

induced sexual behavior (for review: Adkins-Regan, 1983; Balthazart, 1988). Consistent with the implication of aromatization in the control of behavior are the facts that the behavior can be activated directly by systemic injections or stereotaxic implantation in the preoptic area of estrogens such as E2 or diethylstilbestrol (Adkins *et al.*, 1980; Balthazart, 1988).

The Changes in Aromatase Activity

The data summarized above demonstrate that the aromatization of T is required for the activation of copulatory behavior. *In vitro* assays of the enzyme, in addition, suggest that aromatase activity is modulated by the physiological condition of the bird. The enzyme activity appears to be a limiting step in the production of the behavioral effects of T.

In most vertebrates, the preoptic area (POA) represents a critical site in the action of T on copulatory behavior. Accordingly, in quail, stereotaxic implantation of T pellets in the POA activates sexual behavior in castrated males (Watson and Adkins-Regan, 1989; Balthazart *et al.*, 1988). Interestingly, the POA is also a brain area in which very high levels of aromatase activity could be demonstrated by *in vitro* radioenzyme assays (Schumacher and Balthazart, 1984). By detailed morphometric studies, we recently identified a sexually dimorphic area within the POA. The medial preoptic nucleus (POM) is significantly larger in male than in female quail (Viglietti-Panzica *et al.*, 1986). The volume of this nucleus is also T-sensitive; it decreases after castration and is restored to intact level after 2 weeks of treatment with T (Panzica *et al.*, 1987). As the POM is located in the center of the region which controls copulation (POA), a behavior which is itself sexually dimorphic (T activates the behavior in males but not in females: Adkins-Regan, 1983; Balthazart *et al.*, 1983), we hypothesized that POM was the specific site of T action on behavior. Stereotaxic implantation of 27 g needles filled with crystalline T confirmed this. Implants located within POM activated copulation in castrated quail but implants positioned elsewhere in the POA, even a few hundred microns lateral to POM, were ineffective (Balthazart *et al.*, 1988). In addition, electrolytic lesions in the POA suppress copulatory behavior in quail only if they destroy a significant part of the POM. Lesions of similar size in other parts of the POA are ineffective (Balthazart and Surlemont, unpublished).

Microdissection of the preoptic area and hypothalamus using the Palkovits "punch" technique in combination with radioenzyme assays recently demonstrated that aromatase activity is specifically high in the POM compared to the rest of the POA (Schumacher and Balthazart, 1987). It can also be demonstrated that the changes in preoptic aromatase activity and in behavior are highly correlated in a wide range of physiological conditions which supports the idea that the activity of the enzyme actually determines the intensity of the behavioral effects of T. The aromatase activity in the POA is, like the sexual behavior, T-dependent. It is drastically reduced by castration and restored by a treatment with subcutaneous

BRAIN AROMATIZATION OF TESTOSTERONE REGULATES MALE REPRODUCTIVE BEHAVIOR IN BIRDS

Jacques Balthazart

Laboratory of General and Comparative Biochemistry (Bat. L1),
University of Liège, 17 place Dalcour, B-4020 Liège, Belgium.

Pharmacological and physiological studies have demonstrated beyond any doubt that aromatase is intimately implicated in the control of reproduction in both birds and mammals even when the role, if any, played by the estrogens produced in the brain remains a little vague (review by Balthazart, 1988). In particular, the activation by testosterone (T) of copulatory behavior in the male seems to depend on the presence of an active aromatase in the preoptic area-anterior hypothalamus. In addition, the sexual differentiation of reproductive behavior is also modulated by brain aromatase. These notions are particularly well illustrated by studies in birds in which the steroid dependence of reproductive activities is very prominent and the aromatase activity changes markedly as a function of the reproductive condition of the subjects. In this brief review, I should like to summarize the experimental data illustrating these principles using mainly examples selected from studies performed in my laboratory on the Japanese quail (*Coturnix coturnix japonica*) and the zebra finch (*Taeniopygia guttata castanotis*), two widely used models in the study of brain-steroid interactions with reference to the control of reproductive behavior.

ACTIVATION OF REPRODUCTIVE BEHAVIOR IN QUAIL

Evidence for the Implication of Aromatase

Aromatization of T into estradiol (E2) is absolutely required for the activation of copulatory behavior in quail. This is supported by the following facts: aromatizable androgens such as testosterone or androstenedione activate the behavior while non aromatizable androgen such as 5 α -dihydrotestosterone are not or less active; aromatase inhibitors such as androstatrienedione (ADT) inhibit testosterone-induced copulatory behavior in functionally or surgically castrated males; antiestrogens such as tamoxifen or nitromifene citrate (CI-628) block T-

silastic implants (Schumacher and Balthazart, 1986). Additional experiments recently performed using the Palkovits "punch" technique have shown that these regulations are also present within the POM; aromatase activity is approximately four times higher in sexually mature males or in castrated males treated with T than in castrates (Balthazart *et al.*, 1988).

Detailed parametric experiments have shown that the aromatase activity in the POA is a close correlate of the behavioral effects of T in this brain area. The induction of aromatase activity and of sexual behavior by T are both dose- and time-dependent (Balthazart *et al.*, 1986; Balthazart *et al.*, 1989b). The treatment of castrated males with silastic capsules filled with T produce a gradual increase in aromatase activity from 150 to about 500 fmol/mg protein/hour when the length of the implant is increased in the range 2.5 to 40 mm. In parallel, the percentage of birds showing copulatory behavior and the frequency of the sexual behaviors also increases. The minimal dose of T (10 mm implant) which reliably restores copulatory behavior in castrates approximately doubles the aromatase activity in the POA. A significant increase in aromatase activity is observed within 16 hours after the start of the treatment with T and the induction is maximal after 48 hours. Activation of copulatory behavior occurs in parallel but with a delay of 24-48 hours. These results suggest that aromatase activity in the POA is a limiting factor in the activation of copulatory behavior. This idea is confirmed by direct experimentation using an aromatase inhibitor, androstatrienedione (ATD). If T-treated birds receive at the same time silastic implants filled with ATD, the activation of behavior is delayed for at least one week. This behavior inhibition is, as expected, accompanied, and in all probability, caused by the inhibition of the aromatase activity in the preoptic area and anterior hypothalamus.

However, recent studies have questioned the specificity of action of ATD. It was shown that this compound inhibits the 5 α - or 5 β -reduction of testosterone (Alexandre and Balthazart, 1987) and also decreases the binding of androgens to their receptor (Kaplan and McGinnis, 1989). This might potentially explain the behavioral effects of ATD. A new triazole derivative, R76713 (Janssen, Beertse, B) was recently shown to selectively inhibit aromatase without affecting other steroid-metabolizing enzymes and without interacting with estrogen-, progesterin-, or androgen-receptors. This compound was tested for its capacity to interfere with the induction by T of copulatory behavior. In a first experiment, R76713 inhibited, in a dose-dependent manner (range 0.01 to 1 mg/kg), the activation by T silastic implants of sexual behavior and of the hypothalamic aromatase activity in castrated male quail. The 5 α - and 5 β -reductases were not systematically affected. Stereotaxic implantation of R76713 in the medial preoptic area similarly blocked the behavior activated by a systemic treatment with T demonstrating that the central aromatization of T is implicated in the activation of behavior.

AROMATIZATION IN THE ZEBRA FINCH BRAIN

In collaboration with the department of Biology of the University of Bielefeld (A. Vockel and E. Pröve), we have recently initiated a series of studies on the T metabolism in the brain of the zebra finch (*Taeniopygia guttata castanotis*) and this has revealed a number of very interesting differences with the situation described above in quail. In zebra finches as in quail, the activation of several reproductive behaviors appears to depend on the central aromatization of T (Harding *et al.*, 1983; Walters and Harding, 1988). Song production and song learning are controlled in this species by a discrete network of telencephalic and mesencephalic nuclei which are steroid-sensitive and sexually dimorphic. The sexual differentiation of singing behavior and of the brain nuclei controlling its production can be modified by exposure to exogenous steroids during the first days of life. Estrogens and aromatizable androgens masculinize these characteristics in females (Gurney, 1981; Gurney and Konishi, 1980). Although sex differences in the plasma levels of estrogens have been reported in young zebra finches (higher levels in males than in females: Hutchinson *et al.*, 1984; Adkins-Regan, 1988), these are limited in duration and do not completely cover the period during which exogenous estrogens are able to masculinize the brain and behavior of the birds (see Vockel *et al.*, 1989 for discussion). A role of the central aromatization of androgens in the process of sexual differentiation might thus be considered.

In a first study, we could demonstrate that an active aromatase is present in the hypothalamus of both adult and young zebra finches (Vockel *et al.*, 1988). The enzyme activity is clearly detectable at five days post hatch (earlier ages were not studied). The maximum velocity of the aromatase is actually two to three times higher in neonates than in adults. However, contrary to the situation observed in quail, the age-related changes in enzyme activity are accompanied by important modifications of the affinity of the enzyme (Km) for its substrate. The Km of the aromatase is three to four times greater in the young birds which means that the affinity of the enzyme is clearly lower in young than in adult birds. At a same concentration of substrate, more estrogen will thus be produced in the adult compared to the young brain. The change in enzyme affinity occurs around the age of 40 days and is thus in all probability one consequence of sexual maturation which occurs at that age. Whether the age-related differences in the Km result from a change in the type of enzyme which is present or from the synthesis/repression of a specific enzyme inhibitor has not been determined.

The neuroanatomical distribution of the aromatase was recently investigated in young (20 day-old) and sexually mature zebra finches using the Palkovits "punch" technique combined with ultra-sensitive radioenzyme assays. The presence of the enzyme was demonstrated in the preoptic area, in two hypothalamic nuclei (nucleus periventricularis magnocellularis [PVM] and nucleus medialis hypothalami posterioris [PMH]) and in all nuclei of the song system (area X [X], nucleus magnocellularis of the anterior neostriatum [MAN], hyperstriatum ventrale pars caudale [HVc] and nucleus robustus archistriatalis [RA]) with the exception of the nucleus intercollicularis [ICo] (Vockel *et al.*, 1989). Surprisingly the high-

est levels of enzyme activity were observed in the roof of the telencephalon (area parahippocampalis [APH]). Significant sex differences in enzyme activity were detected in HVc, RA, and APH with females showing higher activity than males. The same trend was present (although not significant) in all other brain nuclei (but not significantly so) enzyme activity. This situation is thus very different from that seen in quail in which males always have a higher aromatase compared to females. The sex differences in enzyme activity seen in adult zebra finches were not observed in the young birds.

We are currently investigating the hormonal controls of these sex differences in enzyme activity. Results available at present show that in some brain areas such as the POA or the hypothalamus, the zebra finch aromatase is decreased by castration and induced by T as observed in the Japanese quail. However, in other nuclei such as HVc, RA, and APH these hormonal treatments seem to have little effect on enzyme activity. Some of the sex differences are observed irrespective of the hormonal condition of the birds. This would suggest that these sex differences in aromatase activity are truly differentiated brain characteristics which presumably develop under the influence of neonatal steroids. Additional experiments should then be carried out to test this hypothesis. The physiological meaning of these differences is now difficult to assess because the behavioral role of steroid uptake by the different nuclei of the song system has never been analyzed. The study of the behavioral effects of the stereotaxic implantation of estrogens or androgens in these structures should now be carried out.

CONCLUSION

Important differences have been detected in the mechanisms which regulate the aromatase in quail and zebra finches. All changes in enzyme activity which have been described in quail do not implicate modification of the Km of the enzyme while the reverse has been shown to be true in zebra finches. T exerts a strong control on the enzyme activity in quail and in the POA of the zebra finch but does not seem to control the activity of the enzyme in some song control nuclei. In addition it must be stressed that, in quail, aromatase has only been detected in brain nuclei which contain estrogen receptors to which the estradiol produced locally could bind (Schumacher and Balthazart, 1987; Balthazart *et al.*, 1989a). This coexistence is by no means the rule in the zebra finch song system; aromatase is present and very active in HVc, RA, and MAN where the presence of estrogen receptors is questionable but it is undetectable in ICo where the receptors are very prominent (Gahr *et al.*, 1987; Walters *et al.*, 1988). In both species, it is nevertheless extremely clear that the presence of a functional aromatase in the brain is absolutely required for the full expression of the behavioral effects of T. The changes in enzyme activity also modulate these behavioral effects. Aromatase activity is controlled in a specific manner according to the species, the sex or the brain area considered. Its study contributes substantially to the understanding of

the mechanisms by which steroids act on the brain to activate and differentiate reproductive behavior.

ACKNOWLEDGEMENTS

Work from my laboratory presented in this chapter has been supported by grants from the NIH, Bethesda, MD (HD 22064), the Belgian FNRS (Crédits aux Chercheurs), the Medical School of Liège and the University of Liège (Fonds Spécial pour la Recherche).

REFERENCES

- Adkins EK, Boop JJ, Koutnik DL, Morris JB, Pniowski EE (1980). Further evidence that androgen aromatization is essential for the activation of copulation in male quail. *Physiol Behav* 24:441-446.
- Adkins-Regan E (1983). Sex steroids and the differentiation and activation of avian reproductive behaviour. In Balthazart J, Gilles R (eds): "Hormones and behaviour in higher vertebrates," Berlin: Springer-Verlag, pp 219-228.
- Adkins-Regan E (1988). Sexual differentiation in the zebra finch. Abstracts 20th Conf Reprod Behav, Omaha, NE, USA, p 11.
- Alexandre C, Balthazart J (1987). Inhibition of testosterone metabolism in the brain and cloacal gland of the quail by specific inhibitors and anti-hormones. *J Endocrinol* 112:189-195.
- Balthazart J (1988). Steroid metabolism and the activation of social behavior. In Balthazart J (ed): "Advances in Comparative and Environmental Physiology," vol 3, Berlin: Springer Verlag, pp 105-159.
- Balthazart J, Schumacher M, Ottinger MA (1983). Sexual differences in the Japanese quail: behavior, morphology, and intracellular metabolism of testosterone. *Gen Comp Endocrinol* 51:191-207.
- Balthazart J, Devos F, Dohet A, Foidart A, Hugla JL, Radermaker F, Schumacher M (1986). The induction of aromatase and sexual behavior by testosterone in male and female Japanese quail: a dose-response study. *IRCS Med Sci* 14:1188-1189.
- Balthazart J, Surlemont C, Foidart A, Schumacher M (1988). Induction by testosterone of the aromatase in the sexually dimorphic preoptic medial nucleus and activation of copulatory behavior in quail. *Soc Neurosci Abstr* 14:97.
- Balthazart J, Gahr M, Surlemont C (1989a). Distribution of estrogen receptors in the brain of the Japanese quail: an immunocytochemical study. *Brain Res* (In Press).
- Balthazart J, Foidart A, Hendrick JC (1989b). The induction by testosterone of aromatase activity in the preoptic area and activation of copulatory behavior (Submitted).
- Gahr M, Flugge G, Göttinger HR (1987). Immunocytochemical localization of estrogen binding neurons in the songbird brain. *Brain Res* 402:173-177.

- Gurney ME (1981). Hormonal control of cell form and number in the zebra finch song system. *J Neurosci* 1:658-673.
- Gurney ME, Konishi M (1980). Hormone-induced sexual differentiation of brain and behavior in zebra finches. *Science* 208:1380-1383.
- Harding CF, Sheridan K, Walters MJ (1983). Hormonal specificity and activation of sexual behavior in male zebra finches. *Horm Behav* 17:111-133.
- Hutchinson JB, Wingfield JC, Hutchinson RE (1984). Sex differences in plasma concentrations of steroids during the sensitive period for brain differentiation in the zebra finch. *J Endocrinol* 103:363-369.
- Kaplan ME, McGinnis MY (1989). Effects of ATD on male sexual behavior and androgen receptor binding: a reexamination of the aromatization hypothesis. *Horm Behav* 23:10-26.
- Panzica GC, Viglietti-Panzica C, Calcagni M, Anselmetti GC, Schumacher M, Balthazart J (1987). Sexual differentiation and hormonal control of the sexually dimorphic preoptic median nucleus in quail. *Brain Res* 416:59-68.
- Schumacher M, Balthazart J (1984). Sexual dimorphism of the hypothalamic metabolism of testosterone in the Japanese quail (*Coturnix coturnix japonica*). *Prog Brain Res* 61:53-61.
- Schumacher M, Balthazart J (1986). Testosterone-induced brain aromatase is sexually dimorphic. *Brain Res* 370:285-293.
- Schumacher M, Balthazart J (1987). Neuroanatomical distribution of testosterone-metabolizing enzymes in the Japanese quail. *Brain Res* 422:137-148.
- Viglietti-Panzica C, Panzica GC, Fiori MG, Calcagni M, Anselmetti GC, Balthazart J (1986). A sexually dimorphic nucleus in the quail preoptic area. *Neurosci Lett* 64:129-134.
- Vockel A, Pröve E, Balthazart J (1988). Changes in the activity of testosterone-metabolizing enzyme in the brain of male and female zebra finches during the post-hatching period. *Brain Res* 463:330-340.
- Vockel A, Pröve E, Balthazart J (1989). Sex- and age-related differences in the activity of testosterone-metabolizing enzymes in microdissected nuclei of the zebra finch brain. *Brain Res* (In Press).
- Walters MJ, Harding CF (1988). The effects of an aromatization inhibitor on the reproductive behavior of male zebra finches. *Horm Behav* 22:207-218.
- Walters MJ, McEwen BS, Harding CF (1988). Estrogen receptor levels in hypothalamic and vocal control nuclei in the male zebra finch. *Brain Res* 459:37-43.
- Watson JT, Adkins-Regan E (1989). Activation of sexual behavior by implantation of testosterone propionate and estradiol benzoate into the preoptic area of the male Japanese quail. *Horm Behav* (In Press).

INSULIN-LIKE GROWTH FACTOR RECEPTORS IN DEVELOPING CHICKEN TISSUE

Lluís Bassas¹ and Matías Girbau²

¹Fundación Puigvert and ²Servicio de Endocrinología, Hospital de la Santa Cruz y San Pablo, Apartado 24005, 08080 Barcelona, Spain

Insulin-like growth factors (IGFs) belong to a family of peptides showing structural and functional resemblances to the hormone insulin. Two different peptides, IGF-I and IGF-II, have been fully characterized (Humbel, 1984). They promote cell growth and differentiation, and seem to play important roles in the *in vivo* development of animal species (Zapf *et al.*, 1981). Their effects are exerted by paracrine/autocrine mechanisms, and local synthesis in the tissues correlates with mitotic rate. A number of cell types undergoing differentiation may be able to modulate their own production of IGFs (D'Ercole *et al.*, 1984).

The actions of IGFs are mediated through specific receptors which are present in many tissues. The IGF-I receptor shares structural and functional features with the insulin receptor, including an α -subunit that binds the hormone-like agent and a β -subunit that has a tyrosine-specific protein kinase (Ullrich *et al.*, 1986). The IGF-II receptor has a high affinity for IGF-II, a lower affinity for IGF-I, and typically does not bind insulin. The IGF-II receptor is totally different in structure from the IGF-I receptor and appears to resemble closely a mannose-6-phosphate receptor (Morgan *et al.*, 1987). The functional role of IGF-II receptor and its relation to mannose-6-phosphate recognition are unclear.

EXPRESSION OF IGF RECEPTORS

Expression of IGF receptors also seems to be regulated during *in vivo* development. We have studied the ontogeny, characteristics, and distribution of IGF receptors in the chicken throughout development. Binding assays to constant amounts of membranes from embryos at different stages showed that the density of IGF-I receptors had tissue specific patterns throughout development, characteristic for heart, lens, brain, and liver (Table 1).