidly at 0°C, is not metabolized during the incubation, and exhibits a much stricter steroid specificity. Of 50 tested steroids and antagonists, only medrogestone, and ethynyl oestradiol competed well; medroxyprogesterone acetate, norethinodrell and progesterone competed moderately. However, none approached the efficacy of unlabelled HPO.

In conclusion, it can be stated that there is unequivocal evidence for ER in the rat liver, but that a physiological function is unlikely. There is no evidence whatsoever for the presence of PR. Rats may possess hepatic AR, but they are present in very low concentrations and even a pharmacological role must be seriously questioned. Normal human liver definitely possesses ER, malignant liver tissue contains substantial concentrations of AR, and there is one reported case of PR in a human liver neoplasm. Human hepatic steroid metabolism also differs qualitatively and quantitatively from that in rats. Thus, the mechanisms listed below, which have been elucidated in the rat, must be considered to be models which may or may not be applicable to the human situation.

MECHANISMS BY WHICH SEX HORMONES EFFECT THE LIVER

The discussion in the following paragraphs is limited to outlining the routes and mechanisms by which particular sex hormones *per se* lead to changes in hepatic protein concentrations. I shall not be considering the almost unlimited combinations and permutations of steroid–steroid and steroid–protein interactions that occur at the metabolic level and which undoubtedly play an important role in the pharmacology of these hormones. As mentioned at the start of this paper, sex hormones affect numerous hepatic functions. However, to illustrate exactly how the individual steroid types exert their effects on the liver, I shall be citing data obtained primarily from our own experimental model, namely their effects on the enzymes of hepatic steroid metabolism in the rat. Most of the findings are valid for other parameters as well. In particular, I shall be concentrating on two microsomal enzyme activities, namely 5α-reductase, which is responsible for the reduction of testosterone to DHT, and 3α-hydroxy steroid dehydrogenase (3α-HSDH), the enzyme responsible for the further reductive metabolism of DHT to the respective androstanediol.

Androgens

Microsomal 3α-HSDH activity is a classical example of a continuously androgen-dependent parameter. From sexually indifferent levels in neonatal life, the activity in the male increases rapidly during puberty to reach a level about 2 times higher than in the female in adult life [58]. Testectomy at any stage of life causes the appearance of typically female levels. The normal male activity can be restored by administration of the androgens, testosterone or DHT at a dose of 0.5 mg/day for a period of about 14 days [59, 60]. A similar mas-

culinization can be induced in ovariectomized females, but the induction of male activity by androgens in intact females can only be achieved when DHT is administered [59]. The action of DHT or testosterone can be blocked by simultaneous administration of the nonsteroidal antiandrogen, flutamide (FLU), but not by cyproterone acetate which has a mild androgenic action on some androgen-dependent activities [59].

5α-Reductase is also dependent on androgens for the development of normal male activity. However, this enzyme differs from 3α-HSDH activity in a number of respects. First, it shows the reverse type of sex difference, male activities being very low, female activities being many times higher. However, the major difference lies in the mode of androgenic regulation. 5α-Reductase activity is not dependent on the continuous presence of androgens, but is imprinted by testosterone or related metabolites within the first 24 h of life [11, 61]. In this respect the activity is a metabolic parallel to the many morphological and neurological changes that are imprinted in the same manner in perinatal life. Thus, testectomy on the first day of life leads to the development of the typically high female levels of activity. Administration of a single androgen dose within hours of the operation prevents the development of female activity after the onset of puberty. In an analogous manner, the developmental programme for male activity can be imprinted in females at this stage of life. However, it is only expressed if the ovaries are removed before puberty [62]. To be fair, it must be stated that 5α-reductase activity is not purely neonatally androgen imprinted, but does show some degree of continual androgen dependency. The activity of 5α-reductase in prepuberally ovariectomized rats acts as if it were continuously androgen dependent: the normal female level is present; androgen administration causes transient masculinization. Not only the final male adult activity level is programmed by androgens during the neonatal phase, but also the sensitivity of androgen-dependent parameters to androgens in adult life [12]. Thus testectomized males react to androgens more sensitively than ovariectomized females, and these in turn more sensitively than intact females. This differential sensitivity is reflected in the higher doses of FLU necessary to counteract androgen action [63].

The similarities between the reactions of androgen-dependent and neonatally androgen-imprinted hepatic parameters and those of androgen-dependent morphological parameters, such as prostate gland or seminal vesicle weight, are deceptive. Removal of the pituitary gland, which has no qualitative influence on the actions of sex hormones on classical target organs, not only prevents hepatic sex hormone action, it causes the appearance of typically male levels for normally androgen-dependent enzyme activities in gonadectomized males [60, 61, 64]. Thus the normal androgenic induction of 3α-HSDH or repression of 5α-reductase seen in pituitary-intact animals does not reflect a direct androgen effect on the liver, but rather the release-inhibition of "feminizing factors" from the pituitary. There is now a general consensus of opinion that GH may be the factor involved, since continuous or very frequent administration of GH causes similar changes, as does implantation of an autonomous pituitary under the kidney capsule [65, 66]. There are isolated reports of hepatic parameters that are affected in a similar manner by prolactin (PRL). Strangely enough human GH (hGH) is much more effective than rat GH.

These latter results indicate that the physiological influence of androgens on rat liver is mediated via the CNS. This finding corroborates the fact that the rat liver seems to be deficient in AR. Indeed the work of Gustafsson and Mode and their colleagues suggests that the actual site of action is the hypothalamus [5, 67]. In many ways this seems a very logical way to regulate hepatic parameters by androgens without interference from hepatic androgen metabolism. As might be expected, in the absence of hepatic AR, even pharmacological doses of natural androgens given over a limited time interval do not appear to elicit effects other than those occurring physiologically. Many of the dangers inherent in synthetic androgen or anabolic steroid therapy may be due less to the androgenic characteristics than to the structural modifications performed to prevent heaptic inactivation (e.g. insertion of an acetylene group at 17α).

Oestrogens

The situation for oestrogens is completely different and must be considered under three separate headings, namely: physiological actions of oestrogens, antiandrogenic actions of oestrogens and pharmacological actions of oestrogens.

Physiological actions of oestrogens. Although most of the sexually differentiated activities of hepatic steroid metabolism are either androgen dependent or imprinted, experimentation has revealed a few enzyme activities that are oestrogen dependent. In our studies these were all cytoplasmic enzymes [10]. Ovariectomy led to the appearance of the typically male activity, an effect which could be reversed by administration of small doses (10 μg/day for 14 days) of oestradiol or other oestrogens. This, in turn, could be prevented by simultaneous administration of the nonsteroidal antioestrogen, monohydroxytamoxifen (MHT) [68]. The actions of oestrogens can be inductive (cytoplasmic 3α-HSDH) or repressive (cytoplasmic 17β-HSDH, 5β-reductase).

As with the androgens, there is evidence, although less compelling, that the oestrogenic effects are not direct, but are also mediated at the level of the CNS. Hypophysectomy makes these parameters refractory to sex hormone action. However, it could be argued

that this merely reflects loss of ER following pituitary ablation. Although two of these enzymes (cytoplasmic 3α-HSDH, 5β-reductase) assume typically male levels after hypophysectomy, and can be "feminized" following GH infusion, the third enzyme examined (17β-HSDH) reacts with a shift to the female level and has not as yet been shown to be affected by any known hypophyseal hormone [10]. This enzyme activity shows other regulatory anomalies as well. The relative lack of physiologically oestrogen-dependent enzyme activities of heptic steroid metabolism probably does not reflect the situation for all liver parameters and further studies using other proteins are needed to explore the mechanisms of oestrogen action more thoroughly. The administration of androgens to intact females causes masculinization of physiologically oestrogen-dependent parameters [10] (in contrast to the situation for androgen-dependent parameters). This may simply reflect the suppression of endogenous oestrogen synthesis.

Antiandrogenic action of oestrogens in the liver. The administration of oestrogens to gonad-intact male rats leads not only to a feminization of oestrogen-dependent parameters [10], but of androgen-dependent parameters as well [60]. Although one would expect this shift to occur in males as a result of long-term LH-suppression and depressed testicular testosterone synthesis, there is unequivocal evidence that low doses of oestrogens affect hepatic androgen-dependent parameters by a different mechanism [59]. This is demonstrated by the fact that administration of EE_2 doses between 0.1 and 1 μg/day can block the action of exogenously administered DHT on enzyme activities such as microsomal 3α-HSDH in ovariectomized rats at dosages of 500 μg/day [69]. In this respect the antiandrogenic action of oestradiol resembles that of FLU, with the notable exception that it is about 5000 times more effective on a weight-to-weight basis. However, simultaneous administration of MHT prevents the antiandrogenic action of oestradiol, but not that of FLU [69].

Since these oestrogen doses, in particular those low doses of oestradiol which can antagonize androgen action, are insufficient to activate the hepatic ER [36] and these effects can be mimicked by infusion of hGH [69], it seems almost certain that these actions are mediated at the central level. Indeed, experiments [5, 67] have indicated that the respective ER are probably hypophyseal. MHT does not antagonize the antiandrogenic action of GH in DHT-treated ovariectomized rats [69].

These facts explain why the administration of testosterone to intact female rats does not cause a masculinization of androgen-dependent parameters [60]. The presence of the ovaries permits enough testosterone to be aromatized to block androgenic action. This is not the case for DHT [59]. It is interesting to note that androgen-dependent parameters in the kidney (e.g. renal micrososmal 3α-HSDH), which are independent of hypophyseal control, react to testosterone irrespective of the presence of oestrogens [70]. These antiandrogenic actions of oestrogens on liver parameters can be elicited by very low doses of oestrogen. It is difficult to describe the role of such antiandrogenic actions once higher oestrogen doses are used. Certainly the shift to the female level is more rapid [71], but this could be due to the supplementary loss of testicular androgens (in intact males) or due to pharmacological influences which involve other mechanisms.

Pharmacological actions of oestrogens on the liver. The administration of substantially higher doses of oestrogens than those necessary to elicit physiological and antiandrogenic effects can cause changes in hepatic functions which cannot be attributed to either of the former mechanisms. The influence of oestrogens on the activity of 5α-reductase illustrates this point very aptly. As explained earlier, 5α-reductase activity is both partly continuously androgen dependent and partly neonatal androgen imprinted with androgens acting in a repressive manner. Like any other androgen-dependent enzyme activity, 5α-reductase in males reacts to the administration of low doses of ethynyl oestradiol (<10 μg) with a feminization (i.e. induction of activity) [69]. However, once a threshold dose of 10 μg/day has been surpassed, the activity of the enzyme activity is no longer induced, but is repressed. By doses of 100–1000 μg/day the activity is close to the normal male level. This repression can be demonstrated in intact and ovariectomized females and with other oestrogens such as oestradiol or DES [69]. In fact DES seems to be the most efficient oestrogen in this respect and treatment of ovariectomized rats, with 100 μg/day for 14 days gives a repression of activity similar to that induced by 500 μg DHT/day over the same time period (Fig. 1.)

Since oestrogens bind to AR with moderate affinity, the similarity between pharmacological oestrogen action and physiological androgen effects raises the possibility that such receptors might be involved in mediating DES action. This is obviously not the case since the repressive influence of DES on 5α-reductase activity is not blocked by FLU, but by MHT (Fig. 1), thereby indicating that ER are involved [69]. However, the oestrogenic action is not mediated via GH since DES treatment represses the activity in GH-infused rats. This indicates that the DES may act independently of the pituitary–liver axis, and, indeed, comparison of the DES dose curves for 5α-reductase activity repression [69] and hepatic ER translocation [36] strongly suggest that this effect may be mediated directly at the level of the hepatic receptor protein.

It is not known to what extent physiological oestrogen concentrations affect human hepatic metabolism, nor whether antiandrogenic effects such as those described for the rat have any

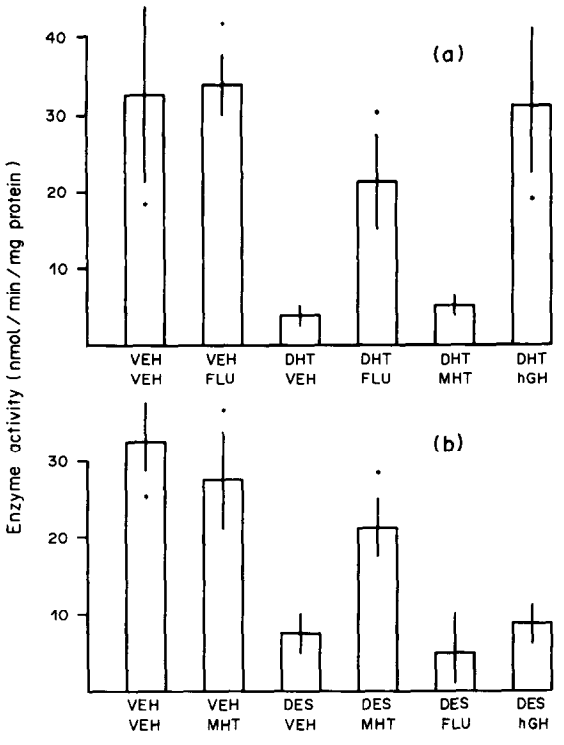

Fig. 1. Influence of FLU, MHT and hGH on the activity of microsomal 5α-reductase in ovariectomized rats treated with DHT or DES. Rats were ovariectomized on day 25 of life. Between day 75 and 89 of life rats received daily injections of either DHT (500 μg) or DES (100 μg) and either vehicle (VEH; 0.25 ml sesame oil–benzoyl benzoate, 4:1, v/v), FLU (5 mg) or MHT (200 μg). Human GH was infused at a rate of 5 μg/h for the last 7 days of treatment. Microsomes were prepared 24 h after the last injection. All results are given as means ± SD ($n = 6$). Significant differences ($P < 0.01$) from values from rats treated with DHT or DES alone are denoted by an asterisk.

significance for man. However, a vast amount of evidence points to the involvement of the human hepatic ER in alterations in liver function following oestrogen administration. It should also be pointed out that exposure to large amounts of oestrogens at critical times of development can seriously influence sexually differentiated hepatic parameters. These can be of an irreversible nature (e.g. oestrogenization) or can cause a prolonged delay in the normal development to mature levels [62].

Progestins

PR have never been detected in normal mammalian liver tissue. How then do progestins mediate their actions on hepatic function? One possibility is that a theoretically "pure" progestin does not affect the liver at all and that the reported changes in function arise as the result of the influence of secondary characteristics of the individual progestin, e.g. their partial androgenic or oestrogenic properties. In a series of experiments we set out to test this hypothesis using 5α-reductase activity as our experimental parameter. Prepuberally ovariectomized female rats were treated with 15 different progestins commonly used in pharmacological preparations at a dose of 5 mg/day for 14 days. Five of these, medroxyprogesterone acetate (MPA), levonorgestrel (LE), cyproterone acetate (CA), lynestrenol (LYN) and norethynodrel (NTY), were chosen for further investigation. The choice of these five lay partly in the fact that all elicited a very significant decrease in 5α-reductase activity at the dosage employed and partly in the fact that the first two steroids are known to be partially androgenic in nature and the last two exhibit definite oestrogenic character. CA is a very potent progestin and antiandrogen with several other steroid hormone agonistic and antagonistic properties. In these further studies, these five steroids were administered to ovariectomized rats simultaneously with FLU or MHT or to rats receiving hGH infusion. Typical results are shown in Figs. 2 and 3. The repressive influence of LE and MPA can be blocked by FLU or hGH, but not by MHT, whereas exactly the opposite situation exists for LYN and NTY. Thus the two progestins with known androgenic properties exert their "progestinic" action on the liver via the hypothalamus–pituitary–liver axis, whereas those two with established oestrogenic properties probably activate the hepatic ER system (Fig. 5).

CA does not fit into this scheme so neatly. Although we were able to demonstrate that the inductive action of CA on microsomal 3α-HSDH could be effectively blocked by FLU, indicating that CA also uses the hypothalamus–pituitary–liver axis to effect its action on this enzyme activity, neither FLU nor MHT had any influence on the repression of 5α-reductase activity by CA even when the dosage regimen was changed. However, when the CA dose

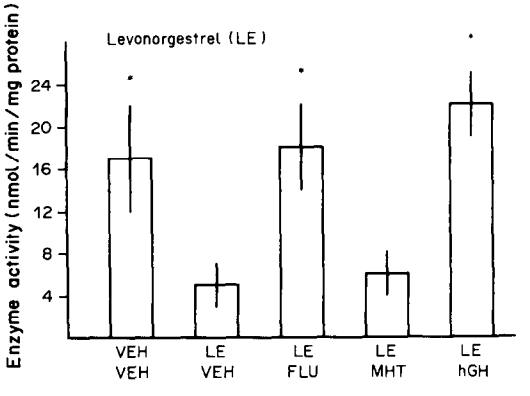

Fig. 2. Influence of levonorgestrel (LE) on the activity of microsomal 5α-reductase in ovariectomized rats. Rats were ovariectomized on day 25 of life. Between day 75 and 89 of life rats received daily injections of LE (5 mg) and either vehicle (VEH; 0.25 ml sesame oil–benzoyl benzoate, 4:1, v/v), FLU (5 mg) or MHT (200 μg). Significant differences ($P < 0.01$) from values from rats treated with LE alone are denoted by an asterisk. All other details are as for Fig. 1.

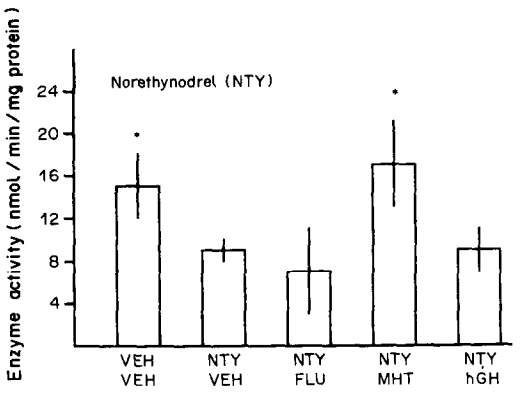

Fig. 3. Influence of norethynodrel (NTY) on the activity of microsomal 5α-reductase in ovariectomized rats. Rats were ovariectomized on day 25 of life. Between day 75 and 89 of life rats received daily injections of NTY (5 mg) and either vehicle (VEH; 0.25 ml sesame oil–benzoyl benzoate, 4 : 1, v/v), FLU (5 mg) or MHT (200 μg). Significant differences ($P < 0.01$) from values from rats treated with NTY alone are denoted by an asterisk. All other details are as for Fig. 1.

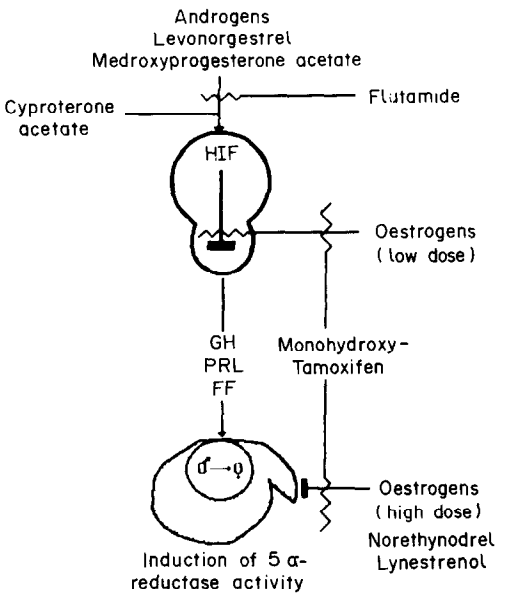

Fig. 5. Schematic representation of possible routes of action of androgens, oestrogens and progestins and their antagonists on hepatic 5α-reductase activity in ovariectomized rats. Abbreviations: HIF, hypothalamic inhibiting factors (including somatostatin); FF, undefined "feminizing factors" including PRL and GH. Arrows denote inductive effects, solid blocks repressive effects; the points of action of FLU and MHT are denoted by serrated lines.

was raised to 20 mg/day, the repression caused by this steroid failed to appear in rats infused with hGH (Fig. 4). Thus although neither AR nor ER appear to be involved in CA action on 5α-reductase activity, it seems very probable that its primary target is located in the CNS and not in the liver (Fig. 5).

To what extent are these findings for progestins on enzymes of steroid hormone metabolism representative of other parameters of hepatic function? A further parameter we have investigated is that of hepatic morphology, in particular the changes in membrane constitution. This is an interesting parameter because, according to one source [72], these changes (increase in smooth ER) occur after progestin administration to hypophysectomized rats, i.e. in a model which lacks an intact pituitary–liver axis as well as hepatic ER. Our initial results have demonstrated that, under our conditions, progestin-induced liver weight increase is always associated with "oestrogenic" progestins and that it can be inhibited by simultaneous administration of MHT. Neither our morphometric nor biochemical studies on the membrane fractions have yet been able to confirm the reported increase in smooth ER at the "lower" progestin dosage of 5 mg/day. However, we have observed differential changes in microsomal phospholipid and cholesterol content following the administration of LYN and NTY. In both cases the effects on the phospholipids, but not cholesterol, were prevented by antioestrogen administration.

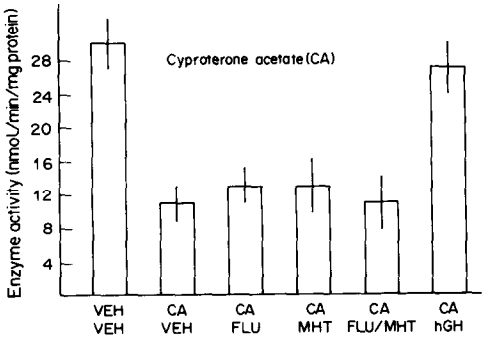

Fig. 4. Influence of cyproterone acetate (CA) on the activity of microsomal 5α-reductase in ovariectomized rats. Rats were ovariectomized on day 25 of life. Between day 75 and 89 of life rats received daily injections of CA (5 mg) and either vehicle (VEH; 0.25 ml sesame oil–benzoyl benzoate, 4 : 1, v/v), FLU (5 mg) or MHT (200 μg). Significant differences ($N < 0.01$) from values from rats treated with CA alone are denoted by an asterisk. All other details are as for Fig. 1.

Extrapolation of these findings for interpretation of the human situation is particularly difficult for a number of reasons. First, there is no real evidence for an androgen → hypothalamus → pituitary → GH → liver route in humans. Second, the secondary characteristics of progestins vary from species to species due to differences in receptor affinities and specificities. Third, the relative dosages applied to obtain these experimental results are extremely high compared to those used in humans. Finally, with respect to contraceptive pill formulations it ought not be forgotten that the interspecies variations in the relative concentrations of sex hormones vary enormously, and the results of exposure of an experimental animal to a combination of sex steroids must be interpreted in light of the normal "milieu". However, there is no doubt that some synthetic

progestins do have inherent oestrogenic properties in humans and may represent an increased oestrogen load for some subjects.

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