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Steroid Metabolism and the Activation of Social Behavior

J. Balthazart

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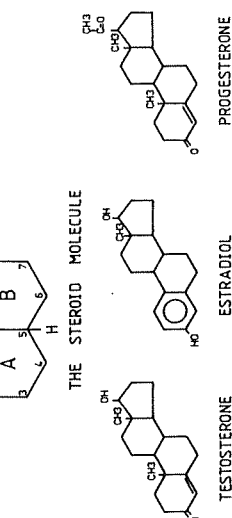
1 Introduction

Within a number of endocrine structures including the gonads, the adrenals, and the placenta, cholesterol is transformed into several compounds that are generally referred to as steroids. Steroids are compounds which have a molecular weight

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Fig. 1. Basic structure of the steroid molecule with the numbering system for carbons and structure of three important examples in the major classes of steroids implicated in the control of social behavior, namely androgens (testosterone), estrogens (estradiol), and progestagens (progesterone)



of about 300 and share with cholesterol the cyclopentano-phenanthrene nucleus (three C6 and one C5 ring; see Fig. 1). Steroid synthesis consists of a series of oxidoreductions and decarboxylations of the cholesterol molecule. Three major classes of steroids implicated in the control of social behavior can be recognized based on the number of carbon atoms they contain: progestins (21 carbons), androgens (19 carbons), and estrogens (18 carbons; see Fig. 1 for a schematic drawing of the major types of steroid hormones).

These steroids are extensively metabolized in the brain. The implications of the central metabolism of steroids in the control of social behavior is best known in the case of androgens. Transformation of estrogens into catecholestrogens and of progesterone into dihydroprogesterone and corresponding diols also probably plays some role in the activation of behaviors such as copulatory behavior in the male or lordosis in the female. Due to space limitations, the data supporting this statement will not be reviewed here but the interested reader can find additional information on these subjects in the literature, namely in Celotti et al. (1984). It is outside the scope of this chapter to review the biosynthetic pathways of all steroid molecules and the principles of steroid nomenclature. This information can be found in most general textbooks of biochemistry and in a form more accessible to investigators with a behavioral orientation in the reviews of Feder (1978, 1981).

The focus here will be on the transformations undergone by testosterone in the brain. A substantial amount of evidence accumulated primarily during the last 20 years indicates that many structures and in particular the central nervous system (CNS) are able to metabolize extensively steroid hormones and that this central metabolism of steroids is important for the expression of their biological activity. The brain is unable to produce significant amounts of steroids because it lacks the enzymes needed to convert cholesterol in pregnenolone (the common precursor). By contrast it contains many of the enzymes involved in the subsequent metabolism of steroid (e.g., the aromatase which converts some androgens into estrogens). These enzymatic conversions of steroids either produce

compounds which are devoid of biological activity (and thus inactivate the steroid) or metabolites which are equally or even in some cases more potent than the parent steroid. In the latter case, the enzymatic transformation amplifies the steroid action. It is thus clear that the balance between these inactivation and activation processes provides a means by which steroid activity is regulated at the level of the target organ, the brain. Data have recently accumulated which show that steroid metabolism plays a critical role in the control of many social behaviors in a wide variety of species. This is especially true in the case of androgens. These data are reviewed in the present chapter.

2 Methods of Study

The evidence for a critical role of central steroid metabolism in the control of social behavior is derived from different types of experiments. First, biochemical studies have demonstrated the presence of steroid metabolizing enzymes in the brain. These experiments were made possible by the availability of radioactive steroid molecules with high specific activity. They were carried out either *in vivo* or *in vitro*. In the former case, trace amounts of radioactive steroid are injected into the animal and radioactive metabolites are analyzed in the brain after sacrifice. This type of experiment, if it demonstrates the production and accumulation in the brain of metabolites of steroid hormones, usually provides no information on the site where metabolic transformation takes place (unless the radioactive substrate is applied stereotactically into a specific brain region and the diffusion of radioactivity is determined). During *in vitro* experiments, small brain areas are dissected out and incubated in the presence of radioactive steroid. The radioactive metabolites produced can then be quantified and this demonstrates the biosynthetic capacity of the brain area under study. The drawback of this procedure is, of course, that the conditions under which metabolism occur markedly differ from those in a living organism and a number of regulatory mechanisms are not present or are drastically altered. By combining both types of approaches, it is, however, possible to demonstrate the presence of specific metabolic pathways in limited brain areas and to obtain quantitative information on the enzymatic activities. Correlations can then be established between the enzyme activity and the biological activity of the steroids in the living animal (e.g., in situation A, steroid X is less active than in situation B and this corresponds to a higher transformation of the steroid into inactive metabolites in situation A). These correlations will then suggest physiological implications of the metabolism which can be tested during *in vivo* experiments. Several strategies are often used in this context. One is to compare the biological activity of the metabolites with the activity of the parent steroid; another is to block the action of metabolizing enzymes with specific inhibitors and evaluate the consequence on the biological activity of the parent steroid; and a third is to specifically block the binding of the parent steroid or its metabolites to their intracellular receptor and compare the resulting inhibition of biological effects. Finally, one can use a number of specific agonists, some of which are not metabolizable and specifically bind to a given type of receptor.

It is only by an approach combining these different techniques that significant progress has been made in the understanding of the implications of steroid metabolism in the control of reproductive and other social behaviors. This will be illustrated in the following sections.

3 Metabolic Pathways for Androgens

Testosterone (T) is the major androgen secreted by the gonads in birds and mammals. In the brain and pituitary gland, T is extensively metabolized into compounds with androgenic or estrogenic properties. This metabolism is highly relevant to the control of reproductive behavior and several metabolites of T formed centrally are able to mimic most if not all behavioral actions of T. At times, it has even been considered that T is only a prohormone used by the brain as a substrate to produce behaviorally active compounds (the metabolites; e.g., Clemens and Pomerantz 1982; Jouan and Samperez 1980). This is probably an exaggeration

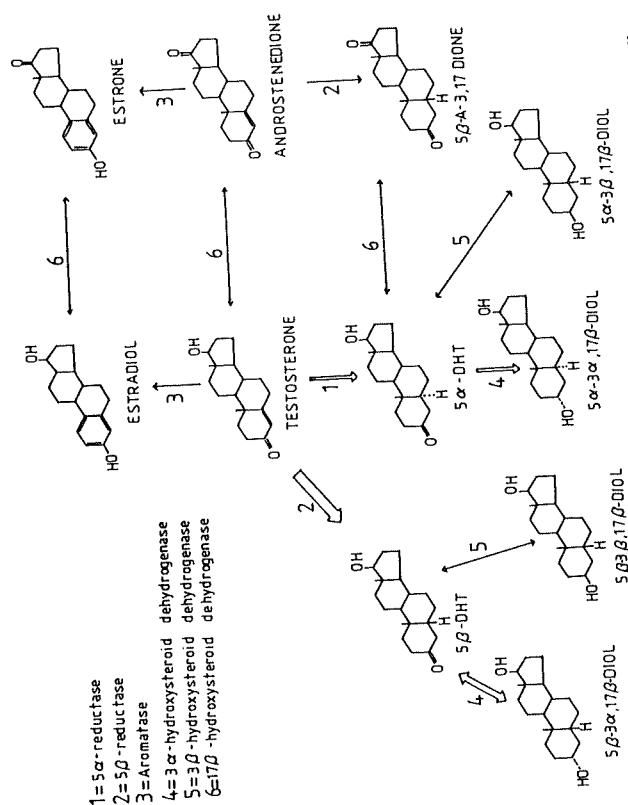


Fig. 2. Metabolic pathways followed by testosterone in the brain as revealed by *in vitro* studies. Widths of arrows between metabolites are roughly proportional to the corresponding enzymatic activities. Enzymes involved are indicated by numbers on the arrows and listed in order at the top left of the figure. DHT = dihydrotestosterone; 5 α (β)-3 α (β),17 β -DIOL = 5 α (β)-androsterane-3 α (β)-diol; 5 β -A-3,17-dione = 5 β -androsterane-3,17-dione

and, as I shall discuss below, T is likely to be active by itself, at least on a number of behavioral activities. It remains, however, that T metabolites, namely estradiol (E2) and 5 α -dihydrotestosterone (5 α -DHT), play a critical role in the activation and differentiation of many reproductive processes. Data supporting this affirmation will be reviewed below after the description of the metabolic pathways implicated and of their physiological regulations.

Numerous studies have been devoted to the analysis of androgen metabolism in the brain. Radioactive substrates which were used are either T or its oxidized form androstenedione (A4). The major biologically relevant metabolites which were detected in these experiments are illustrated in Fig. 2. These metabolic pathways are briefly described below.

3.1 Aromatase

Aromatase is a microsomal enzyme which irreversibly transforms T into E2 or A4 into estrone (E1). T and A4 bind to the same active site of the enzyme but A4 would have an affinity slightly higher than T for the human placental aromatase (apparent Km are 0.1 and 0.4 μ M for A4 and T, respectively; Reed and Ohno 1976). This difference, however, was not confirmed in a more recent study of the ovarian aromatase (same Km when T and A4 are used as substrate; George and Ojeda 1987). The process of aromatization is the result of a series of reactions (at least four) during which 3 mol O₂ and 3 mol NADPH₂ are used to produce 1 mol E2 (or E1 according to the androgen used as substrate; see Siiteri and Thompson 1975; Akhtar and Skinner 1968; Skinner and Akhtar 1969). Aromatization entails the hydroxylation of carbon 19 and its loss to form an 18-carbon steroid. A number of intermediates are produced including 19-hydroxytestosterone and 19-oxotestosterone. The latter compound is transformed into E2 by a process during which 1 mole formic acid is produced. The transformation of 19-oxotestosterone into E2 is the rate-limiting step of the reaction (Siiteri and Thompson 1975; Thompson and Siiteri 1974a). The whole enzymatic process is linked to cytochrome P450 (see Thompson and Siiteri 1974b; Siiteri and Thompson 1975; Lehninger 1970 for more detail). It is important to note here that during the last step of aromatization, two hydrogen atoms located in beta position on the carbons 1 and 2 are lost from the androgen molecule and incorporated into water (Osawa and Spaeth 1971). The stereospecific loss of these 1 β and 2 β hydrogens is the basis of one commonly used assays for aromatase activity. In this assay, tritiated androstenedione bearing all or most of the radioactivity in 1 β , 2 β is incubated with the enzyme and the aromatization is evaluated by the quantity of ³H₂O released in the medium.

Aromatization occurs in several regions of the CNS and has been demonstrated in the brain of many species belonging to all classes of vertebrates from fishes to mammals (for review: Callard et al. 1978a, 1978b; Callard 1984). Interestingly, the neuroanatomical localization of the enzyme has been subjected to major changes in the course of evolution. While the enzyme is present throughout the brain in cartilaginous and bony fishes (elasmobranch and teleost), it is found only in the forebrain and diencephalon of birds and only in the limbic

system of mammals (hypothalamus, preoptic area, septum, amygdala, and hippocampus; Callard et al. 1978a, b).

3.2 The 5α -Reductase Pathway

The enzyme 5α -reductase converts by a reduction process testosterone (or androstene-17 β ol, 3 one) into 5α -androstene-17 β ol, 3 one also called 5α -dihydrotestosterone (5α -DHT). This enzyme is essentially microsomal also. Its action results in the addition of two hydrogen atoms on the testosterone molecule after cleavage of the double bond between carbons 4 and 5. Under "normal" conditions (i.e., thermodynamic conditions which are compatible with life) this reaction is not reversible. It must also be mentioned that 5α -reduced compounds are no longer aromatizable by the brain, although aromatization of 5α -DHT has been reported in the embryonic ovary of the chicken (Weniger and Zeis 1982; the metabolic pathway has not, however, been fully elucidated).

From a phylogenetic point of view, 5α -reductase may have an origin even more ancient than the aromatase (Callard et al. 1978a, b). It has been identified in the cerebral ganglion of the lobster and in all classes of vertebrates from fishes to mammals (see Callard et al. 1978a, b for more details on the distribution of the enzyme in different species). The 5α -reductase has been identified in a large number of central and peripheral structures. In male mammals very high enzymatic activities are present in the prostate, seminal vesicles, and anterior pituitary. In the brain, the enzyme is widespread but its activity varies from one region to another with the highest levels being observed in decreasing order in the hypothalamus, the midbrain, the amygdala, the hippocampus, the cerebellum, and the cerebral cortex (see Celotti et al. 1979 for detail). The enzyme localization has been studied by the "Punch technique" (Palkovits 1973) combined with radioenzymassay in the quail diencephalon: the highest levels of activity are found in the area lateralis hypothalami, in the bed nucleus of the pallid commissure, and in the medial septum (Schumacher and Balthazart 1987).

3.3 The 5β -Reductase Pathway

The saturation of the double bond between carbons 4 and 5 of the testosterone molecule can be obtained in two different ways according to the localization in space of the two hydrogen atoms which are added on the testosterone molecule. The hydrogen added on carbon 5 can be located above or below the plane in which the molecule is represented (beta and alpha isomers, respectively). This also corresponds to a rotation of the A versus the B carbon ring of the steroid molecule to generate two isomers with representations in space which are quite different despite the similarity of their projections in the plane (cis and trans configuration, respectively; see Feder 1981 of a detailed presentation). These differences in spatial configuration have important biological consequences because they determine the affinities for specific receptors. In the brain of many vertebrates, a 5β -reductase catalyzes the reduction of testosterone into a form of

dihydrotestosterone with the cis (beta) configuration (the 5β -dihydrotestosterone or 5β -DHT). Contrary to 5α -reductase and aromatase, this enzyme is essentially cytosolic. The affinity of the 5β -reductase for T is markedly lower than that of the 5α -reductase or aromatase (about micromolar versus 10 nanomolar range), although direct comparisons within a single species and a single experiment are rarely possible (see, however, Schumacher et al. 1984). The maximum velocity of the 5β -reductase is by contrast much higher (about ten times) than that of the two other enzymes. Contrary to aromatase and 5α -reductase, which are found throughout the class of vertebrates, the 5β -reductase has only been characterized in a few species, most of which are birds (Nakamura and Tanabe 1974; Massa et al. 1977; Schumacher et al. 1984; Steimer and J.B. Hutchison 1981b). The presence of the enzyme has not been, however, researched systematically in lower vertebrates. Its presence in mammals is still disputed. It is probably present in the brain of the hamster (*Mesocricetus auratus*, Callard et al. 1978a, b; Callard et al. 1979 and perhaps also occurs in other species, including the rat and the human (fetus). In these species, the 5β -reduction of progesterone produces anaesthetic compounds (Holzbauer 1976) which have been identified but the affinity for testosterone of the enzyme responsible for the reduction of progesterone appears not to have been studied. In the avian brain, the highest levels of 5β -reductase activity are found in areas not directly connected to androgen-dependent reproductive processes. The activity is minimal in the hypothalamus and preoptic area (Steimer and Hutchison 1981b; Schumacher et al. 1983). Combined with the low affinity of the enzyme for testosterone, with the absence of activation effects of 5β -DHT on behavior and with the fact that enzyme activity is high under all physiological conditions in which testosterone action is not important (e.g., in juvenile or in castrated animals), these data suggest that 5β -reductase of testosterone is an inactivation pathway for the steroid. This will be further elaborated below.

No specific inhibitor of the 5β -reductase has been identified so far due probably to the lack of clinical significance of this process which has been mainly studied in birds. It must be noted, however, that most of the 5α -reductase inhibitors also suppress very efficiently the activity of the 5β -reductase in the quail brain or cloacal gland (Balthazart et al. 1982; Alexandre and Balthazart 1986).

3.4 The 3α - and 3β -Hydroxysteroid Dehydrogenases

The enzymatic transformations of testosterone previously described are all irreversible. In the brain, other enzymes are also capable of reversibly catalyzing transformation of T or its metabolites. Testosterone, androstenedione, and the two dihydrotestosterones (5α -DHT and 5β -DHT) all contain a ketone function on carbon 3. This function can be reversibly oxidized to form an alcohol. In each case, two isomers can be produced depending on whether the hydroxyl function is located above or below the plane of the molecule (3β or 3α ol; see Fig. 2). Again a more detailed description of these enzymatic pathways can be found in Feder (1981). The physiological significance of these enzymatic transformations is very difficult to appreciate for a number of reasons. First, a good specific

inhibitor of these enzymes (3α - 3β -hydroxysteroid dehydrogenases) is not available. Second, the transformations they catalyze are reversible so that it is difficult to compare during *in vivo* experiments the biological activity of the parent molecule and of its metabolite (transformation in both directions are possible and one can never know whether the observed effects result from the action of one or the other compound). Finally, it appears that several enzymes with different subcellular localizations (cytosolic, microsomal), different coenzyme requirements (NADPH, NADH), and different affinities for the substrates (T, DHT, E2) are present (see Jouan and Sempere 1980 for more detail). All these reasons explain why our knowledge of the physiological significance of the activity of these enzymes is still so primitive. A number of physiological studies have nevertheless been devoted to the action of 5α -androstane- 3α , 17β -diol (5α , 3α -diol) and 5α -androstane- 3β , 17β -diol (5α , 3β -diol) and these will be reviewed below. The enzymes responsible for the reversible transformations of DTHs into diols have been found in a variety of species of mammals and birds (Callard et al. 1978a). It must also be mentioned that the 3α - and 3β -HSDH can use both 5α - and 5β -DHT as substrate. Like 5β -DHT, the 5β -androstenediols seem to be devoid of any biological activity as far as the control of reproductive activities is concerned (Balthazart 1983).

3.5 The 17β -Hydroxysteroid Dehydrogenase

In most biological systems tested so far, testosterone can also be reversibly converted into androstenedione under the action of the 17β -hydroxysteroid dehydrogenase (17β -HSDH). The same enzyme is also responsible for the interconversion of estradiol and estrone (see Fig. 2 and below). This enzyme has been detected in all classes of vertebrates from fishes to mammals (Callard et al. 1978a). For the same reasons as those described in the case of 3α - and 3β -HSDH, the biological significance of the 17β -HSDH is poorly understood. Androstenedione appears in most biological systems as a steroid with androgenic properties approximately equivalent to testosterone (sometimes a little more sometimes a little less active) but it is difficult to discriminate between direct actions of A4 and indirect ones involving the reduction of A4 into testosterone. The amounts of A4 produced from testosterone during *in vitro* enzymoassays are dramatically affected by the kinetic conditions and it is very likely that they give a poor representation of the enzyme activity in the living organism. In many assay systems, high quantities of A4 are produced at the start of the incubations with radioactive testosterone and then they are converted back to testosterone when the substrate concentration decreases. Before considering a physiological interpretation for a difference in 17β -HSDH activity it is critical to demonstrate that it was measured under conditions in which product formation is linear with respect to duration of incubation and substrate concentration. Otherwise the apparent changes in 17β -HSDH activity could only reflect alterations in the activity of other enzymes through the modification of substrate availability.

4 Control of Male Copulatory Behavior

In almost all species of vertebrates which have been studied, orchidectomy suppresses or at least strongly decreases male sexual behavior and this decrease can be reverted by treatment with testosterone (see, however, Crews and Gartski 1982; Crews et al. 1984 for a striking exception in snakes). The fact that testosterone is extensively metabolized in the brain has stimulated numerous studies designed to evaluate the role of this metabolism in the control of male reproductive behavior. Two enzymatic transformations appear to be of paramount importance in this context: the aromatization and the 5α -reduction. Important species differences seem, however, to exist at this level so that it is impossible to draw general conclusions. We shall thus present first the main body of evidence showing that estradiol and 5α -DHT alone or in combination are critical hormones in the activation of copulatory behavior and afterwards we shall point to a number of exceptions (or apparent exceptions) to this rule.

4.1 Aromatization

In many species of mammals and birds, it is usually accepted that the activation of male sexual behavior by testosterone requires its aromatization into estradiol. This notion is based on the following experimental results:

1. Estrogens *per se* can restore most aspects of copulatory behavior in the male of many species including rats, mice, hamsters, quail, chicken, ring doves etc. (Beach 1942; Pfaff 1970; Södersten 1973; Christensen and Clemens 1974; Davis and Barfield 1979; Adkins et al. 1980, see also Celotti et al. 1979 for review).
2. Aromatizable androgens such as androstenedione, androstenediol, or 19 -hydroxytestosterone also activate sexual behavior, while nonaromatizable androgens such as 5α -DHT and the derived diols have little or no effect (Adkins et al. 1980; McDonald et al. 1970; Feder 1971; Whalen and Luttge 1971).
3. Aromatase inhibitors such as androstatrienedione (ATD) inhibit testosterone-induced sexual behavior in castrated males (Adkins et al. 1980; Morali et al. 1977; Christensen and Clemens 1975). This effect is probably specific and does not result from toxic properties of the drug because ATD does not block sexual behavior directly activated by estrogens.
4. Antiestrogens such as tamoxifen or nitromifene citrate (CI-628) (compounds which prevent the action of estrogens at the receptor level) block testosterone-induced sexual behavior (Adkins and Nock 1976; Alexandre and Balthazart 1986; Beyer et al. 1976; Yahr 1979). This later set of data actually concern the strongest version of the "aromatization hypothesis" which is the "estrogen-receptor hypothesis" proposing that the activation of male sexual behavior by E2 depends on binding of E2 to the estrogen receptor.

All these data support the aromatization theory which says that, ultimately, the restoration by testosterone of male sexual behavior in castrated animals results

from some action of estrogens (derived from T by aromatization). This interpretation, however, ignores a number of facts:

1. Exogenous estrogens only activate behavior when injected at fairly high doses which are likely to have toxic effects. This, of course, could result only from the fact that in the physiological situation, aromatization of T occurs close to the site where estrogens act to activate behavior. A limited area of the brain only is exposed to the estradiol compared to the whole organism when E2 is given systemically. This can explain the requirement for high, probably non-physiological, doses of E2 (peripheral catabolism must be important and consequently only a small fraction of the injected doses ever reaches the target areas in the brain) and the associated toxicity of the treatments.
2. 5 α -DHT and the 5 α -androstanediols are not completely devoid of activity. Even in the male rat in which 5 α -DHT is usually considered inactive, conflicting results have been obtained and it has been found that under some circumstances and/or in some strains of rats 5 α -DHT possesses a low activity in restoring male sexual behavior (see Martini 1982 and Yahr 1979 for a more complete discussion of 5 α -DHT behavioral effects).
3. The antiandrogen, flutamide is able to reduce testosterone-induced mounting in castrated male rats (Gladue and Clemens 1980). This effect is, however, limited: the drug can block the T-induced initiation of mounting behavior but may not affect T-dependent maintenance of mounting. In addition, another study reported only minimal effects of flutamide on sexual behavior in rats (Södersten et al. 1975).
4. Methyltrienolone or R1881, a synthetic androgen which is not metabolizable (Bonne and Raynaud 1976a, b) and in particular is not aromatizable (Doering and Leyra 1984a, b), restores sexual behavior in castrated rats (Baum 1979; Södersten and Gustafsson 1980b; Mode et al. 1984), hamster (Lisciotto and Debold 1984), and Japanese quail (Balthazart et al. 1985; Schumacher et al. 1987a). Considering that R1881 binds with high affinity to the androgen but not to the estrogen receptor, it seems likely that restoration of sexual behavior involves, at least in part, some androgen-dependent mechanisms.

4.2 5 α -Reduction

The effects of 5 α -DHT on male sexual behavior are somewhat controversial. In some species, treatment of castrates with 5 α -DHT efficiently restores sexual behavior (e.g., guinea pig: Alsum and Goy 1974; rabbits: Beyer et al. 1975), while in other species, 5 α -DHT has little or no effect depending on the experiment, strain of animals, and form of treatment (type of solvent used for injection, use or not of propionated forms: Södersten and Gustafsson 1980a; Södersten et al. 1985; Yahr 1979; Adkins 1977; Adkins and Pniwski 1978; Deviche and Schumacher 1982). Several authors (ignoring the large number of negative results) have found that 5 α -DHT and other 5 α -reduced androgens have a weak activity in restoring sexual behavior in rats and CD1-mice (see Martini 1982 for detail). These effects are, however, observed only after a prolonged treatment (Södersten 1975) or a treatment with doses sufficiently elevated (Paup et al.

1975). The same observation has been made in the Japanese quail in which 5 α -DHT was usually considered as inactive in the restoration of sexual behavior in castrates (Adkins 1977; Adkins and Pniwski 1978; Wada 1982): treatment for several weeks with high doses of 5 α -DHT restores sexual activity in a small but reproducible percentage of the birds (Deviche and Schumacher 1982; Schumacher and Balthazart 1983; Balthazart et al. 1985).

It must be stressed, however, that results obtained following systemic administration of 5 α -DHT cannot be taken as conclusive. This is because the poor efficiency of 5 α -DHT in stimulating behavior, which was reported by several investigators, might be due to the fact that 5 α -DHT is rapidly metabolized by the liver and many other structures including the brain into 5 α ,3 α -, and 5 α ,3 β -diols (3 α - and 3 β -HSDH activities, see above). These steroids have only weak androgenic properties as far as activation of sexual behavior is concerned. It must, however, be mentioned that 5 α ,3 β -diol is able to bind with relatively high affinity to the estrogen receptor (Vreeburg 1975; Thieuland et al. 1981, 1982) and, in this way, it could weakly activate copulatory behavior (rat: Baum and Vreeburg 1976; quail: Balthazart et al. 1985). This conclusion has, however, been disputed (Doering and Gladue 1982). This metabolism of 5 α -DHT perhaps explains why the steroid can induce lordosis behavior, an estrogen-dependent response, in the ovariectomized rat (Feder et al. 1979; Beyer et al. 1971). Stimulation of a behavior by 5 α -DHT thus provides no absolute proof of its androgen dependence because 5 α -DHT can be transformed into 5 α ,3 β -diol which is potentially capable of activating estrogen-dependent responses (see also Thieuland et al. 1983).

In Swiss Webster mice, 5 α ,3 β -diol has even been found highly effective in activating sexual behavior (Lutge et al. 1974; Lutge 1979). In general, the reduction of 5 α -DHT into diols can, however, be considered as a pathway of relative inactivation and considering the very high capacity (maximum velocity) of the 3 α - and 3 β -HSDH, this metabolic pathway contributes to an explanation of the relatively weak activity of 5 α -DHT itself on behavior. This is especially true when the steroid is administered in the periphery and consequently undergoes an extensive catabolism before reaching the brain (3 α - and 3 β -HSDH are extremely active in the liver: e.g., Deviche et al. 1987). In support of this interpretation is the finding that 5 α -DHT activated (at least in one experiment) sexual behavior when implanted into the preoptic area of castrated male rats (Johnston and Davidson 1972). Even in this study, 5 α -DHT was still less effective than T in restoring copulatory behavior.

4.3 Interaction of E2 and 5 α -DHT

Initial attempts to induce sexual behavior in castrated rats (but also in other species) with E2 used very high, probably nonphysiological doses of hormone (e.g., 50–100 μ g estradiol benzoate: Davidson 1969; Södersten 1973). In view of the fact that E2 significantly interacts with the androgen receptors in the brain, especially if present in supraphysiological concentrations (Sheridan 1983; Harding et al. 1984; Lieberburg and Nottelbohm 1979; Bonneau et al. 1987), these results may be of little biological interest. However, it has been repeatedly

reported that the effects of 5 α -DHT on behavior are greatly enhanced by the simultaneous administration of low doses of E2 which are behaviorally ineffective per se (Parrott 1975; Baum and Vreeburg 1973; Larsson et al. 1973; Wallis and Lutge 1975; see also Martini 1982 for additional references).

These findings were originally interpreted by suggesting that 5 α -DHT was only assuring the normal development of peripheral structures which are required for copulatory behavior (such as penile spines), while estrogens would be activating behavior at the central level. It is now clear that this interpretation is not sufficient to explain the interaction of androgens and estrogens in the activation of sexual behavior as suggested by the following facts.

1. In birds, which have no copulatory organ (penis), the additive (or synergistic) effects of estrogens and of nonaromatizable androgens have also been clearly demonstrated (e.g., in quail: Adkins and Pniwski 1978; Balthazart et al. 1984b; in zebra finches: Harding et al. 1983).
2. It is now evident that central actions of androgens and estrogens are implicated in behavioral activation. It is indeed possible to facilitate copulatory behavior by brain implants of 5 α -DHT in animals receiving systemically subthreshold doses of estrogens (Baum et al. 1982) as well as by brain implants of estrogens (Christensen and Clemens 1974; Davis and Barfield 1979).

Taken together, these results suggest that both androgen and estrogen receptors present in the brain (see Chap. 2 this Vol.) are implicated in the activation of male sexual behavior. Estrogen derived from testosterone aromatization would in this interpretation occupy the estrogen-binding sites while it remains uncertain whether androgen-binding sites would be occupied by 5 α -DHT derived from testosterone 5 α -reduction or by T itself.

One alternative interpretation of the synergistic effects of E2 and 5 α -DHT has, however, been suggested. As mentioned above, one problem when testing the effects of 5 α -DHT is its rapid transformation into 5 α ,3 α - and 5 α ,3 β -diols which are weak androgens (or even show some estrogenic properties, see above). It has also been shown that the nonmetabolizable androgen, methyltrienolone (or R1881) is able to activate sexual behavior by itself in castrated rats (Baum 1979; Södersten and Gustafsson 1980b; Mode et al. 1984) presumably in the absence of estrogens. This suggested that estrogen receptor occupancy is not required for behavioral activation. Södersten and Gustafsson (1980a) then proposed that the effects of E2 on sexual behavior were not a consequence of its binding with estrogen receptors but rather that E2 acts in the brain to decrease 5 α -DHT catabolism. Some evidence supporting this interpretation has been presented recently (Södersten et al. 1985, 1986). It was indeed shown that testosterone-induced sexual behavior is blocked by treatment with the aromatase inhibitor, ATD, but restored by concurrent treatment with the 5 α -reductase inhibitor, 4-MA, or with small doses of estrogens. This was interpreted as supporting the claim that ATD prevents testosterone from inducing male sexual behavior because it permits the metabolism of testosterone into inactive compounds via the 5 α -reduction pathway. Estradiol derived from testosterone aromatization would normally inhibit this rapid inactivation of the androgen and the 5 α -reductase inhibitor, 4-MA would have acted by the same mechanism. At present, this interpretation

remains questionable essentially because E2 was never identified as a good 5 α -reductase inhibitor except when present in very high, probably nonphysiological, quantities (as in Södersten et al. 1986). In addition, we failed to reproduce in quail the restoration by 4-MA of sexual behavior in birds treated with testosterone and the aromatase inhibitor, ATD (Alexandre and Balthazart 1986).

It must finally be mentioned that recently the specificity of action of R1881 has been seriously questioned. Several authors, on the one hand, reported that this synthetic steroid is never able to restore copulatory behavior to the precastration level (Parrott 1986; Baum et al. 1987). It was also pointed out that very high doses of R1881 had to be used to produce behavioral effects (Nyby and Simon 1987) which raised the possibility that the behavioral action of R1881 represented a pharmacological interaction with receptors different from those of testosterone (possibly estrogen receptors). This idea was tested experimentally and it could be demonstrated that the behaviorally effective doses of R1881 indeed reduced the cytosolic estrogen-binding sites by as much as 91% (Nyby and Simon 1987). Although R1881 has no affinity for the estrogen receptors in standard *in vitro* competition studies (Raynaud et al. 1980), it thus seems capable of interaction with estrogen receptors *in vivo*. The nature of this interaction is not well defined at present and it cannot be ascertained that it is the basis of the behavioral activation. Nevertheless, these data demand that caution be used in the interpretation of experiments using R1881 as a probe to confirm the androgen dependence of a behavioral response.

In conclusion, it must be admitted that after at least 15 years of active research on the subject, we still do not know exactly how estrogens and androgens interact at the molecular level to stimulate male sexual behavior in mammals or in birds. The major routes of actions and some pharmacological agents frequently used in behavioral studies are shown in Fig. 3. Biochemical tools to study

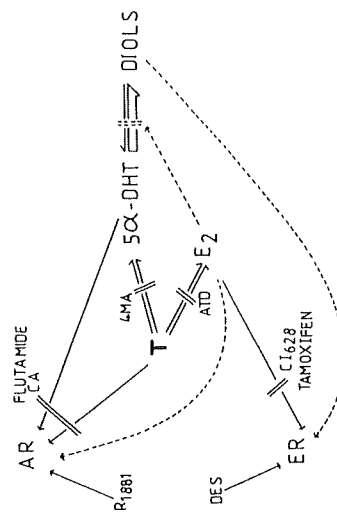


Fig. 3. Summary of the different ways in which testosterone (T) can activate social behavior and examples of pharmacological agents which can be used to manipulate various aspects of this action. AR Androgen receptors; ER estrogen receptors; 4MA 5 α -reductase inhibitor; 17 β -N,N-diethylcarbamoyl-4-methyl-4-aza-5 α -androstan-3-one; ATD aromatase inhibitor; 1,4,6 androstatriene-3,17-dione; CA cyproterone acetate; C1628 nitrophenyl citrate; R1881 methyltrienolone; DES diethylstilbestrol. See also text for additional details

steroid metabolism and receptor characteristics are now available and should allow decisive progress in the study of this question if effective collaborations can be established between behavioral endocrinologists and brain biochemists.

4.4 Effects of 5 β -Androstanes

In the avian brain, T can also be 5 β -reduced to produce the 5 β -DHT (an isomer of 5 α -DHT) and the corresponding diols (essentially 5 β -androstane-3 α ,17 β -diol). The 5 β -reductase has been identified in every bird species studied so far (starling: Massa et al. 1977; chicken: Nakamura and Tanabe 1974; Balthazart and Hirschberg 1982; quail: Balthazart et al. 1979; duck: J. Willems and J. Balthazart unpublished; dove: Steimer and Hutchison 1981b; zebra finch: Balthazart et al. 1986a) but has never been studied in detail in mammals with the exception of the hamster, *Mesocricetus auratus* (Callard et al. 1978, 1979b; see also Sect. 3.3).

Quantitatively, the 5 β -reduction is the main enzymatic pathway for the transformation of T in the avian brain. However, 5 β -DHT is, as far as we know, almost completely devoid of any hormonal activity. It does not depress plasma gonadotropins, nor stimulate the growth of secondary sexual characteristics such as the cloacal gland in quail or the comb in chicken (Adkins 1977; Massa et al. 1980; Mori et al. 1974; Davies et al. 1980; Balthazart and Hirschberg 1979). More important, in relation to the topic of this chapter, 5 β -DHT has no activation effect on the reproductive behavior of quail (Adkins 1977) and dove (Steimer and J. B. Hutchison 1980; Saad et al. unpublished). In addition, 5 β -DHT seems to be devoid of organizational effects on copulatory behavior. Injection of 2 mg 5 β -DHT into quail eggs on day 9 of incubation (an age when similar treatment with T totally suppresses male copulatory behavior; Adkins 1979) had no effect on the reproductive behavior of the birds which hatched from these eggs (Balthazart unpublished). All these facts strongly suggest that the 5 β -reduction of T is an inactivation pathway for the hormone which regulates the concentration of active steroid in the target organ (Steimer and J. B. Hutchison 1981b). It must, however, be mentioned that some limited androgenic effects of 5 β -DHT were detected when the compound was injected in high quantities (growth of chicken comb or quail cloacal gland: Balthazart and Hirschberg 1979; Balthazart et al. 1981; Deviche et al. 1982) or in association with subthreshold doses of T (activation of copulatory behavior in quail: Deviche et al. 1982). These effects can probably be explained by the low affinity of 5 β -DHT for the androgen receptor in birds (Lieberburg and Nottebohm 1979).

In contrast to all these data, it was shown that in young chicks, 5 β -DHT injections or silastic implants have the same efficiency as T in activating sexual responses in a hand test situation similar to that described by Andrew (1975). The responses activated by 5 β -DHT in chicks included the sexual repertoire of the adult cock including behaviors such as mounting on the hand, crouching, treading, circling, and pelvic thrusts. They occurred with the same frequency as in chicks treated with T (Balthazart and Hirschberg 1979; Balthazart et al. 1981). This behavioral effect of 5 β -DHT in young chick contrasts very much with the inactivity of the steroid in all other biological systems studied so far. This

discrepancy could be explained by the specific test situation (hand test versus copulation with a live female) or the young age of the birds correlated with a reduced specificity of the steroid receptors or a higher permeability of the blood-brain barrier (see Balthazart et al. 1981 for a more detailed discussion). More experiments are needed to answer these questions. In conclusion, it seems that the 5 β -androstanes are devoid of hormonal effects on reproductive processes. They are not, however, only a biological waste, down-regulating the action of T. In the young animal, the 5 β -androstanes play a key role in the hematopoiesis (Levere et al. 1967; Irving et al. 1976; Garavini and Cristofori 1984).

4.5 Species Differences and Similarities

In most, if not all, species of vertebrates which have been studied so far, the activational effects of T on copulatory behavior can be mimicked, at least in part, by the administration of either estradiol (E2) or 5 α -DHT or by a combined treatment with both steroids. Reviews of the available evidence supporting this statement are available for mammals (Luttge 1979; Celotti et al. 1979) as well as for the other classes of vertebrates (Adkins-Regan 1981a).

These data have often been used to imply that testosterone metabolism is a critical step in the activation of behavior in all these species. It must be stressed here that this reasoning proceeds only by analogy with what is known in only a few species, such as the rat and the Japanese quail. In these few cases, the implication of T metabolism in the activation of copulatory behavior has been demonstrated by the use of antihormones and inhibitors of metabolism (see above) in parallel with the demonstration that the naturally occurring T metabolites mimic the behavioral effects of the parent steroid. In the other species, it has usually been proved only that E2 or 5 α -DHT reproduce some (all) effects of T.

The relative effectiveness of E2 and 5 α -DHT in restoring copulatory behavior has been found to vary according to the species, suggesting a different importance of the aromatization or the 5 α -reduction of T in the activation of behavior. For example, E2 is quite active in rats (Davidson 1969; Baum and Vreeburg 1973; Larsson et al. 1973; Södersten 1973), mice (Edwards and Burge 1971; Wallis and Luttge 1975), chicken (Guhl 1949; Balandier et al. 1977), and quail (Adkins and Pniowski 1978; Adkins et al. 1980; Balthazart et al. 1985) but not in rabbits (Agmo and Södersten 1975) or guinea pigs (Alsum and Goy 1974). On the other hand, 5 α -DHT given alone effectively restores copulation in guinea pigs (Alsum and Goy 1974), hamsters (Whalen and Debold 1974), rabbit (Agmo and Södersten 1975), rhesus monkeys (Phoenix 1974), in mice of the Swiss Webster strain (Luttge and Hall 1973), and in the green anole (*Anolis carolinensis*, Crews et al. 1978; Adkins and Schlesinger 1980), while it has little or no effect in rats (Beyer et al. 1973; Parrot 1974, 1975; Feder et al. 1974; Agmo and Södersten 1975; Feder 1971) or mice of the CD-1 strain (Wallis and Luttge 1975; Luttge 1972; Luttge and Hall 1973). The behavioral effects of the combined treatment with E2 and 5 α -DHT have been analyzed in quite a few species. A significant difference between the effects of E2 + 5 α -DHT and the treatment with either steroid alone has been shown in several species such as the rat (e.g., Baum

and Vreeburg 1973; Larsson et al. 1973), the CD-1 mice (Wallis and Luttgé 1975), the hamster (Noble 1974), the rabbit (Agmo and Södersten 1975; Beyer et al. 1975), and the Japanese quail (Balthazart et al. 1985).

In those cases where many studies on the same species are available, there are sometimes discrepancies between results concerning the effectiveness of one or the other metabolite (see Yahr 1979 for a detailed example concerning effectiveness of 5α -DHT in rats). Generalizations on species where only one or a few studies have been performed must consequently be made cautiously and the classification of copulation in one animal as E2- or 5α -DHT-dependent should not be done on the basis of a single study. Nevertheless, the mass of results mentioned above clearly point to the existence of true species differences in the hormonal dependence of copulatory behavior. These can find no definite interpretation at present. It has been speculated (Adkins-Regan 1981a) that the patterns of hormone responsiveness could be related to several factors such as:

1. Relative levels of circulating steroids (i.e., species with high plasma levels of 5α -DHT would have a DHT-dependent copulation).
2. The relative catabolism of one or the other hormone.
3. The relative activity of testosterone-metabolizing enzymes in the brain.
4. The presence or absence of androgen or estrogen receptors in the brain areas involved in the control of behavior.

However, none of these interpretations can be taken as definitive at present either because there are numerous exceptions and counterexamples or because the relevant data are simply not available.

5 Control of Other Social Behaviors

Besides copulatory behavior, many other reproductive activities such as pre- and postcopulatory displays, aggressive behavior, territorial defense, avian vocalizations, and some aspects of maternal behavior are also activated by testosterone. In a number of cases, evidence exists to support the idea that testosterone metabolism is also implicated in the control of these testosterone-dependent activities. Generally, this evidence is, however, limited to the demonstration that the behaviors are activated by E2 as well as by T (in which case, a role for aromatization is implied) or by 5α -DHT as well as T (in which case a role for 5α -reduction is postulated). Additional evidence as would be available from studies using metabolism inhibitors or antiandrogens and antiestrogens is usually lacking. The conclusion that T metabolism is implicated in the control of these activities must consequently remain tentative because alternative explanations can be offered. Activation by T as well as by E2 can indeed result from a cross-reaction of E2 with the androgen receptor, while activation by T and 5α -DHT does not imply that the 5α -reduction needs to take place under physiological conditions for the behavior to be activated. This probably explains why some studies have identified behaviors which can be activated by E2 as well as by 5α -DHT (e.g., copulatory behavior in young domestic chicks: Young and Rogers 1978; scent marking in gerbils: Nyby and Thiessen 1971).

Space limitations do not permit one to list all the behavior types and species which have been studied from this point of view. Behaviors stimulated by T and E2 or by T and 5α -DHT are found in many species of mammals, birds, reptiles, and amphibians (see Adkins-Regan 1981a and Callard 1984 for extensive lists of references). In the first category one can find, for example, urine marking in rats, Flehmen behavior in sheep, rutting in red deer, wing-flipping and nest-cooing in ring doves, warbling in budgerigars, and production of long calls in laughing gulls (see Adkins-Regan 1981a for detail). In the second category (activation by 5α -DHT and T) behaviors such as establishment of dominance in rhesus monkeys, crowing and strutting in quail and chicken, bow-cooing and hopping in ring doves, challenging and courting in the green anole, and clasping in newts and frogs (Adkins-Regan 1981a) can be found. Numerous papers and reviews are available which have dealt with the problem of behavioral activation by T, E2, and/or 5α -DHT. The interested reader can, for example, find additional information in Adkins-Regan (1981a: metabolism in general in all classes of vertebrates), Luttgé (1979: metabolism and sexual behavior in mammals), Brain et al. (1983: metabolism and aggressive behavior), Balthazart and Schumacher (1983: metabolism and sexual differentiation of reproductive behavior; 1985: metabolism and social behavior in birds), Martini (1982: 5α -reduction of T and behavior), Celotti et al. (1979: general review on all classes of steroids), Silver et al. (1979: androgens in birds), Crews (1979), and Crews et al. (1978: behavioral endocrinology in reptiles). I shall thus limit myself here to the description of a number of principles which emerge from all these studies.

It is interesting to note that one can often find within the same species behavior patterns which can be activated by E2, while others are stimulated by 5α -DHT. These behaviors showing different hormonal specificities can occur during the same stage of the reproductive cycle as it is the case in Japanese quail (the precopulatory display, strutting is activated by 5α -DHT, while copulation is activated by E2; Adkins and Pniowski 1978) or in the zebra finch (Harding et al. 1983). In the adult zebra finch, some behavioral effects of T can be reproduced by treatment with the androgenic and/or estrogenic metabolites of the hormone. Some behavioral components (high intensity aggressive displays, defense of the nest site) can be activated only by E2 and by aromatizable androgens such as androstenedione. In these cases, concurrent treatment with the aromatase inhibitor, androstatrienedione (ATD) significantly suppresses the behavioral effects of androstenedione, suggesting that aromatization of this androgen is a critical step in its action. This is, however, surprising in view of the very high plasma levels of E2 which were observed in adult males during that study (Harding et al. 1985).

Androgen- and estrogen-dependent behaviors can also be separated in time as in the ring dove. In this species, the male directs predominantly aggressive behaviors (bow-cooing and hop-charging) toward its partner immediately after pairing and these are androgen-dependent (they can be activated by 5α -DHT). After 2–3 days, these behaviors decline in favor of the nest-oriented behavior (nest demonstration, nest-cooing, wing-flipping) which are estrogen-dependent (J.B. Hutchison 1970; Cheng and Lehrman 1975; Adkins-Regan 1981b). We shall discuss later in more detail this transition in ring doves from 5α -DHT to E2-

dependent behaviors during the reproductive cycle. At this point, it must only be mentioned that this coexistence of both types of hormone dependence in the same species clearly excludes the justifications of these hormonal specificities based on species differences in relative levels of circulating steroids or the relative catabolism of one or the other hormone or even relative activity of testosterone-metabolizing enzymes or amounts of receptors in the brain (provided it can be shown that E2- and 5 α -DHT-dependent behaviors are controlled by the same brain areas, which is the case at least for the ring dove: J. B. Hutchison 1971).

Those cases where the same response has been shown to depend on both E2 and 5 α -DHT raise the important question of the mechanisms by which both androgens and estrogens exert facilitatory central effects on a wide array of physiological responses. Based on the actual knowledge of hormone action on the brain, several models may be proposed to explain this interaction.

Androgens and estrogens might act on the same or on different brain cells. In favor of the second possibility are results from studies using brain implants of hormones or describing the precise neuroanatomical distribution of aromatase and 5 α -reductase activity. Stereotaxic implants of E2 facilitate male sexual behavior when located in the POA (Christensen and Clemens 1974; Davis and Barfield 1979). By contrast, implants of 5 α -DHT propionate were shown to be behaviorally effective when located in the septum or medial amygdala (Baum et al. 1982). When T metabolism was studied by the Palkovits punch technique in small brain nuclei, highest levels of 5 α -reductase activity were not found in the same nuclei as highest levels of aromatase activity (Schumacher and Balthazart 1987; Selmanoff et al. 1977; Lieberburg and McEwen 1977). In quail, for example, aromatase activity is highest in the nucleus preopticus medialis (POM), whereas 5 α -reductase activity is highest in the area lateralis hypothalami (LHY) and in the nucleus septalis medialis (SM). Both LHY and SM contain only little aromatase activity (Schumacher and Balthazart 1987). Some observations are, however, in agreement with the hypothesis that androgens and estrogens exert their central effects by acting on the same brain cells. Even if highest levels of aromatase and 5 α -reductase activity do not correspond, there is often an important neuroanatomical overlap of both enzymes. Thus, in the nucleus preopticus medialis of quail, where aromatase activity is highest, 5 α -reductase activity is far from being negligible (Schumacher and Balthazart 1987). Autoradiographic studies of the neuroanatomical distribution of androgen and estrogen receptors show that sites of androgen target cells overlap in part with those of estrogen target cells (Stumpf and Sar 1978; Watson and Adkins-Regan 1988). Although brain implants of 5 α -DHT have been reported to facilitate male sexual behavior in estrogen-treated rats when located in the septum and in the medial amygdala (Baum et al. 1982), in another study brain implants of 5 α -DHT propionate activated copulatory behavior in about 40% of castrated male rats when located in the anterior hypothalamic-preoptic area which is the main site of estrogen action for the activation of male sexual behavior (Johnston and Davidson 1972). However, even if androgen and estrogen receptors and aromatase and 5 α -reductase are located within the same nuclei, this does not prove that androgens and estrogens act on the same cells. Techniques which allow simultaneous determina-

tions of estrogen and androgen receptors such as immunohistochemistry will provide an answer to this question.

The fundamental question of the molecular mechanisms by which androgens and estrogens exert their effects at the brain level still remains. Numerous brain functions seem to require the simultaneous activation by androgens and estrogens, suggesting that this is a basic principle of CNS physiology. Both androgens and estrogens control the vasopressinergic innervation of the adult rat brain (De Vries 1985), the induction of hypothalamic aromatase activity (Schumacher et al. 1987a), the activation of male sexual behavior (see above), as well as numerous other behaviors such as olfactory behavior in the male hamster (Steel and Hutchison 1986), aggression in guinea pigs (Goldfoot 1979) and male mice (Finney and Erpino 1976), and singing in canaries (DeVoogd 1984). Both androgens and estrogens are also involved in the process of sexual differentiation (Booth 1977; Hart 1979; Van der Schoot 1980; see also below). However, it is not yet established that in each case both androgens and estrogens act at the central level. For example, in the control of male aggression, it has been proposed that androgens might stimulate the secretion of an aggression-arousing pheromone, whereas estrogens would facilitate expression of the behavior by acting at the brain level (Beatty 1979).

Biochemical studies have revealed several molecular and physiological mechanisms by which androgens and estrogens might exert their combined effects. Both types of steroids might exert synergistic actions at the level of gene transcription. Indeed, in the chick oviduct, 5 α -DHT probably enhances the number of RNA polymerase molecules transcribing genes already activated by E2 (Palmiter and Haines 1973). It has also been suggested that in the presence of E2 the metabolism of DHT by the brain is modified so that the rapid conversion of 5 α -DHT to 3 α -diol and 3 β -diol is prevented. As a consequence, 5 α -DHT could bind to the androgen receptor and activate male sexual behavior (Södersten and Gustafsson 1980a; see also above for a detailed coverage). According to this view, estrogens activate male sexual behavior only by preventing the catabolism of androgens.

Another mode of interaction between both types of steroids is also illustrated by the control of vocalizations in oscines and their sexual differentiation. The song nuclei (see Nottebohm and Arnold 1976 for their anatomical description) are steroid-sensitive: their total volume, the size of their neurons, and/or their dendritic branching are modified by androgens in adulthood (DeVoogd and Nottebohm 1981a,b; Gurney 1981; Nottebohm 1980). In female canaries, adult treatment with testosterone (T) can transform some morphological aspects of the song nuclei so that they can no longer be distinguished from males (DeVoogd and Nottebohm 1981a). In particular, dendritic fields of females are significantly smaller in females than in males in the nucleus robustus archistriatalis (RA). However, if females are ovariectomized in infancy, treatment with testosterone in adulthood results in an increase of dendritic field radius to nearly the size seen in intact adult males. This effect is partly reproduced by treatments with either E2 or 5 α -DHT (DeVoogd and Nottebohm 1981a). However, other morphological characteristics, e.g., proximal dendritic segments, demonstrate a stable sexual dimorphism which cannot be suppressed by hormonal manipula-

tions in adulthood (DeVoogd and Nottebohm 1981a). It has been shown that the neonatal steroid environment is responsible, at least in part, for this sexual dimorphism in the brain. This is especially true in zebra finches in which adult treatment of females fails to masculinize morphological aspects of the song control nuclei (Arnold 1980; Gurney 1982; Gurney and Konishi 1980). Gurney and Konishi (1980; Gurney 1981, 1982) have demonstrated profound masculinizing effects of sex steroids given during the neonatal period to female zebra finches. E2 increases as much as fivefold the volumes of the hyperstriatum ventrale pars caudale (HVc), the robustus archistriatalis (RA), the magnocellular nucleus of the anterior neostriatum (MAN), and the area X of the lobus parafac-torius. Treatment with 5 α -DHT has more modest effects (doubling of volume at most) and they are observed in a different subset of brain nuclei (HVc, RA, and the dorsomedial part of the nucleus intercollicularis, ICo-DM). A closer look at E2 and 5 α -DHT action on RA morphology reveals other differences in the mechanism of action. Neonatal treatment of females with E2 increases the size and density of neurons within RA close to values typical for males but causes only a modest increase in neuron number. By contrast, females which received a treatment with 5 α -DHT just after hatching have a nearly masculine number of RA neurons but the size of these neurons and their density in the nucleus have been little or not at all modified. Testosterone treatment affects all three measures (neurons number, size, and density). The increase in RA volume which is observed after treatment with both E2 and 5 α -DHT thus reflects totally different mechanisms at the cellular level.

Thus, it seems that both E2 and 5 α -DHT can have separate effects at the level of the brain. How these two types of steroids interact in the control of behavior remain, however, largely unknown and will probably remain so until the precise biochemical effects of these hormones have been elucidated.

6 Control of the Activity of Testosterone Metabolizing Enzymes

The body of data which is reviewed above clearly supports the notion that the metabolism of testosterone in the brain is an important step in the central action of the steroid on behavior. This idea is supported by the studies of the activity of T-metabolizing enzymes. It has indeed been shown repeatedly that these activities are not constant but rather that they are modulated by the physiological condition of the animals. Variables like age, sex, sexual maturation, or social environment all modulate the activity of these enzymes in a biologically relevant way. By this, it is meant that the production of active metabolites of T (e.g., E2 and 5 α -DHT) is increased in physiological situations where testosterone is supposed to play an important role in the control of behavior, while inactivation of T, for example through 5 β -reduction, is enhanced in situations where no effect of T is supposed to take place. All these regulations usually take place specifically in the brain sites involved in the control of behavior which strengthen the idea that they are biologically relevant. These changes in enzyme activity in relation to social behavior will be reviewed below. First, I shall, however, present the proximal causes by which enzyme activities are modulated. These changes according to the

sex, age, or physiological status of the animal are, in fact, brought about at a more mechanistic level by effects of steroids, peptides, protein hormones, and neurotransmitters. These effects will first be reviewed to permit a better understanding of the biologically relevant variations which are described afterwards.

Quite often, steroids originating from the gonads or the adrenals are the major regulators of their own metabolism in the target organs. This regulation involves either a genomic action and a change in synthesis of the enzyme or a direct modification of enzyme activity. The available data will be summarized for each enzyme successively.

6.1 Aromatase

The initial study on aromatase activity conducted in the rabbit hypothalamus suggested that the castration increases and testosterone treatment decreases the enzyme activity (Reddy et al. 1973; Nafolin et al. 1975). This type of result was never reproduced and could be an artifact due to the technical limitations at the time of the study or to the small number of determinations which were carried out. Another study also claimed that castration does not influence aromatase activity in the hypothalamus of the rat (Dessi-Fulgheri et al. 1983a) but this negative result is probably explained by the fact that the whole hypothalamus was studied and local variations of enzyme activity within this very heterogeneous structure could not be detected.

In fact, all subsequent studies performed with improved techniques agree to show that, in adult animals, castration decreases and testosterone treatment increases aromatase activity in specific nuclei of the hypothalamus. In the castrated ring dove (*Streptopelia risoria*) testosterone increases the aromatase activity in the POA but not in the anterior hypothalamus or in the area basalis (Steimer and Hutchison 1981a). Similarly, it was shown later in rats that this effect is observed at the level of the preoptic area (POA) (which is implicated in the control of male reproductive behavior) but not in the amygdala or in the cerebral cortex (Roselli et al. 1984; Roselli and Resko 1984). The neuroanatomical specificity in the regulations of aromatase could suggest the presence of different forms of enzymes in different parts of the brain. The alternative explanation is, of course, the existence of neuroanatomically specific mechanisms of control.

At the present time, it is not clear whether T or one of its metabolites controls aromatase activity. In addition, we do not know whether T directly activates the enzyme or acts indirectly through a number of intermediate compounds. Aromatase activity can indeed be modulated by several chemicals which are present in the brain. Norepinephrine increases aromatase activity in the Sertoli cells of the testes (Verhoeven 1980) as well as in the pineal gland of the rat (Cardinali et al. 1982). Considering that NE exerts most of its effects through changes in cyclic AMP (cAMP) it is then not surprising that cAMP also stimulates aromatase activity in the Sertoli cells (Verhoeven 1980), in the pineal gland (Cardinali et al. 1982), and in the brain of the turtle, *Chrysemys* (Callard 1981). It is thus conceivable that testosterone increases aromatase activity through a modification of the catecholaminergic activity in the brain. It must also be men-

tioned that the increase of aromatase activity following T treatment has not been observed in studies on fetal monkeys (Roselli and Resko 1986) and ferrets (Tobet et al. 1985). It is therefore possible that the control mechanisms of aromatase activity show major changes with age and are only fully developed in the mature animal.

Other groups of substances also activate aromatase activity. The pituitary hormone FSH has been shown to act synergistically with T (but not E₂) in the ovarian follicles of the rat. The fact that antiandrogens such as SCH 16423 block the effect of T shows that the steroid acts through its receptors (Hillier and De Zwart 1981; Daniel and Armstrong 1980). This has been confirmed for the rat brain in which flutamide significantly blocked the T-induced increase in aromatase activity in the hypothalamus-preoptic area (Roselli and Resko 1984). FSH also stimulates the aromatase in the Sertoli cells (Verhoeven 1980). By contrast, it would be inactive in the human adipose tissue (Folkerd and James 1983) and in the brain of the turtle *Chrysemys* (Callard 1981). We do not know whether FSH is active in the brain of mammals or birds.

Cortisol increases aromatase activity in the placenta of sheep (Rickerts et al. 1980) and in human adipose tissue (Folkerd and James 1983).

It is thought that T increases the enzyme activity by a nuclear mechanism implying genomic effects. This is suggested by the observation that one single injection of T has little effect on the preoptic aromatase in the ring dove, while daily injections for 12 days are extremely active and even superior to a similar treatment performed over 4 days (Steimer and Hutchison 1981a). These injections of T only affect the maximum velocity of the enzyme (V_{max}) but not its affinity for the substrate ($K_m = 2 \times 10^{-8}$ M), suggesting that the change in activity is due to an increase in enzyme quantity and not to a direct regulation of activity (Steimer and Hutchison 1981a). Similarly, in quail, the increase in aromatase activity following the initiation of T treatment in castrates is a relatively slow process. Detectable effects are, however, already observed after 8 h and the maximum increase is obtained after about 2 days. This slightly faster reaction could be due to the more efficient mode of testosterone administration: daily injection in the ring dove and silastic implants in the quail (Balthazart and Foidart unpublished).

It is also unclear whether T or one of its metabolites is the primary regulator of aromatase activity. T increases the activity of aromatase in the preoptic area (POA) of adult doves (Steimer and Hutchison 1981a), rats (Roselli et al. 1984), and quail (Schumacher and Balthazart 1986; Schumacher and Hutchison 1986). In the dove, E₂ but not 5 α -DHT also stimulates preoptic aromatase activity (J. B. Hutchison and Steimer 1986). By contrast, 5 α -DHT but not estrogen induces preoptic aromatase activity in the rat (Roselli and Resko 1984; see Fig. 4).

Whether these contradictory results reflect species differences or different methodologies has yet to be determined. In the periphery (e.g., human placenta), 5 α -DHT at high concentration (0.8 mM) is able to inhibit aromatase by a direct action on enzyme activity (Schwarzel et al. 1973). A similar effect has also been observed in the turtle brain (Callard et al. 1980a). A potential mechanism underlying these effects has been suggested by Siiteri and Thompson (1975). 5 α -DHT binds to the same cytochrome P450 as T and in this way could compete for the active site of the aromatase. By contrast, 5 β -DHT would not inhibit the

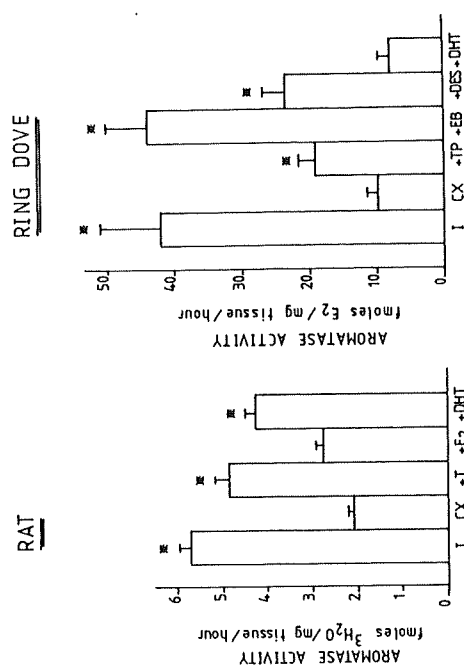


Fig. 4. Effects of steroid treatments on the aromatase activity in the preoptic area of rats and ring doves. Enzyme activity was measured by in vitro assays (production of tritiated water in the rat, production of radioactive estradiol in the dove) in the brain of intact (I) and castrated (CX) animals and in castrates treated with testosterone (+T), estradiol-17 β (+E₂), 5 α -dihydrotestosterone (+DHT), testosterone propionate (+TP), estradiol benzoate (+EB), or diethylstilbestrol (+DES). Data are means and SEM. Within a species, aromatase levels significantly higher than in castrates are indicated by an asterisk above the corresponding column. (Redrawn from data in Roselli and Resko 1984 and J. B. Hutchison and Steimer 1986)

aromatase activity in vitro even at concentrations as high as 0.8 mM (Schwarzel et al. 1973) and it does not bind to the P450 cytochrome (Siiteri and Thompson 1975). More recent data, however, show that 5 β -DHT at micromolar concentrations can inhibit aromatase activity in the ring dove (J. B. Hutchison and Steimer 1983; Schumacher and J. B. Hutchison unpublished).

At high concentration (0.8 mM) progesterone weakly inhibits (about 10%) aromatase activity in the human skin (Schwarzel et al. 1973). This steroid is quite active in the rat ovarian follicles: aromatase inhibition observed under these conditions is, however, probably indirectly obtained through a reduction of the stimulating effects of FSH (Fortune and Vincent 1983).

6.2 5 α -Reductase

The regulations of 5 α -reductase are at least as complex as those of aromatase and have been the subject of numerous studies (for review see Jouan and Sampedez 1980; Martini 1982). A number of studies suggest that the hypothalamic 5 α -reductase activity is not sensitive to castration in the rat (Denef et al. 1973, 1974; Massa et al. 1972; Jouan and Sampedez 1980; Dessi-Fulgheri et al. 1983a). However, all these experiments were performed on whole hypothalamic and local

effects on specific nuclei may have been overlooked. Several studies have demonstrated that 5α -reductase is not evenly distributed within the hypothalamus (e.g., Lieberburg and McEwen 1977; Selmanoff et al. 1977).

In support of this interpretation, castration increases 5α -reductase activity in the anterior pituitary of the rat (Ghrat et al. 1982a; Jouan and Sampedez 1980; Denef et al. 1974). This increase is paralleled by a proliferation of pituitary gonadotropic cells which contain most of the enzyme (Denef 1979). The mechanism by which the 5α -reductase activity is increased has not been totally elucidated at present (see Jouan and Sampedez 1980 for a detailed discussion). 5α -Reductase is also sensitive to LHRH. This peptide increases enzyme activity in the intact rat but decreases it in the castrated animal (Has et al. 1975). No satisfactory explanation has been given for these paradoxical effects.

Injections of T, 5α -DHT, or E2 inhibit 5α -reductase activity in the anterior pituitary of the castrated rat (Ghrat et al. 1982a, b). These effects can be antagonized by the simultaneous injection of antiandrogens (flutamide) or antiestrogens (tamoxifen), respectively, which suggests receptor-mediated responses (Ghrat et al. 1982a, b). There is probably no direct effect of the steroids on the enzyme: even at high concentrations (up to 10 μ M) E2 and 5α -DHT do not inhibit the 5α -reductase of the human skin during *in vitro* incubations (Voigt et al. 1970).

By contrast, progesterone (P) competitively inhibits the 5α -reduction of testosterone (Voigt et al. 1970; Tan et al. 1974). Indeed 5α -reductase is able to reduce T into 5α -DHT but also P into 5α -pregnane-3,20 diene (5α -DHP). The affinity of P for the enzyme is slightly higher than the affinity of T (Voigt et al. 1970; see also Martini 1982).

Contrary to what is observed in the pituitary gland, castration decreases and testosterone injections increase the 5α -reductase activity in the rat prostate. This effect involves the synthesis of new mRNA and proteins (for review see Tenniswood et al. 1982) which suggests a genomic action. However, E2 inhibits the enzyme in this tissue (Jenkins and McCaffery 1974) and also in liver microsomes (Lee et al. 1973). This demonstrates the tissue specificity of the regulation of testosterone metabolizing enzymes.

In the male quail, surgical castration as well as photic castration (transfer in short days which inhibits testicular activity) reduces 5α -reductase in the cloacal gland and this effect can be reversed by treatment with testosterone (Massa et al. 1979; Balthazart et al. 1983). A similar effect is observed in the hypothalamus but only in a restricted part at the anterior level (Schumacher and Balthazart 1986).

The microsomal and cytosolic enzymes which are responsible for the reduction of 5α -DHT into diols is even more complex. The activities of the *microsomal* 3α -hydroxysteroid dehydrogenases (3α -HSDH) of the rat kidney or pituitary which use NADPH₂ or NADH₂, respectively, as cofactors are increased by castration and decreased by injections of E2 or 5α -DHT (Ghrat et al. 1979a, 1982a). These inhibitory effects are not blocked by antiestrogens (tamoxifen) or antiandrogens (flutamide), respectively, which suggests actions not mediated by the classical steroid receptors (Ghrat et al. 1982a, b). In contrast, E2 injections increase the activity of the *cytosolic* 3α -HSDH in the kidney male rats (Ghrat et al. 1979b). The effects of E2 are thus differentiated at the subcellular level.

Dihydroprogesterone (5α -DHP) also competitively inhibits the reduction of 5α -DHT into diol (Giacomini and Wright 1980). 5α -DHP is indeed reduced into 5α -pregnane-3 α -ol-20-one by the 3α -HSDH and this process competes for the enzyme with the reduction of 5α -DHT (Celotti et al. 1979).

NADPH₂ plays a key role in the control of 3α -HSDH activity. When its intracellular concentration is low, this stimulates the transformation of 5α -DHT into diol. On the other hand, when the NADPH₂ concentration is high, the reversed transformation (diol into 5α -DHT) is promoted (Thien et al. 1974; Jouan and Sampedez 1980).

6.3 5β -Reductase

Castration increases the 5β -reductase activity in the preoptic area (POA) of the ring dove but has no effect in the adjacent lobus parolfactorius (J.B. Hutchison and Steimer 1981). Injections of testosterone propionate or diethylstilbestrol (DES) in the castrated bird decrease the enzyme activity specifically in the POA. The effect of DES is not directly on the enzyme, because *in vitro* even a 100-fold excess of DES by comparison with T (the substrate) does not modify the 5β -reductase activity (J.B. Hutchison and Steimer 1981). Similarly, it is only at very high and probably pharmacological doses (10 μ M) that E2 decrease the 5β -reduction of T in the quail brain (Balthazart et al. 1982). In addition, it has been shown that DES inhibits in a noncompetitive way 5β -reductase in the pig liver but only at nonphysiological concentrations (1.25 or 2.50 μ M; Van Doorn and Clark 1973). It thus appears that E2 inhibits the 5β -reduction by a genomic mechanism.

By contrast, P decreases the 5β -reduction of T at concentrations similar to those of the substrate (Balthazart et al. 1982). In this case also it appears to be a competitive inhibition which is explained by the fact that both T and P can be 5β -reduced by the same enzyme (see same reasoning for the effects of P on 5α -reductase).

7 Changes in the Activity of T-Metabolizing Enzymes in Relation to Social Behavior

All these biochemical controls of the T-metabolizing enzymes concur to adjust the levels of activity to the actual requirement of the individual in its particular environmental and social situation. A vast array of studies have indeed demonstrated that T-metabolizing enzymes in the brain not only provide the metabolites required to activate behavior but also adjust their production so that the central action of T is fine-tuned at its site of action. Many of these studies have used birds as subjects, although there is evidence that similar processes take place in mammals. These correlations between changes in enzyme activity and changes in behavior provide strong support to the notion that T metabolism is a key step in the activation behavior by the steroid. They are reviewed below.

7.1 Correlation Between the Neuroanatomical Distribution of Enzymes and Brain Areas Implicated in Behavioral Activation

Soon after the discovery of brain aromatase, it was observed that in mammals it is essentially localized in the hypothalamus and the limbic structures (Naftolin et al. 1975) which are key areas in the activation of social behavior by steroids. This localization is not so clear in other classes of vertebrates (especially in cold-blooded animals; see above), but nevertheless the enzyme remains primarily located in the telencephalon and diencephalon from fishes to mammals (Callard et al. 1978, 1979a; Callard 1984).

The neuroanatomical localization of T-metabolizing enzymes in the rat was first analyzed by *in vivo* techniques. Lieberburg and McEwen (1977) analyzed the brain cell nuclear retention of estradiol and 5 α -DHT after injection of tritiated testosterone. Both unchanged T and its metabolites were recovered from the nuclear fractions of various brain areas 2 h after the injection of the radioactive substrate. Estradiol was found almost exclusively in the limbic and hypothalamic areas (with highest levels in the amygdala), while 5 α -DHT was recovered in all brain areas studied with maxima being detected in the hypothalamus and septum. An *in vivo* uptake and metabolism study also demonstrated regional specificity in the distribution of metabolites in the brain of the rhesus monkeys (*Macaca mulatta*). Highest levels of estrogens were recovered in the septum and amygdala but also, in contrast to rats, in the lateral hypothalamus. The highest concentrations of 5 α -DHT were found in the septum and quite surprisingly in the cerebellum (Sholl et al. 1982).

Interestingly, the *in vivo* rat study (Lieberburg and McEwen 1977) showed that the nuclear distribution of radioactive estradiol was not the same after direct injection of tritiated E2 (highest levels in the preoptic area) as after injection of tritiated T (highest levels of E2 derived from T aromatization in the amygdala). This points to a weakness of the *in vivo* technique in the studies of T metabolism. If it shows that aromatization or 5 α -reduction has occurred under physiological conditions, it cannot ascertain when the enzymatic reaction takes place. In addition, the amounts of metabolites which are recovered in the nuclear fractions depend not only on the activity of T-metabolizing enzymes, but also on the concentrations of steroid receptors which must be present to retain the newly formed steroids.

To differentiate between these interpretations, Selmanoff et al. (1977) quantified *in vitro* the aromatization and 5 α -reduction of testosterone in microdissected brain regions of male and female rats. The medial preoptic-anterior hypothalamic area exhibited the highest levels of aromatase activity and the second highest 5 α -DHT production. The lateral preoptic and hypothalamic areas by contrast showed little aromatase activity but demonstrated high rates of 5 α -DHT synthesis.

More recently, the precise localization of aromatase in the rat brain has been studied by the Palkovits punch technique (Palkovits 1973; Palkovits and Brownstein 1983). Highest levels of activity were detected in several preoptic, hypothalamic, and limbic nuclei including the bed nucleus of the stria terminalis, the medial and cortical amygdala, the periventricular preoptic nucleus, and medial

preoptic nucleus (Roselli et al. 1985). By a similar procedure, Schumacher and Balthazart (1987) have found a similar distribution for aromatase activity in the brain of the Japanese quail, although highest levels of enzyme activity were found here in the preoptic area (medial preoptic nucleus) rather than in the avian equivalent of the amygdala, that is the archistriatum (nucleus taenia, archistriatum ventrale; Schumacher and Balthazart 1987). In both species, large differences (up to tenfold) in enzyme activities are found between adjacent nuclei indicating a very discrete localization of the enzyme.

In conclusion, all these studies show that the hypothalamus and the limbic system are very heterogeneous structures from a structural but also functional point of view. They are involved in the control of a great number of behaviors (such as sexual and aggressive behavior, maternal behavior (Numan 1983), and play behavior (Beatty 1984), but also of autonomic functions such as thermoregulation (Mills and Heath 1972) and heart rate change (Cohen and Macdonald 1976). If T metabolism is studied in large heterogeneous parts of the brain such as the whole hypothalamus, local changes in enzymatic activity can be masked by the dilution of responsive tissue by surrounding areas which do not show the same type of regulation. Several studies in rat (Roselli et al. 1984; J. B. Hutchison and Steimer 1981; Steimer and J. B. Hutchison 1981a), and quail (Schumacher and Balthazart 1986) have now shown that the regulation of T metabolism is specific to the brain region considered. It is not unusual to find tenfold differences in enzyme activity between closely related brain areas.

Most studies show that aromatase activity is higher in the POA than in more caudal parts of the hypothalamus (quail: Schumacher and Balthazart 1986; dove: Steimer and J. B. Hutchison 1981a; rat: Roselli et al. 1984; Selmanoff et al. 1977). This fits in well with the well-established role of POA in the activation of male copulatory behavior. In most studies on mammals, the highest levels of aromatase activity have, however, been found in the amygdala (Kobayashi and Reed 1977; Lieberburg and McEwen 1977; Roselli et al. 1985). In the rat, the amygdala has been implicated in the control of male sexual behavior (Baum et al. 1982; Harris and Sachs 1975) and gonadotropin release (Velasco and Taleisnik 1969). In birds, the archistriatum has also been related to agonistic behavior (Maley 1969; Phillips and Youngren 1971). This again points to relationships between enzyme activities and control of behavior.

Interestingly, the highest levels of 5 α -reductase activity are usually not observed in the same nuclei as the highest levels of aromatase activity (Lieberburg and McEwen 1977; Selmanoff et al. 1977; Schumacher and Balthazart 1987). In rats, high 5 α -reductase activity has been found in the septum and lateral hypothalamic nucleus (Lieberburg and McEwen 1977; Selmanoff et al. 1977). In quail, the production of 5 α -DHT was higher in the area lateralis hypothalamic (LHV), nucleus septalis medialis (SM), and bed nucleus of the pallid commissure (BPC) than in the other nuclei studied here and in particular in the preoptic area. In rodents, the septum is involved in the activation of male sexual (Paxinos 1976; Baum et al. 1982) and aggressive behavior (Owen et al. 1974) as well as in the inhibition of female receptivity (Nance et al. 1975). It has been shown that stereotaxic implants of 5 α -DHT propionate located in the septum or in the medial amygdala activate male sexual behavior in rats primed by peripheral treat-

ment with low doses of E_2 (Baum et al. 1982). In the same study, similar implants located in the POA or in the hypothalamus were behaviorally ineffective. By contrast, estrogens act mainly on the POA to activate mating behavior (Christensen and Clemens 1974, 1975; Davis and Barfield 1979). Thus, it is possible that E_2 and 5α -DHT exert their synergistic effects on male sexual behavior (Larsson et al. 1973; Balthazart et al. 1985) by acting on different parts of the brain. This agrees well with the results of the enzymatic studies showing that the neuroanatomical localization of the enzymes producing these two metabolites of T is quite different. However, the presence of a substantial 5α -reductase activity in aromatase-rich nuclei such as the POM leaves open the possibility that androgens and estrogens also exert synergistic effects by acting in the same brain nuclei or even in the same neurons.

7.2 Changes in T Metabolism and Their Behavioral Effects During Ontogeny

7.2.1 The Perinatal Period and Sexual Differentiation of Behavior

It is well established that the activity of testosterone-metabolizing enzymes is deeply modified according to the age of the animal. Detailed studies on these processes are available in a number of mammalian and avian species. In particular, the changes in aromatase, 5α - and 5β -reductase activities have been quantified and seem to play a key role in the control in the determination of processes such as sexual differentiation of reproductive behavior and sexual maturation and puberty. These data are briefly reviewed below.

In general, aromatization is more active in the hypothalamus of fetal or newborn mammals than in the hypothalamus of adults (Reddy et al. 1974; Weisz and Gibbs 1974). This decrease with age has been particularly well studied in the brain of the rat (George and Ojeda 1982) and the rabbit (George et al. 1978). In the rabbit diencephalon, aromatase activity increases tenfold from day 16 to day 19 of gestation, then remains high until day 25 and falls at the time of delivery to the same level as that observed on day 16 (George et al. 1978). In the rat, aromatase activity was first detected in the diencephalon on day 16 of gestation and it reached maximal levels on days 18 to 20. It then rapidly decreases to minimal levels which were obtained between postnatal days 16 to 20 (George and Ojeda 1982). This high embryonic aromatase activity which is present in the hypothalamus and preoptic area is probably involved in the sexual differentiation of reproductive behavior. It is indeed well accepted now that during the fetal and neonatal period, the sexual behavior of rats is organized in a male direction (masculinization and defeminization) by the action of testicular androgens. Similar processes are supposed to take place in many, if not all, species of mammals probably including men (Schumacher et al. 1987b). With the possible exception of primates, the vast majority of the available data points, to estrogens as the major metabolites of T involved in the sexual differentiation of the brain. Data supporting this idea are of the same nature as those supporting the aromatization hypothesis in the context of the activation of sexual behavior in adult animals (see above). Namely, it has been shown that aromatizable androgens

mimic the masculinizing effects of T, while nonaromatizable androgens are not or less effective, that estrogens have a masculinizing effect at doses much lower than those of T, and finally that treatment with antiestrogens or aromatase inhibitors block the differentiating effects of T. Since testosterone is present in the blood of fetuses and newborn rats (Weisz and Ward 1980; George and Ojeda 1982) and aromatase activity is extremely high in the hypothalamus, it is very likely that the experimental manipulations described above (injections of antiestrogens and aromatase inhibitors) reveal a biologically relevant process and that the local formation of estrogens in the brain mediates the testosterone-induced masculinization of sexual behavior.

Some data, however, conflict with this theory and suggest that androgens are also involved in this process (Martini 1982). Recent studies have also more carefully differentiated between the effects of embryonic and neonatal steroids on defeminization (loss of the lordotic response in the female) and masculinization (enhancement of male copulatory behavior) of behavior. In particular the synthetic nonaromatizable androgen, R1881 (see, however, above for potential problems in the interpretation of R1881 action) has been shown to cause a partial defeminization and masculinization in rats and hamsters (reviewed in Olsen 1985). In addition, 5α -reductase is present and very active in the rat fetus and in the newborn rat so that potentially 5α -androstanes can be synthesized and also take an active part in the differentiation of behavior.

At this point, the available evidence can thus be summarized by saying that the defeminization of behavior is controlled mainly by estrogens derived from central administration of T with a possible accessory role of androgens, while masculinization is mediated by androgens alone or in combination with estrogens. More experimental data are, however, required to more precisely characterize these conclusions and test their applicability to different species (see namely Baum et al. 1985 and Tobet et al. 1985 for studies in ferret and Roselli and Resko 1986 for studies in monkeys). The fetal and neonatal brain is capable of transforming testosterone into both 5α -androstanes and estrogens and the high enzymatic velocities observed potentially amplify the actions of T on the developing brain.

7.2.2 Puberty

During the first few weeks after birth in rats, there is a continuous decrease in the hypothalamic and pituitary 5α -reductase activity (Massa et al. 1975; Degtiar et al. 1981). It is probable that this process participates in the control of the onset of puberty (Martini 1982). According to current theories, puberty in the male results either from an increased sensitivity of the testes to the gonadotrophic hormones or from a decrease of the sensitivity of the hypothalamus-pituitary unit to the feedback effect of testosterone. According to the second hypothesis, lower levels of androgens would be required to inhibit gonadotropin secretions in juvenile than in adult rats. Massa et al. (1972) postulated that the higher sensitivity of the young rat to the feedback effects of T is due to their higher capacity to metabolize the hormone into 5α -androstanes (5α -DHT and $5\alpha,3\alpha$ -diol) which would be

more active than T in inhibiting LH secretion. The fact that formation of 5α -androstanes is higher before puberty (see above) and that these compounds actively inhibit LH and FSH secretion when injected or implanted into castrated rats (Martini 1982) clearly provides a mechanism by which the sensitivity of the central gonostat can be altered and initiate puberty (see Martini 1982 and Jouan and Samperez 1980 for a more detailed treatment). This change in T metabolism is of course affecting behavior only indirectly by modifying the secretion of testicular steroids.

As mentioned previously, 5α -DHT can also be metabolized into $5\alpha,3\beta$ -diol. This probably represents an important aspect of 5α -DHT metabolism because the $5\alpha,3\beta$ -diol could also be involved in the process of sexual maturation. This compound, when injected into immature female rats, indeed induces precocious puberty (Eckstein 1975; David and Eckstein 1976). The mechanisms underlying this effect remain, however, obscure at present. As mentioned previously, $5\alpha,3\beta$ -diol binds with the estradiol receptor and its biological effects are certainly worth additional investigation.

7.2.3 The Japanese Quail

In the Japanese quail, a series of studies have investigated in detail the ontogeny of the brain metabolism of T and its relationship with behavior control.

Masculine sexual behavior is strongly differentiated in quail. Testosterone treatment restores copulation in castrated males but is without effect in females (Adkins 1975; Balthazart 1983). Adkins and collaborators have established that this differential responsiveness to the activating effects of T results from the demasculinization of female embryos by ovarian steroids. Males are usually considered as a "neutral" or "anhormonal" sex as their phenotype apparently develops in the absence of hormonal control (Adkins-Regan 1983). These conclusions are based on the finding that estradiol benzoate (EB) injection into male eggs demasculinizes the future adults (they will not copulate in response to T: Adkins 1975, 1979), while blockade of estrogen action in female embryos by the injection of an antiestrogen (CI-628) prevents their demasculinization (Adkins 1976). Surprisingly, testosterone propionate (TP) injections also demasculinize male embryos (Adkins 1975). Knowing that the embryonic testes and/or adrenal glands secrete significant amounts of T (Ottinger and Bakst 1981; Schumacher et al. unpublished) raises the intriguing question as to why males are not demasculinized by their testicular secretions. It was first demonstrated that demasculinization of male embryos requires larger doses of androgen than of estrogen: 2 μ g of E₂, E₁, or estril but not of T injected on day 9 of incubation significantly decreases the copulatory behavior of male quail (Whitsett et al. 1977), which was later confirmed by detailed dose-response experiments (critical doses are 1 and 500 μ g for EB and TP, respectively: Adkins 1979). It can thus be reasoned that presumably plasma levels of T are too low to demasculinize male embryos. This argument is strongly reinforced by the analysis of T metabolism in the brain. An extensive series of experiments by Adkins-Regan et al. (1982) has shown that T-induced demasculinization is mediated by or at least requires T

aromatization. This is supported by the observations that aromatizable androgens (T, TP, androstenedione) but not nonaromatizable ones (5α -dihydrotestosterone propionate, androstene) are active agents in the demasculinization process, that the antiestrogen, tamoxifen blocks the T-induced demasculinization and that androstatrienedione, an aromatase inhibitor inhibits the TP-induced but not the EB-induced demasculinization. Considering that rates of aromatization are extremely low (a few fmol/mg tissue/h) in 4-day-old chick (Callard et al. 1978, 1979a), in newly hatched or adult quail or ring doves (Steimer and J. B. Hutchison 1980; Schumacher et al. 1984; J. B. Hutchison and Schumacher 1986), and also in embryonic quail (Schumacher et al. 1988), it can be expected that this is a limiting step in the demasculinization induced by T. This would explain the requirement for large doses of exogenous hormones as well as protect male embryos from their endogenous secretions.

In the avian brain, T is also reduced into 5β -androstanes (mainly 5β -DHT and 5β -diol). As far as we know, these compounds are devoid of androgenic activity in adult birds (see above; for review: Balthazart 1983) and 5β -DHT is apparently devoid of differentiating effects in the quail (Balthazart unpublished). It is thus usually considered that 5β -reductase is an inactivation pathway for T which controls within the target cells the biological activity of the hormone. We showed that 5β -reductase activity is extremely high in the hypothalamic area of male quail throughout embryonic life (Balthazart and Ottinger 1984). This enzymatic activity is at least ten times higher in the embryos than in the adults and only decreases when birds reach sexual maturity at 4–5 weeks posthatch. This is relatively specific to the hypothalamus: 5β -reductase activity decreases markedly in the cerebellum around hatching and even earlier in the cloacal gland (a sexually dimorphic androgen target organ (Sachs 1967) which also differentiates under the influence of embryonic steroids (Adkins 1975, 1979; Adkins-Regan et al. 1982). Considering that 5β -reduction irreversibly transforms T into behaviorally inactive compounds which are in addition not aromatizable, it appears that the high enzymatic activity present in the embryonic hypothalamus should protect males from being behaviorally demasculinized by converting aromatizable androgens to nonaromatizable metabolites.

In a study of cockerel, Massa and Sharp (1981) showed that sexual maturation is contingent with a sharp decrease in the 5β -reductase activity in the brain and pituitary gland. We have extended these studies to the Japanese quail and, in addition, asked whether the changes described in the cockerel were anatomically and biochemically specific (do they occur in neuroendocrine structures only and do they exclusively affect the 5β -reductase?). Testosterone metabolism was thus studied in the hypothalamus, hyperstriatum, pituitary gland, and cloacal gland from quail of different ages between hatching and sexual maturity (Balthazart and Schumacher 1984). A sharp decrease in 5β -reductase activity was observed in the brain but not in the pituitary or cloacal gland. This change also appeared specific to 5β -reductase and did not concern the 5α -reduction of T. These data suggested that perhaps the relative insensitivity of young birds to the behavioral effects of T (see, e.g., Schleidt 1970) could result from the presence in the brain of the active 5β -reductase causing a too rapid inactivation of the hormone. For a number of technical reasons (see Balthazart et al. 1984b for detail) this idea

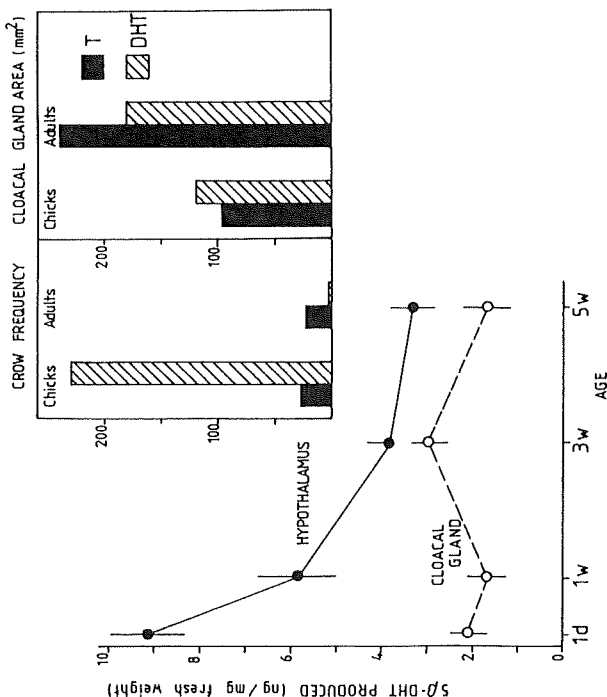


Fig. 5. During the first 5 weeks posthatch, a sharp decrease is observed in the activity of the 5β -reductase in the brain but not in the cloacal gland. Testosterone is thus more inactivated into 5β -dihydrotestosterone in the brain of chick than in adults. This difference is not observed in the cloacal gland. In parallel, the behavioral effects of T compared to those of 5α -dihydrotestosterone (DHT), which cannot be inactivated by 5β -reduction) are affected by the age of the birds but the activation of cloacal gland growth does not show this age-related change in the relative efficiency of T and DHT. Drawn from data in Balthazart and Schumacher (1984) and in Balthazart et al. (1984b)

cannot be tested directly. However, it is possible to obtain a measure of the relative action of T in adult and young quail by comparing the behavioral and morphological effects of T with those of 5α -DHT in both age groups. Both T and 5α -DHT activate crowing and promote cloacal gland growth in adult castrated quail (see above). As 5β -reductase decreases with age in the brain but not in the cloacal gland, 5β -androstanes have little or no androgenic action in quail (see above), and 5α -DHT is protected from inactivation by 5β -reductase while T is not, it could be predicted that 5α -DHT would be more potent than T at activating crowing in young than in adult quail while no such age-related difference would be found in the activation of cloacal gland growth. Experimental data confirmed this prediction (Balthazart et al. 1984b; see Fig. 5). A number of reports had established that T and 5α -DHT stimulate crowing and cloacal gland growth in adult quail with the same efficiency at least when hormones are given in unsterilized form (Balthazart et al. 1980; Deviche and Schumacher 1982; Wada 1982; Schumacher and Balthazart 1983). On the contrary, we found that in

young quail 5α -DHT was much more potent than T in the induction of crowing but this difference was not observed in the activation of cloacal gland growth. These observations are best explained by referring to the fact that 5β -reductase inactivates T more in the brain of young than adult quail and that 5α -DHT is protected from this inactivation (see Balthazart 1984b for a more detailed discussion). The ontogenic changes in 5β -reductase activity thus probably play an important role in the control of hormone action. These changes appear now as a common feature of birds as they have been demonstrated also in ring doves (J. B. Hutchison and R. E. Hutchison 1985) and in zebra finches (Balthazart 1986a), in addition to the cockerel and quail which were already mentioned.

It appears also that generally in birds 5α -reductase and aromatase activity are lower in young animals than in adults while in mammals, the reverse is rather observed. This conclusion is supported now by studies on one precocial (quail) and two altricial species (ring dove and zebra finch). The biological implications of this taxonomic difference are presently unknown. Kinetic studies carried out with quail hypothalamic tissue suggest that differences between chicks and adult metabolic enzymes are not caused by ontogenetic changes in affinity of the enzymes for their substrate. The apparent K_m for each enzymatic activity is similar in adult and newly hatched quail (J. B. Hutchison and Schumacher 1986). This is in contrast to the data obtained in zebra finches where the K_m of 5β -reductase and aromatase seem to decrease (increased affinity) between hatching and adulthood (Vockel et al. 1988). Whether this represents a general difference between precocial and altricial species (the latter being more immature at hatching) and has biological consequences at the level of behavior control remains to be determined.

7.3 Changes in Time During Adult Life

7.3.1 Seasonal Changes and Changes Related to Photoperiod

In wild animals which exhibit annual breeding cycles, there is an associated cycle in the activity of T-metabolizing enzymes, especially in aromatase. This provides additional evidence for a functional implication of these enzymes in the control of reproductive behavior. These effects can either be direct in that they modify T action in the preoptic area-anterior hypothalamus or indirect in which case they affect the secretion of hormonal steroids via a change in their feedback action on the hypothalamus-pituitary axis. These changes are well documented in a number of avian species but also in some mammals whose reproduction is heavily dependent on photoperiod such as the hamster.

In the European starling, Bottomi and Massa (1981) showed that the 5α - and 5β -reductases of T by the hypothalamus and pituitary gland are quite different in the breeding season (May) compared to the beginning (July) and the end (November) of the photorefractory period. Specifically, the formation of 5β -androstanes by the hypothalamus was greater in May than in July or November. The opposite changes were detected in the pituitary gland. These enzymatic changes have unfortunately no obvious role in the control of reproduction. By

contrast, in Japanese quail, it was found recently that short photoperiods which inhibit all reproductive processes in this species, induce a decrease in hypothalamic aromatase and 5α -reductase activities (production of behaviorally active metabolites) but an increase in the production of 5β -androstanes (Schlinger et al. 1984; Callard 1984). These enzymatic changes should amplify the effects on behavior of the variations in plasma T resulting from the exposure to short days.

In general, it seems that the hypothalamic aromatase is modulated to a larger extent than the reductases. For example in the red grouse (*Lagopus lagopus scoticus*), aromatase activity in the anterior and posterior hypothalamus was shown to be directly related to gonadal function during a photoinduced reproductive cycle (Sharp et al. 1986). Enzyme activity increased within 3 weeks of transfer to long days and stayed high during about 1 month during which birds were reproductively active. When they became photorefractory and as a consequence gonadal activity declined, the aromatase activity returned to baseline level. These enzymatic changes are probably controlled directly by the variations in plasma testosterone (T induces brain aromatase activity, see above) and should amplify the behavioral effects of T through an increased production of active estrogenic metabolites.

This type of regulation is also present in photoperiodic mammals. In the little brown bat (*Myotis lucifugus*), aromatase in the preoptic-hypothalamic region is higher in spring than in autumn (Callard et al. 1983). Similarly, in hamsters short days decrease the hypothalamic aromatase activity and this has more obvious direct behavioral consequences. In hamsters, exposure to short days decreases the responsiveness to the behavioral effects of T. Following T injections, male hamsters maintained in short days show relatively low rates of copulatory behavior in comparison to long day controls (Campbell et al. 1978; Morin and Zucker 1978). When aromatase was measured by in vitro radioenzyme assay in discrete brain regions of individual hamsters, it was found that enzyme activity is lower in the hypothalamus of short day than in long day animals. The effects were observed in both castrated and intact animals, suggesting that it is not controlled by circulating testosterone. The same trend was seen in the preoptic area but failed to reach significance. Interestingly, it could also be shown that in vivo after injection of aromatizable androgens, nuclear estrogen receptor levels are higher in long day than in short day hamsters (seen both in intact animals and castrates). This difference is not due to the different availability of the receptors themselves because such a difference is not seen after the injection of estradiol (Callard et al. 1986). It can thus be concluded that the difference in aromatization rates observed in vitro is actually a rate-limiting step controlling the concentration of nuclear estrogen receptors in the hamster brain. The decrease in behavioral effectiveness of T observed in short days can thus be explained at least in part by the modulation of aromatase activity and the consequent decrease in behaviorally active metabolites of T. More studies should, however, be undertaken to precisely determine the neuroanatomical relationships between the brain areas showing a clear modulation of the aromatase and those directly implicated in the control of copulatory behavior.

7.3.2 Aging

Aging in animals and in man coincides with a decrease in sexual activity associated with a host of endocrine modifications including changes in the patterns of secretion of testosterone and pituitary gonadotropins. These phenomena are also observed in the Japanese quail in which a marked decrease in copulatory behavior is already observed after about 1 year of age. In old animals, two subpopulations can thus be distinguished which either show or do not show copulatory behavior in standard test situations (Ottinger et al. 1983). It was demonstrated that, in parallel, testosterone metabolism in the preoptic area-anterior hypothalamus is also affected by age and that these changes are differential in sexually active and inactive birds. The major metabolic change was observed in the 5α -reductase activity which increased markedly in old, active males but did not change or slightly decreased in old, inactive birds in comparison with the young controls. There was also a general decrease in testosterone 5β -reductase with age but this change was not different in active and inactive birds (Balzhart et al. 1984a). Considering that 5α -DHT is an active metabolite of T which alone or in combination with E2 activates the copulatory behavior and sexual displays in quail while the 5β -DHT is devoid of any behavioral effects, the enzymatic changes observed in aging animals can thus be considered as neuroendocrine adaptations (production of more active and less inactive metabolites) which could be causally related to the maintenance of sexual activity in old birds.

7.3.3 The Reproductive Cycle of Ring Doves

For many years, the breeding cycle of the ring dove (*Streptopelia risoria*) has been a classical model for the analysis of the interactions between steroids and the external environment in the regulation of reproductive behavior (Lehrman 1965; Cheng 1979). When a male ring dove is paired with a female, the male directs predominantly aggressive courtship (bow-cooing, chasing) toward its partner. After a few days, these are progressively replaced by nest-oriented behaviors such as nest-cooing and wing-flipping (Lovari and J. B. Hutchison 1975; Cheng 1979). All these behaviors are dependent on testicular secretions: they are absent in castrated males and can be restored by testosterone treatment. However, the aggressive components of courtship can be activated by non-aromatizable androgens, while the nest-oriented courtship requires either estrogens or aromatizable androgens (J. B. Hutchison 1970; Cheng and Lehrman 1975; Adkins-Regan 1981b). For some time, the hormonal mechanism underlying this behavioral transition remained obscure because, although it was postulated that there was a shift from an androgen-dependent to an estrogen-dependent brain mechanism, no change in the plasma concentration of estradiol over the breeding cycle could be detected (Korenbrodt et al. 1974). In fact, estradiol was not detected in the plasma of the males at any stage in the reproductive cycle but in view of the low levels of circulating estrogens in male birds and the relative lack of sensitivity of the assay used in that study, this is probably an experimental artifact. If the behavioral transition between aggressive and nest-oriented court-

ship had to be controlled by estrogens, it was thus necessary to postulate that the steroid was formed in the target areas within the brain. Using *in vitro* radioenzymoassays, Steimer and J. B. Hutchison (1980) were able to demonstrate the conversion of T to E₂ in the preoptic area of the ring dove. They showed that the aromatization also occurs *in vivo* by showing the presence of a radioactive peak with the chromatographic mobility of estradiol in nuclear extracts from the preoptic area (Steimer and J. B. Hutchison 1980, 1983). The analysis of the neuroanatomical distribution of the aromatase showed that the enzyme activity is preferentially located in the preoptic area, a known target for estradiol action on nest-oriented courtship (J. B. Hutchison 1971; Steimer and J. B. Hutchison 1980, 1981a).

Shortly thereafter, the same group of researchers demonstrated that testosterone had a striking effect on the preoptic aromatase. Treatment of birds with behaviorally active doses of testosterone propionate increased the enzyme

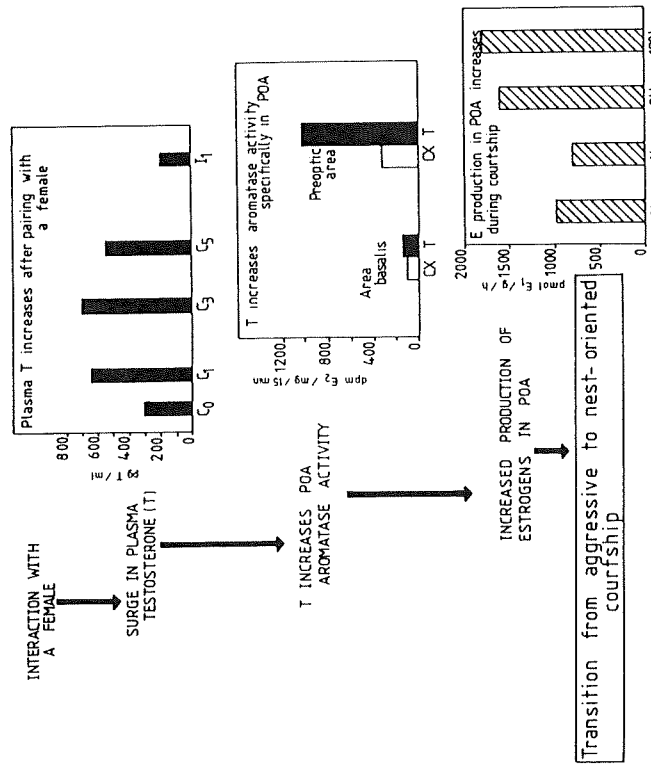


Fig. 6. Sequence of endocrine and neuroendocrine events which are supposed to control the transition from aggressive to nest-oriented courtship during the reproductive cycle of the ring dove. C0 to C5 Courtship days 0 to 5; male and female were paired on day 0; 0 to 120 h number of hours since male and female were paired; POA preoptic area; E1 estrone; E2 estradiol. Based on data from Feder et al. (1977), Steimer and J. B. Hutchison (1981a), Dudley et al. (1984), and J. B. Hutchison and Steimer (1983). See also text for additional details.

activity between three- and fivefold (Steimer and J. B. Hutchison 1981a). This provided a molecular mechanism which could control the behavioral transition from aggressive to nest-oriented courtship. It has also been shown that during the reproductive cycle of male ring doves plasma testosterone reaches a peak 1 or 2 days after the initial interaction with the female (Feder et al. 1977). It is actually the interaction with the female which results in a massive surge of testosterone as studied in great detail by O'Connell et al. (1981). This testosterone peak roughly coincides with the behavioral transition and it had been hypothesized that it was the hormonal signal responsible for the behavior changes through increase in preoptic aromatase and local production of estrogens which would then bind to their specific receptors (J. B. Hutchison and Steimer 1983; see Fig. 6). Recently, experimental evidence supporting this interpretation has been obtained. Dudley et al. (1984) showed that 24 h after the introduction of a female dove into the cage of a male, there is a twofold increase in aromatase activity confined to the ventromedial preoptic area. This increase in enzyme activity is sustained through day 5 of courtship and presumably provides the estrogens needed to activate the nest-oriented behavior. The preoptic aromatase activity then declines during the incubation phase of the cycle (Lea and Armstrong 1986). The preoptic aromatase system thus appears to play an important part in modulating the action of androgens during the cycle of the ring dove. Together with the injection and implants, experiments demonstrating the steroid specificity of the different courtship behaviors (J. B. Hutchison 1970, 1971; Cheng and Lehrman 1975; Adkins-Regan 1981b), this represents one of the best examples illustrating the critical role played by the neural metabolism of T in the control of behavior.

7.4 Sex Differences and Sexual Differentiation

Another significant contribution of changes in testosterone metabolism is related to the explanation of sexual differences in behavioral responsiveness. In mammals and in birds, the process of sexual differentiation results in two types of adults with profound differences in their behavioral responses to steroids, in particular to testosterone. The same treatment with testosterone always elicits more vigorous and more frequent copulatory behavior in the castrated male than in the ovariectomized female. In some species such as the Japanese quail, the latter are even totally insensitive to the activating effects of T on male sexual behavior (Adkins 1975; Adkins and Adler 1972). This behavioral difference raises the question of the mechanisms which underlie this differential sensitivity to T. The different biochemical steps between the entry of testosterone into the brain and the production of the behavior have been investigated in this respect (e.g., binding to steroid receptors, modulation of neurotransmitter levels, or turnover) and many biochemical and even morphological differences between the male and the female brain have been demonstrated during the last decade. Considering that the metabolism of T into estradiol and/or 5 α -DHT and possibly its inactivation by transformation into 5 β -androstanes play a key role in the induction of the behavioral effects (see above), it was also legitimate to research whether the sexually differentiated responses to T did not result from a sexually

differentiated neural metabolism (production of more active metabolites and/or less inactive metabolites in males in comparison with females). Data are now available in a number of species to support this interpretation.

In several species it has been shown that the preoptic-hypothalamic aromatase activity is higher in males than in females (e.g., rat: Naftolin et al. 1972; Reddy et al. 1974; Roselli et al. 1985; rabbit, mouse, and human: Naftolin et al. 1975; ferret: Tobet et al. 1985; little brown bat: Callard et al. 1983; monkey: Roselli and Resko 1986; quail: Schumacher and Balthazart 1984a, 1986; Schlinger and Callard 1987; zebra finch: Vockel et al. 1988).

By contrast, very few sexual differences in 5α -reductase activity have been identified in brain areas that could be directly related to behavioral dimorphism. This enzymatic activity shows major changes during ontogeny (see above) but the patterns are usually quite similar in males and females. Minimal differences were found at specific ages in development (Massa et al. 1975; Degtiar et al. 1981). These were, however, transient and were no longer present in the adult age. In addition, all these sex differences in 5α -reductase point to a higher activity in females which can hardly be invoked to explain their lowered sensitivity to the behavioral action of T. It must, however, be remembered that in females, the 5α -reductase activity is primarily devoted to the metabolism of progesterone (Jouan and Sampere 1980) which probably explains the higher activity in females.

Using a microdissection of the hypothalamic and adjacent areas, we (Schumacher and Balthazart 1986) recently demonstrated the presence of a sexually dimorphic (higher in males than in females) 5α -reductase activity in the region of the anterior hypothalamus. This report also showed that the 5β -reductase activity is higher in females than in males for brain regions located in the lobus parafactorius and in the preoptic area.

All these data thus can be taken to support the original hypothesis according to which the behavioral insensitivity of the females to activating effects of T would result from an insufficient transformation of the steroid into active metabolites or from an excessive inactivation by 5β -reduction. However, it must be stressed here that all these studies had been performed on gonadally intact animals so that the enzymatic differences observed could have been the result of the exposure to a different hormonal milieu rather than represent a neonatally differentiated sexual dimorphism. In this context, it is interesting to relate the observation that almost all sexual differences in T metabolism which have been detected concern a higher aromatase activity in males with the well-established fact that aromatase activity is inducible by T (see above). It is thus possible that the higher aromatase in males only reflects their higher levels of plasma testosterone. That this could be the case was suggested by the discovery that gonadectomy not only decreases aromatase activity, but also suppresses its dimorphism (rat: Roselli et al. 1985; ferret: Tobet et al. 1985; quail: Schumacher and Balthazart 1986). One study in rat suggested, however, that when animals were gonadectomized and then submitted to the same replacement therapy with T, there was a trend to higher aromatase levels in males compared to females (Roselli et al. 1984). This had already been suggested by a study on gonadectomized-adrenalectomized male and female rats showing that aromatization was higher in males than in females. However, in that study, the small number of

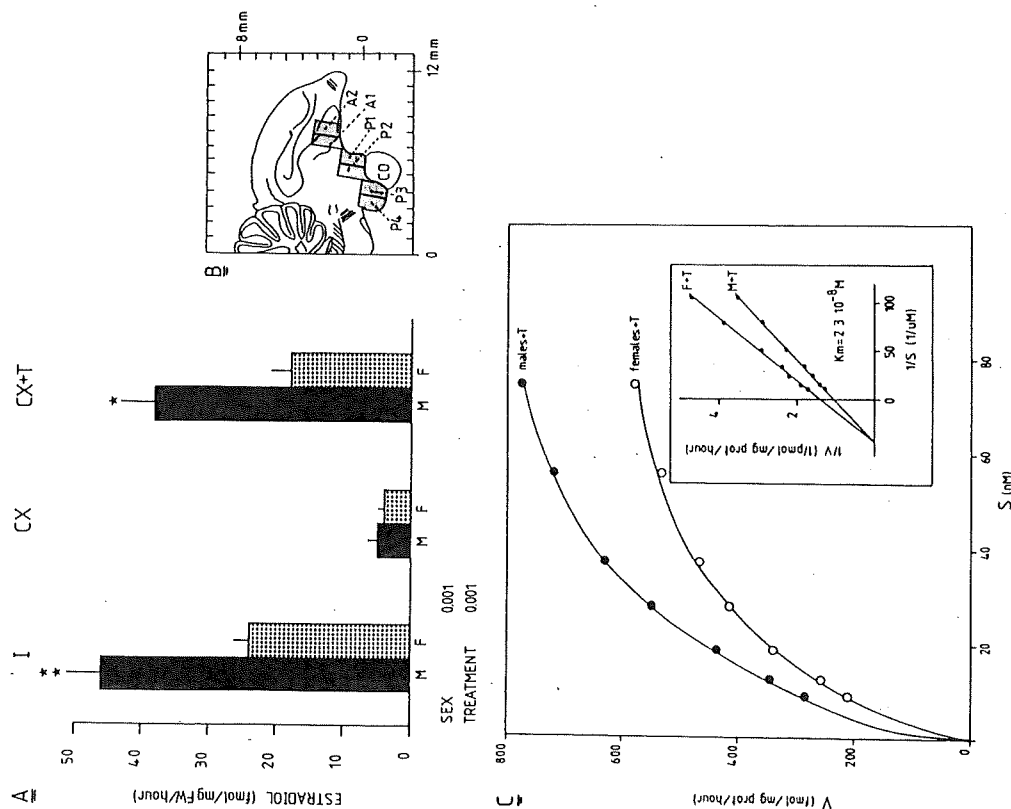


Fig. 7. A Aromatase activity observed in the preoptic area of male (M) and female (F) Japanese quail under different physiological conditions (I intact; CX gonadectomized; CX + T gonadectomized, testosterone-treated birds). B Sagittal view of the quail brain showing the brain samples which were studied for testosterone metabolism. The preoptic area corresponds to sample P1. C Effect of substrate concentration (tritiated T) on the aromatase activity in the hypothalamus of gonadectomized testosterone-treated male (closed circles) and female (open circles) quail. The insert shows the double reciprocal plot of these data demonstrating the sex difference in maximum velocity but identity of enzyme affinity for its substrate (K_m) in males and females. Data from Schumacher and Balthazart (1986).

data precluded any statistically supported conclusion (Selmanoff et al. 1977). All this prompted us to reinvestigate the sexual dimorphism of T metabolism in male and female quail either gonadally intact or gonadectomized with and without testosterone therapy (Schumacher and Balhazart 1986). In this study, the sexual differences in 5 β -reductase activity which had been found in intact birds were confirmed but they disappeared in gonadectomized birds and were not restored by T treatment. It also appeared that the sex difference in 5 α -reductase activity which had been observed in the anterior hypothalamus was only the result of a differential induction by T. 5 α -DHT production was decreased by castration in males and increased by T treatment in both sexes. This is probably the only known case of a regulation by T of the 5 α -reductase activity in the brain but this mechanism probably plays no part in the control of behavior considering that 5 α -reductase activity is no longer different in males and females after treatment with the same dose of T while their behavior remains dimorphic.

By contrast, the study of the aromatase revealed true sexual differences which are probably implicated in the control of behavior (see Fig. 7). As seen previously in quail and in other species, aromatase activity was much higher in intact males than females. This difference disappeared in gonadectomized animals. The enzymatic activity was then induced in both sexes by testosterone but the extent of this induction was significantly larger in males than in females, in particular in the preoptic area. This was confirmed during kinetic experiments in which the aromatase of testosterone-treated males and females was estimated in the presence of increasing concentrations of tritiated substrate. The enzyme activity was higher in males than in females for all concentrations of tritiated testosterone between 10 and 80 nM (Schumacher and Balhazart 1986). Lineweaver Burke (double reciprocal) plots of these data showed that the affinity (Km) of the enzyme was similar in both sexes and it is only the maximum velocity which was different. This suggests that males and females differ by the quantity of aromatase which is present in their preoptic area but that the characteristics of the enzyme are the same in both sexes. This sexual difference was recently confirmed and it was shown in addition that the differential induction of the aromatase by T can be observed for a wide range of testosterone doses (Balhazart et al. 1986b). Interestingly, the sexually differentiated inducibility of the aromatase by T does not seem to be established during the early posthatching period when the mechanisms regulating the copulatory behavior are not themselves fully differentiated (Schumacher and Balhazart 1984b; Schumacher and J. B. Hutchison 1986). Considering that the activation of copulatory behavior by testosterone requires the aromatization of the steroid into estradiol (see above), the enzymatic difference which has been observed in the quail preoptic area could contribute substantially to the causation of the behavioral sex differences in response to testosterone.

7.5 Individual Differences in Behavior and Brain Metabolism of Testosterone

The causation of individual differences in behavior is poorly understood. Although it is clear that the previous history of an individual and a host of develop-

ment factors can contribute to their explanation, it could also be expected that the endocrine systems plays some part in their control. Quite often though, it has been impossible to relate individual levels of reproductive behaviors which are known to be androgen-dependent to plasma levels of testosterone (see Balhazart and Schumacher 1985 for review). This failure could of course result from a number of technical artifacts but could also express the fact that ultimately the behavioral effects of T are modulated at the brain level. Individual differences in intracellular metabolism (relative activation/inactivation) could then obscure the relationship of the behavior with plasma testosterone levels and play a causal role in the genesis of the behavioral differences.

This idea was first suggested by a work of Dessi-Fulgheri et al. (1976) showing that individual levels of fighting behavior in mice were positively correlated with the activity of the 17 β -hydroxysteroid dehydrogenase in the brain, while a negative correlation was observed between the behavior and the aromatizing enzymes in the nuclear fraction of the brain. The fighting behavior was by contrast not related to plasma testosterone or estradiol levels. This prompted us to investigate whether similar correlations could be observed in the quail.

During a first experiment carried out in collaboration with R. Massa and his group in Milan we found that in a group of 20 male quail transferred from short days to long days, there was no correlation between the reproductive behavior that had been recorded and plasma testosterone. By contrast, significant correlations were observed between behavior and brain testosterone metabolism: aggressive actions were negatively correlated with the 5 β -reductase in the hypothalamus (Balhazart et al. 1979). More recently, a series of experiments was carried out to confirm the existence of relationships between individual variations in reproductive behavior and brain testosterone metabolism in quail (Delville et al. 1984). Various aspects of aggressive and sexual behavior were measured during three different types of tests and it was found that the individual variations between all types of behaviors in the different situations were strongly intercorrelated. In one experiment, the behavioral measures were significantly correlated with plasma testosterone, while in another one performed under slightly different conditions they were not. Significant correlations were also found between behavior and the hypothalamic 5 β -reductase activity (productions of 5 β -DHT and 5 β -diol) but in the first experiment, these correlations were positive, while they were negative in the second one. These correlations were quite specific because they were observed with the metabolism in the anterior but not posterior hypothalamus.

It thus seems warranted to conclude that individual variations in behavior are always related to some aspects of T metabolism in the brain, particularly the 5 β -reductase in the hypothalamus. As the direction of this correlation was observed to change from one experiment to another (twice negative and once positive), the functional significance of these relationships is difficult to establish. It could be that the negative correlation between hypothalamic 5 β -reductase and behavior has a causal meaning and that the birds which are behaviorally more active are so because their brain inactivates less efficiently the steroid (5 β -reduced compounds are behaviorally inactive). The experiment showing the reverse correlation is then difficult to interpret in this context although potential explanations have been

proposed which should be experimentally tested (Delville et al. 1984). Nevertheless, the existence of these correlations whatever their direction points to a biologically relevant role of the brain testosterone metabolism. It is of course possible that these correlations indicate an effect of behavioral activity on the metabolism as discussed below.

7.6 Modulation of Brain Testosterone Metabolism by Social Stimuli

The possibility that social interactions could influence the brain metabolism of testosterone has been investigated by F. Dessi-Fulgheri and co-workers. It was already known that social isolation in rodents is responsible for many behavioral and endocrine modifications concerning aggressive, sexual, and open-field behavior as well as adrenal and gonadal physiology (see references in Dessi-Fulgheri et al. 1975). It was first confirmed that in rats, long-term isolation increased aggressive behavior and at the same time increased plasma testosterone levels without affecting the circulating concentrations of estradiol (Dessi-Fulgheri et al. 1975). In this first set of experiments, brain aromatase was also decreased by social isolation (Lupo di Prisco and Dessi-Fulgheri 1975). In a subsequent series of experiments carried out on much younger animals (2–3 versus 15 months at sacrifice), opposite effects were observed. Within that series, a consistent effect of manipulations of the social environment on the brain aromatase was detected. First, it was determined that brain aromatase is much lower in male rats which had been raised in heterosexual groups than in those raised in total isolation or contact isolation (male and female separated by a wire mesh screen) or even in unisexual groups (Lupo di Prisco et al. 1978). The type of signals which decreased aromatase activity in the rats kept in heterosexual groups was investigated in subsequent experiments. It was found that anosmia induced by surgical destruction of the olfactory epithelium mimics the effect of isolation from the females and increases the aromatase in the brain (Dessi-Fulgheri et al. 1980). This effect was observed in rats which had been housed with receptive females (sexually experienced group) but not in rats kept in social isolation which suggested that it was due to olfactory stimuli emanating from the females and not to a nonspecific effect of the surgery. Again, the increase in brain aromatase was associated with decrease in plasma testosterone. This interpretation was confirmed in a last experiment during which male rats were housed with other males or with females or were housed with males and at the same time exposed to olfactory stimuli (bedding) coming from males or females. All males kept in the absence of females showed increased aromatase levels in the hypothalamus. Aromatase activity was decreased significantly in males exposed to female bedding, although not to the level seen in rats kept in heterosexual groups (Dessi-Fulgheri and Lupo di Prisco 1982). All these data are thus strong support to the notion that the social environment strongly modifies the hypothalamic aromatase activity. The olfactory stimuli emanating from the female seem to be involved in this effect but other types of stimulation (visual, tactile) are not excluded. It can even be speculated that the performance of sexual behavior by the males housed with females is itself responsible for the increased enzyme activity.

Theoretically this hypothesis could then be tested by researching the existence of correlations between individual levels of behavior and enzyme activity as demonstrated in the previous section. Whatever the mechanism involved in the generation of this effect, it can probably be considered that the increased aromatase activity social isolation and it has been speculated that the increased aromatase activity could be a regulatory mechanism compensating olfactory deprivation. This could lower the threshold for the stimuli necessary to elicit some of the sexual behaviors (Dessi-Fulgheri et al. 1983 b). This interpretation now awaits experimental testing.

8 Conclusions

All the data reviewed in this chapter lead to the unequivocal conclusion that the metabolism of testosterone by the central nervous system plays an important role in the control of a wide variety of social behaviors. By shifting from the production of behaviorally active metabolites to the synthesis of inactive ones, the target areas of steroid action regulate at the local level the final action of the hormone.

The evidence for the implication of T metabolism in behavior control comes from essentially two types of approaches. On the one hand, it is possible to mimic the behavioral effects of T by injections of some of its metabolites and conversely to block the effects of T with compounds which inhibit its central metabolism. On the other hand, the activity of the brain enzymes which catalyze T metabolism is precisely regulated and is affected in a biologically relevant way by factors such as age, sex, castration, or social environment. These two types of evidence thus leave no doubt as to the functional role of T transformations in the brain. Despite years of active research, many questions remain, however, unanswered. For example, we still do not know how estrogens and androgens interact at the molecular level to activate copulatory behavior. New biochemical tools are now available to study these questions and this should permit important progress in the near future.

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