INTRODUCTION: A REVIEW

It has been a long standing interest of a good number of investigators to explore the specific biological actions of sex steroids in homeotherms as well as in poikilotherms. Extensive investigations have been made to throw light on the importance of sex hormones in a large number of cellular processes (Jensen and DeSombre, 1972, 1973; Chester Jones et al., 1972; Williams-Ashman and Reddy, 1971, 1972; McCorquodale and Mueller, 1958; Gorski and Gannon, 1976; Katzenellenbogen, 1980; Liao et al., 1980; Feldman et al., 1981 Sherman, 1984; Hutson et al., 1985). The actions of these hormones have been studied mainly with two objectives: What the hormones do to the tissues and what the tissues do with the hormones. These two kinds of experimental approach have brought into light considerable information on the endocrine control and mechanisms during the last two decades.

The various effects of sex hormones comprise mainly metabolic, morphological, and behavioral changes (Green et al., 1970; Stack and Gorski, 1985; Gehring, 1987; Gorski and Jacobson, 1981). Their hormonal specificity, at least in part, lies in the fact that different tissues contain proteins that specifically bind only certain classes of physiologically active steroids. The tissue, thus, selects a particular class of steroid hormones with which it will interact by high affinity binding proteins or receptors (Katzenellenbogen, 1980; Chan and O'Malley, 1976; Gorski and Gannon, 1976; Katzenellenbogen and Gorski, 1975; Yamamoto and Alberts, 1976, Rosner et al., 1986, Evans, 1988; Rajendran et al., 1987, Carlstedt-Duke et al., 1988). The cellular sensitivity to hormones
may be affected by several factors - endocrine, metabolic, genetic, state of differentiation or cell cycle (Baxter and Funder, 1979). The steroid hormones can also regulate the levels of receptors for other classes of hormones (Lippman and Allegra, 1978; Leavitt et al., 1978). The collection of studies on steroid hormone - target tissue interactions has led to an increasing refinement of the concepts of target tissue and receptor. Prolonged hormone retention was considered as the initial hallmark of a target tissue (Glascock and Hoekstra, 1959; Jensen and Jacobson, 1962; Clark et al., 1980), and it still remains as an important characteristic of target tissues. Increasingly sensitive hormone-binding assays have revealed, however, that some tissues, previously considered as non-target tissues, do contain low but significant level of receptors, and that such tissues do respond to hormone under certain circumstances. Hence, there appears to be a spectrum in terms of receptors content and the responsiveness of different tissues to a given category of steroid hormones.

The interaction of steroid hormones with intracellular receptors in target cells has been best explained on the basis of a "two-step-mechanism" proposed independently by Jensen et al. (1968) and Gorski et al. (1968). According to this concept, target cells for steroid hormones contain specific high affinity receptors. Binding of the steroid causes the cytoplasmic receptors to be activated and translocated to the nucleus where it stimulates the transcription of responsive genes by interacting with regulatory DNA sequences. Thus, the interactions of steroid hormone-receptor complexes with their target cell nuclei are thought to be essential steps in the mechanism of action of these hormones (Clark et
The exact nature of these interactions may not be very clear, although binding sites for steroid hormones-receptor complexes are reported to be associated with the nuclear membrane (Jackson and Chalkley, 1974), ribonucleoprotein particles (Liang and Liao, 1974), nuclear matrix (Barrack and Coffey, 1980), histone and non-histone proteins (Puca et al., 1975; Kallos et al., 1981), DNA and chromatin (Yamamoto and Alberts, 1976; Mels Sluyser 1983, Gehring, 1987; Beato, 1989).

Earlier studies have shown that estrogens increase water, protein, nucleoproteins, glycogen (Brody and Westman, 1958; Notides and Gorski, 1966; Telfer and Hisaw, 1957; Leathen, 1958), β-glucoronidase (Leathen, 1958), succinoxidase (Telfer and Hisaw, 1957), isocitricdehydrogenase (Ville, 1968), lactic dehydrogenase-DPN oxidase system and DPN-cytochrome-C-reductase (Bever, 1958) in the uteri of mice, rat, hamster and guineapig. The modulation of specific protein synthesis by estrogen in the uterus has been observed (Skipper et al., 1980; Kuivanen and DeSombre, 1985, Wheeler et al., 1987). The concentration of contractile proteins in human myometrium is enhanced by estrogens (Cretius, 1947). The basal phospholipid is reduced but the apical phospholipid is increased by estrogens in the endometrium of castrated rats (Eltman, 1958). The growth-promoting effect of estrogens on the uterus has been shown (Velardo, 1958; Wicks and Seligal, 1956). The ontogeny of estrogen receptors during the early uterine development has also been observed (Clark and Gorski, 1970). During the estrogen-mediated tissue differentiation, protein biosynthesis is found on the chick oviduct polyribosomes (Means et al., 1971). The hyperplasia of the connective tissue in the seminal vesicles of
immature rats is induced by estradiol (Howard and Allen, 1957). Regarding the development of the urinogenital system and of its responsiveness to estrogen, detailed studies have been made, such as the development of the embryonic müllarian duct, the response of the organ to estrogen, the ontogeny of the estrogen receptor system, the composition and activity of chromatin, and on ovalbumin gene expression and tubular gland cell differentiation (Teng and Teng, 1975a, 1975b, 1980a, 1980b). A stimulatory influence of estrogen on the formation of ovalbumin in the tubular gland and development and function of hen oviduct has been found (Palmiter et al., 1970; Oka and Schimke, 1969a, 1969b).

Besides the usual target organs, such as reproductive organs, sex steroids have some effects on other organs. The sex steroids have marked influence on melanogenesis (Snell, 1964, 1967; McGill and Tucker, 1967; Kupperman, 1944; Mills and Spaziani, 1966; Hall, 1969; Wilson and Spaziani, 1969). Estradiol dipropionate stimulates RNA synthesis, but not to affect the formation of protein and enzyme activity in liver (Sergeev et al., 1971a) or to first inhibit and then stimulates protein synthesis by the liver microsomal fraction (Sergeev et al., 1971b). There are reports on the effects of estrogen on the synthesis of phosphoprotein in liver (Greengard et al., 1965), and the synthesis and accumulation of fat in the adipose cells (Gassner et al., 1958). A decrease of all normal serum components with a significant increase in the production of yolk protein in liver takes place in the laying hens or in both sexes following estrogen treatment (Schjeide, 1963). The development of estrogenic responses in embryonic chick liver has also been studied (Lazier, 1978, 1980; Lazier et al., 1982).
The liver thus appears to be an advantageous target tissue for relatively restricted and reversible nature of the responses evoked (Tata and Smith, 1979). As part of the process of vitellogenesis, estrogen stimulates the hepatic production of specific proteins and lipids, which are eventually incorporated into the developing oocyte. The proteins include vitellogenin, a large phospholipoglycoprotein precursor of yolk phosphovitin and lipovitellin (Carinci et al., 1974a; Tata and Smith, 1979), the apoproteins B and II of very low density lipoprotein (Williams, 1979; Chan et al., 1976), and vitamin and mineral binding proteins (Murthy and Adiga, 1978; Lee et al., 1978). In chicken embryo liver cultures, estradiol-17β decreases the synthesis of secreted proteins (Carinci et al., 1974b).

The induction of lipovitellin synthesis and elevation of RNA synthesis are effected by estrogen in amphibian liver (Wittliff and Kenney, 1972; Wittliff et al., 1972). Estrogen has also been found to induce the appearance of lipophosphoprotein in serum of male Xenopus laevis. The initial event leading to vitellogenesis in the liver must be triggered by an interaction between estradiol and a receptor. Developing oocytes of oviparous vertebrates sequester egg-yolk precursor proteins from the blood during the period of ovarian recrudescence. One such group of proteins, the vitellogenin gene family, is synthesized by the liver in response to circulating estrogen (Ho.S.M., 1987; Mommsen and Walsh, 1988). The informations regarding the nature of the receptor and vitellogenin gene expression in all types of oviparous animals, viz., bird, amphibia and fish, etc., were not adequate. One difficulty was that the putative receptor in chicken or frog liver is extremely labile unlike the estradiol receptor present in rat utorus or chick oviduct. The chick liver cytosol
receptor for estradiol has been discussed by Arias and Warren (1971). Others have suggested that the hormone interacts with the nucleus directly without first binding to a cytosol component (Mester and Baulieu, 1972; Lebeau et al., 1973). It has also been reported that estradiol binds directly to liver chromatin in rooster with a high affinity and its interaction with cytosol protein is weak (Gschwendt and Kittstein, 1974; Gschwendt, 1977).

Vitellogenin has been demonstrated in the plasma of a wide variety of female teleostean and non-teleostean fishes. (Wallace, 1985; Wallace and Selman, 1981; Wiegand, 1982; Campbell and Idler, 1976, 1980; Sumpter, 1985; Van Bohemen and Lambert, 1981; Idler et al., 1979; Campbell and Jalabert, 1979; Hara et al.; 1980; Nath and Sundararaj, 1981, de Vlaming et al., 1980; Terkatin-Shimony and Yaron, 1978; Plack et al., 1971; Hara et al., 1983; Selman and Wallace, 1983). Little was known about the fish vitellogenin genes or their transcripts except the studies on vitellogenin mRNA (Chen et al., 1982; Roach and Davies, 1980; Valotaire et al., 1984). It has been proved that estrogen is the inducer of vitellogenesis in fishes (Sundararaj et al., 1982; Scott and Sumpter, 1983). Vitellogenesis can be induced in male and non-vitellogenic female fishes by the administration of estradiol (Emmersen and Petersen, 1976; Idler and Campbell, 1980; Korsgaard et al., 1983; Wallace and Bergink, 1974; Sundararaj and Nath, 1981).

Apart from vitellogenesis, other hepatic changes associated with estrogenization of fishes include liver tissue hypertrophy (Aida et al., 1973; McBride and VanOverbeeke, 1971), augmented rate of protein
synthesis (Medda et al., 1980; Ng et al., 1984; Olivereau and Olivereau, 1979), an increase in protein synthesizing capacity (Emmersen and Korsgaard, 1982), changes in enzyme activities (Whiting and Wiggs, 1978, Sand et al., 1980), and an accumulation of hepatic RNA (Korsgaard, et al., 1983; Medda et al., 1980; Emmersen and Emmersen, 1976).

The gonadal steroids are responsible for development of the secondary sex characters in each of the major groups of fishes prior to breeding (Hoar, 1969; Liley, 1969). The cyclical and seasonal changes in gonadal steroidogenesis in fish are triggered by changes in the gonadotropic activity of pituitary. The levels of androgens and estrogens have been found to alter during the seasonal reproductive cycle in male and female fish (Hoar, 1969). Androgens and estrogens have anabolic effects on different fishes (Donaldson et al., 1979). The physiological role of gonadal steroids in fish has also been discussed with emphasis on gametogenesis (Fosteir et al., 1983). Both in teleosts and amphibians, gonadal steroids exert both morphogenetic and integrative actions on the germinal cells (Colombo et al., 1979). In these animals, steroids may be responsible for the resumption of meiotic maturation of the postvitellogenic oocytes (Colombo et al., 1979; Nagahama, 1987). Accumulating evidence suggests that oocyte maturation in these organisms is regulated by a series of interdependent hormonal actions (Wasserman and Smith, 1978; Masui and Clarke, 1979; Kanatani and Nagahama, 1980; Schuetz, 1979; Maller and Krebs, 1980). The source of maturational steroids in Hereto- pneustes fossilis is located in the interrenal gland (Sundarraraj and Goswami, 1977). In other species, the follicular cells appear to be the most likely producers (Hirose, 1972; Jalabart et al., 1973; Redshaw, 1972).
tissues and may also play a role in the estrogen-dependent processes in some organs related to reproductive functions in the female. Its antagonism to estrogen is known (Courrier, 1950), and it has been demonstrated that the same hormone can potentiate, amplify or sometimes mimic estrogenic activity (Mester and Baulieu, 1984). The tissues connected with female reproduction have intracellular proteins or receptors with high affinity for progesterone and related molecules. The specificity for binding the progesterone receptors can be correlated with the biological activity of the respective compounds, supporting the hypothesis that the receptors are involved in the mediation of hormone action (Raynaud et al., 1980). The progesterone receptor from the avian oviduct has been studied extensively. The monoclonal antibodies to the chick and rabbit progesterone receptors have been prepared for rapid purification and characterization of the progesterone receptors (Sullivan et al., 1985; Logeat et al., 1985). Estrogen treatment induces the synthesis of progesterone receptors in most of the female reproductive tissues (Milgrom et al., 1973; Wheeler and Lyttle, 1985). The amplitude of the response of the tissue of progesterone depends in turn on the preceding estrogen priming of the tissue with resulting synthesis and accumulation of progesterone receptor. In their target cells progesterone and estrogen thus display a complex pattern of actions on a number of biochemical parameters (Kalkhoff, 1982; Wahl et al., 1983; Calderon et al., 1987). The synergism and antagonism of estrogen and progesterone seem to be related to the regulation they exert on the concentration and subcellular distribution of their respective receptors (Ksueh et al., 1975; Bhakoo and Katzenellenbogen, 1977; Okulicz et al., 1981a, 1981b; Mester and Baulieu, 1984).
In most vertebrates, full grown post-vitellogenic oocytes in the ovary resume their first meiotic division involving breakdown of the germinal vesicle, under appropriate hormonal stimulation. In teleosts, a variety of $C_{21}$-steroids have been shown to be potent in this connection. The levels of plasma progestogens increase in conjunction with the preovulatory increase in gonadotropin, reaching peak levels at or after the period of oocyte maturation (Campbell et al., 1980; Scott et al., 1982, 1983; Young et al., 1983). It is also evident that there is a significant decrease in estradiol levels. The ovarian production of progestogens has been confirmed in several teleosts by in vitro incubation studies (Young et al., 1982; Young et al., 1983).

Regarding the sex steroid actions on different biochemical and physiological parameters in lower vertebrates, it has been reported from our laboratory that sex steroids have some effects on liver and muscle of fresh-water fish (Medda et al., 1980; Dasmahapatra 1980; Dasmahapatra and Medda, 1982). Moreover, it has been observed that extrogen action is temperature-specific (Dasmahapatra et al., 1981). This female sex hormone has no effect on the gonad (Medda et al., 1980; Dasmahapatra and Medda, 1982). The inhibition and potentiation of estrogen action by progesterone in fish have been found to be dependent upon the reproductive stage of the animal (Dasmahapatra et al., 1983; Dasmahapatra and Medda, 1986). But progesterone alone has no effect on the serum vitellogenin level (Dasmahapatra et al., 1983). In Heteropneustes fossilis Bloch, vitellogenin synthesis is induced by only estrogens, while testosterone, progesterone and cortiol fail to exhibit such action (Sunderaraj and Nath, 1981). The effects of estrogen, testosterone and progesterone on the hematological parameters and various cellular constituent of liver
and ovary of fish and toad have also been reported from our laboratory (Pal, 1985). Further, there are some reports from our laboratory on the influence of estrogen and testosterone on different cellular components of the liver of toad, *Bufo melanostictus*. The changes in melanin, protein, RNA, DNA glycogen and ascorbic acid contents, RNAase, DNAase, alkaline and acid phosphatase activity of liver, and protein and free aminoacids of blood plasma of toad have been observed after treatment with male and female sex steroids (Bhattacharya, 1978; Bhattacharjee and Medda, 1977a and 1977b; Medda et al., 1983a and 1983b; Dasmamaptra et al., 1984).

Besides the involvement of sex steroids in the function of the sex organs in case of animals and man as well as in the function of liver in case of oviparous vertebrates, the results of animal research clearly document an important role of the steroid environment in the development and differentiation of brain function and morphology. The fact that the brain undergoes sexual differentiation is of paramount importance to our understanding of the development of the reproductive system (Gorski and Jacobson, 1981). The brain, regardless of the genetic sex of the animal, has the potential to develop functional and morphological characteristics recognized as feminine in the adult. The development of masculine functional and morphological characteristics requires the exposure of the brain to effective levels of gonadal steroids particularly during a critical period of development (Gorski and Jacobson, 1981). Although neuronal genomic factors in the sexual differentiation of the brain cannot be completely ruled out, such genomic factors, if they exist, have been suggested to have lesser importance than the hormone environment (Gorski and Jacobson, 1981).
In higher vertebrates, studies on the effects of steroid hormones on the brain have progressed to a point where specific mechanisms and brain sites of action are recognized. Steroid hormones of the gonads and adrenals influence the brain and alter the effective state as well as overt behaviour and neuroendocrine function (McEwen and Parsons, 1982). The activity of the nerve cell can be altered by steroid hormones. The actions may be directly on the membrane as well as indirectly at the genomic level which are mediated by intracellular receptors (McEwen et al., 1978). The direct effects are of short latency and duration, and this can be shown by inhibition of cell firing within milliseconds by 17-estradiol when applied iontophoretically in the preoptic area and hypothalamus (Kelly et al., 1977). The indirect effects are of longer latency and duration; for example, activation of sexual receptivity in female rats by estradiol, which has 18-24 hour onset latency (Green et al., 1970; Parsons et al., 1980) and which outlasts the removal of the estrogen stimulus by 24-36 hours (Parsons et al., 1980), is blocked by inhibitors of protein and RNA synthesis (McEwen et al., 1979; Rainbow et al., 1980; Parsons et al., 1981). Moreover, estradiol treatment stimulates RNA polymerase II activity in the rat hypothalamus for several hours (Kelner et al., 1980).

Steroid effects, whether direct or indirect, primary or secondary, on neural tissues must be regarded also in terms of their relation to neuronal electrical activity and synaptic transmission, which constitute the common currency by which the mechanisms of brain function and behaviour are analyzed (McEwen and Parsons, 1982). Various aspects of nerve cell structure and function are influenced by steroid hormones. In
the adult, gonadal steroids influence the growth of neuronal processes during brain development and after brain damage. Both testosterone and estradiol stimulate outgrowth of neurites in the explants of hypothalamus and preoptic area from newborn mice (Toran - Allerand, 1976, 1978, 1980). Estrogen treatment prior to puberty increases the number of axodendritic synapses in the arcuate nucleus region of rat hypothalamus (Arai et al., 1978). Moreover, an increasing effect of estradiol on the formation of synapse in the arcuate nucleus is seen as a result of hypothalamic differentiation of adult rats. It has been found that new synapse occupy the sites vacated by degenerating affreants cut by the surgery (Arai et al., 1978). There is an instance of changes in neuronal size in hormone-sensitive cells of undamaged adult brain, although the adult brain is not believed to grow markedly. Excess steroids may also damage the adult brain irreversibly. Injection of 2 mg estradiol valerate into young cyclic female rats induces delayed and prolonged degenerative changes in the arcuate nucleus and leads to disrupted gonadal cyclicity (Brawer et al., 1978).

Neuronal electrical activity is altered by sex steroids. The firing rates of the neurons and their responses to electrical stimulation are different in gonadectomized rats receiving hormone-replacement therapy than in gonadectomized controls (Paff and Pfaffman, 1969; Kubo et al., 1975; Bueno and Pfaff, 1976; Kendrick and Drewett, 1979). Such effects are found in the preoptic area and hypothalamus and consists of increased as well as decreased basal firing rates, changes in the absolute refractory period, alterations in magnitude and even direction of the electrical response to sensory stimulus. Estradiol decreases firing in preoptic area
and hypothalamus of ovariectomized female rats but has no effect when applied to male rats. Testosterone increases unit activity in preoptic area and hypothalamus of intact male rats, in which estradiol is ineffective (Yamada and Nishida, 1978; Yamada, 1979). The involvement of sex steroids, particularly estrogen and progesterone, in the turnover of monoamines in certain brain areas has been reported (McEwen and Parsons, 1982).

A number of neural enzyme activities alter after gonadal steroid treatment. These effects are found in the regions of the brain containing intracellular steroid receptors (McEwen and Luine, 1978; McEwen et al., 1978). It should be noted that some of the reported effects represent an increase in enzyme activity while others consist of a decrease. The increase of one enzyme, cholineacetyltransferase has been analyzed by immunotitration and has been shown to involve an increase in the number of immunoreactive enzyme molecule (Luine et al., 1980). The estrogen-dependant decrease of type-A monoamine oxidase has been analyzed in terms of synthesis vs degradation and has been shown to be due to an increased rate of degradation rather than decreased rate of synthesis (Luine and McEwen, 1977). The alterations in enzyme activity may change as a function of the time after estrogen treatment. A single injection of estradiol benzoate into ovariectomized rats causes a transient increase in tyrosine hydroxylase activity in medial basal hypothalamus, whereas repeated injections of estradiol benzoate for one week lead to decreased medical basal hypothalamus tyrosine hydroxylase (Luine et al., 1977).
It is not so surprising that steroid hormones also influence the neurotransmitter receptor systems of the brain. Estrogen treatment of ovariectomized rats increases the level of putative B-adrenergic, muscarinic, cholinergic and serotonergic (5HT₁) receptors in hypothalamus, but not in other brain regions, which lack estrogen receptors (McEwen and Parsons, 1982). In cases, where the effects have been localized within hypothalamus, they appear to occur in known estrogen-concentrating cell grouping: ventromedial, anterior hypothalamic, medial preoptic and bed nucleus for muscarinic receptors (Rainbow et al., 1980; McEwen et al., 1981), and arcuate nucleus, lateral preoptic area near bed nucleus of striaterminalis and ventrolateral septum for 5HT₁ receptors (Biegon et al., 1981; Fischette et al., 1981). Various direct effects of estradiol or a metabolite, 2-hydroxyestradiol, occur on putative neurotransmitter receptor systems. The estrogens are competitive inhibitors of binding of H³⁻ligands to ¹ adrenergic and dopaminergic receptors but are without effect on ² adrennergic binding. Estradiol and 2-hydroxyestradiol are also able to affect in vitro binding to 5HT₁ receptors. However, these effects reduce the number of available receptors, rather than altering apparent affinity via competitive effects (McEwen and Parsons, 1982). Another receptor phenomenon, in which gonadal steroids are involved is the imipramine induced decrease in 5HT₂ receptors in cerebral cortex. This decrease does not occur in ovariectomized rats and it is reinstated by replacement therapy with their estradiol or progesterone (Finidori-Lepicard et al., 1981).
Estrogens influence the dopaminergic systems of the corpus striatum and median eminence and the dopamine response system of the pituitary, and there are many mechanisms proposed to account for these effects (Weiner and Ganong, 1978; Hope and Woolley, 1980). The concentration of DA in hypophyseal portal blood is increased by estrogen treatment (Gudelsky et al., 1981). This may be the result of increased prolactin secretion, since estrogen treatment increases circulatory prolactin and prolactin stimulates DA release into hypophyseal portal blood (Gudelsky and Porter, 1980; Cramer et al., 1979) and from isolated hypothalamic nerve endings (Foreman and Porter, 1981). Estrogen treatment has also been reported to be facilitative, e.g., estrogen replacement elevates DA-dependent adenylate cyclase activity (Kumakura et al., 1979), it potentiates stereotyped behavior elicited by dopamine-agonists (Chiodo et al., 1981; Hruska and Silbergeld, 1979; Nausieda et al., 1979; Koller et al., 1980), and it prolongs the duration of ipsilateral rotation elicited by amphetamine in rats with unilateral 6-hydroxydopamine lesion of nigrostriatal projections (Hruska et al., 1980; Hruska and Silbergeld, 1979). On the other hand, estrogen treatment can also apparently antagonize dopaminergic function, e.g. estrogen treatment potentiates catalepsy resulting from spiperon administration (Chiodo et al., 1979). It reduces the increase in striatal acetylcholine levels elicited by dopamine agonists (Euvrard et al., 1979). Estrogen reduces the amount of apomorphin-induced rotation in unilaterally-lesioned rats (Euvrard et al., 1980; Bedard et al., 1978) and also attenuates phenylethylamine and amphitamine-induced stereotypy in mice (Naik et al., 1978). The reduction of the magnitude of apomorphine-elicited stereotyped behaviour by estrogen occurs in female rat chronically pre-treated with haloperidol.
Estradiol and monoamines interact in many ways in the control of anterior pituitary hormones secretions, reproductive behaviour and onset of puberty. The anatomical basis for the interaction between estradiol and catecholamines has been provided by the observations that neurons containing estradiol receptors in hypothalamus, amygdala, septum and brain stem also contain catecholamine neurotransmitters and are closely surrounded by catecholnergic neuron terminals (Thompson and Woolley, 1983). It is also reported that if individual neurotransmitters are able to modulate steroid receptors binding, the effectiveness of steroids at a given time in a given brain area would be regulated by the type and frequency of synaptic input (Nock and feder, 1981). The acute dopaminergic stimulation should intensify at least the short term effects of estradiol and that the major mechanism for that effect is via increased estradiol receptor levels (Thompson and Woolley, 1983; Grant and Stumpf, 1975; Sar and Stumpf, 1981; Geitzen et al., 1981).

A major advantage of using steroid hormones as tools for probing brain function is the opportunity they afford for localizing sites of hormone action within the brain (McEwen and Parsons, 1982; Rosner et al., 1972; Pfaff and McEwen, 1983; McEwen et al., 1979). Estradiol action on feminine sexual behaviour involves an important contribution from the ventrolateral portion of the ventromedial nuclei of the hypothalamus (Barfield and Chen, 1977; Davis et al., 1979; Rubin and
Barfield, 1980; McGinnis et al., 1985). In *Xenopus laevis* and other vertebrates, there are specific brain areas that appear to mediate the behavioral action of gonadal steroids, such as the preoptic area, medial hypothalamus, limbic structures, and an area in the mesencephalon (Kelley and Pfaff, 1978; Morrell and Pfaff, 1980). The gonadal steroid hormones act at all stations of the neural pathway controlling the behaviour. The centrolateral portion of the ventromedial nuclei of the hypothalamus is implicated in progesterone action in estrogen-primed rats. Estradiol action on preovulatory LH-release appears to involve an important contribution of neural targets within the preoptic area (Goodman, 1978).

By studying relevant brain regions and using minimally sufficient hormonal stimuli to evoke neurochemical changes in those regions, it remains to be seen which parameters of synaptic function are most critical for the hormonal control of behaviour and neuroendocrine function. There are indications on the estradiol-regulated changes in several parameters even in one discrete brain region, such as the ventromedial nucleus, e.g., progestin receptors (McEwen and Parsons, 1982), muscarinic receptors (Rainbow et al., 1980). Type-A monoamine oxidase, and glutamic acid decarboxylase (Wallis and Luttrell, 1980). The direct actions of estradiol and progesterone contributing to the regulation of feminine sexual behaviour and ovulation has been suggested for direct estrogen effects on 5HT₁ receptor levels, based on observation of decreased 5HT₁ binding at the time of elevated estradiol secretion (Biegon et al., 1980). At the same time, it appears that indirect, genomically-mediated actions of estradiol and progesterone are primarily responsible for the
appearance of sexual behaviour and ovulation at precise times during the estrous cycle (McEwen et al., 1981).

Thus, there are many indications of the kinds of effects which steroid hormones have on brain at the cellular and biochemical levels. The steroid effects range from alterations in electrical activity of brain regions which contain appropriate hormone receptors to effects on amino-acid incorporation and nucleic acid synthesis and the induction of a number of enzymes; and they include a variety of effects on both the biosynthesis, inactivation and reuptake of transmitter candidates (Lieberburg and McEwen, 1979). Estrogen has also marked effects on the cellular and biochemical events in pituitary. Briefly, the effects of estrogen on hypothalamus include neuronal electrical activity (Bueno and Pfaff, 1976), neuronal thresholds for stimulation of ovulation (Kubo et al., 1975), release and/or synthesis of LHRH (Araki et al., 1975; Kalra, 1976), turnover of neurotransmitter (Cardinali and Gomez, 1977), induction of enzymes (McEwen and Luine, 1978), aminoacid incorporation (Litteria and Thorner, 1974), and RNA synthesis (Peck, 1976). The effects of estrogen on pituitary include DNA polymerase activity (Mastro and Hymer, 1973), levels of RNA (Robinson and Leavitt, 1971), decrease in FSH synthesis (Miller et al., 1977), increase in pre-prolactin and sensitivity to LHRH (Stone et al., 1977; Vilchez-Martinez et al., 1974; Tang and Spies, 1975; Drovin et al., 1976), and sensitivity to TRH (DeLean et al., 1977).

A large body of evidence indicates that estrogens act on the central nervous system, specially the hypothalamus in regulating pituitary
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function (Kato, 1977). Specific uptake and accumulation of estrogens in the brain tissues have been demonstrated after administration of radioactive estradiol and hexestrol to rat (Harris, 1959; Michael, 1962; Attramedal, 1964; Trunnel, 1957). It has been reported that a preferential uptake of radioactive estradiol occurs in the hypothalamus, preoptic region, brain stem and cerebrum, but not in the cerebellum of rat (Eisenfeld and Axelrod, 1966). Following injection of a physiologic dose of 3H-estradiol into adult ovariectomized rats, it has been found that the anterior hypothalamus, the median eminence and the anterior hypophysis are labeled. So there are specific uptake and retention of radioactivity similar to those shown by the uterus and vagina of rats, namely, high uptake and retention of non-metabolized estradiol (Jensen and Jacobson, 1962; Kato and Villee 1967a). Moreover, little or no transformation of 3H-estradiol is observed in the hypothalamus and the anterior hypophysis. On the other hand, 3H-estradiol is intensively metabolized into estrone and other metabolites in the liver and blood (Kato and Villee, 1967a).

The selective uptake and retention of estradiol by the anterior hypothalamus, median eminence and hypophysis is postulated to suggest a means by which the hormone could initiate the sequence of reactions of its feedback control of the central nervous system. Additional characteristics of the uptake of estradiol by brain have also been studied. (Attramedal, 1964; Eisenfeld and Axelrod, 1965, 1966; Kato and Villee, 1968, 1967a, 1967b; Kato et al., 1968; Kato, 1971).

These evidences suggest the presence of a specific estradiol-binding receptor of limited capacity and with stereospecificity for 17\(\beta\)-estradiol in the hypothalamus and the anterior pituitary, and therefore,
a hypothetical concept of its role in the mechanism of feedback action of estrogen was proposed. After the intravenous injection of $^3$H-17$\beta$-estradiol, radioactive estrogen is found to be incorporated in all subcellular fractions of rat hypothalamus and hypophysis, but more is found in the nuclear and cytoplasmic fractions (Kato, 1977). In hypothalamus, both cytoplasmic and nuclear receptors for estrogen have been demonstrated (Kato, 1977). Estrogen is an important factor in the maturation process of the brain-pituitary unit in developing female animals and in the regulation of the onset of puberty (Everett, 1961; Greep, 1961; Donovan and Van der Werfften Bosch, 1959; Critchlow and Bar-Sela, 1967; Ramirez and Sowyer, 1965; Smith and Davidson, 1968). The negative feedback action of estrogens seems to be lacking in the immature female rats at early stages of development (Kato, 1977).

Estradiol receptors are localized mostly in the preoptic-anterior hypothalamus, the median eminence and the anterior hypophysis (Kato, 1977). Three possible sites of the feedback action of estrogen are present in the central nervous system: the preoptic-anterior hypothalamus, median eminence and anterior hypophysis. The estrogen receptors in the preoptic-anterior hypothalamus may play a major role in cyclic regulation of reproductive function in rats. Interaction of estrogen with the receptors in the median eminence and the anterior hypophysis also may be the basis of feedback action. In the molecular mechanism of the feedback action of estrogen on the brain, the hormone may interact with the cytoplasmic and nuclear receptors at two steps. This interaction with the receptors may be an initial step to initiate subsequent processes, leading to changes in reproductive function, i.e., the secretion of gonadotropin-releasing factors and pituitary gonadotropin.
Receptors for all classes of steroid hormones: estrogen, progesterin, androgen, glucocorticoid and mineralocorticoids have been identified biochemically in the brain (McEwen et al., 1982). These receptors resemble those found in the non-neuronal target tissues. The steroid hormones cross the plasma membrane of target cells and bind to high affinity intracellular receptors. The hormone-receptor complex acquires a strong affinity for the cell nucleus where it controls the expressions of a limited number of specific genes, which are responsible for the physiological response of the cell to the hormone. Thus, the interaction of steroid hormone-receptor complexes with their target cell nuclei are thought to be essential steps in the mechanism of action of these hormones (O'Malley and Birnbaumer, 1978). The exact nature of these interactions may not be very clear, although binding sites for steroid-hormone-receptor complexes are reported to be associated with the nuclear membrane (Jackson and Chalkley, 1974), ribonucleoprotein particles (Liang and Liao, 1974), nuclear matrix (Barrack and Coffey, 1980), histone and non-histone proteins (Puca, et al., 1975; Kallos et al., 1981), DNA and chromatin (Yamamoto and Alberts, 1976). Characterization of the estradiol receptors have been done in hamster brain (Paul and Callard, 1985). There is a regional sex differences in progesterin receptor induction in the rat hypothalamus at various doses of estradiol benzoate was observed (Brown et al., 1987). The synergism between progesterone and estrogen for the maintenance of the receptive behaviour apparently results from estradiol stimulating the formation of progesterone receptors in the hypothalamus preoptic area of female frog (Roy et al., 1986).
The topography of steroid hormone-receptors in the brain and some of their functional implications have been reviewed (Warembourg, 1985). Concentration of radio-activity in neuron nuclei is found in widely distributed areas of the overiectomized mouse brain 1 hour after administration of $^{3}$H-estradiol (Warembourg, 1985). A similar distribution of estrogen-concentrating neurons is also found in the brain of guineapig, with only some differences in the intensity and extent of the labeling within regions being recorded. With minor exceptions, the neuroanatomical pattern of estradiol-binding cells in the squirrel-monkey brain closely resembles that observed in rodents. A very low index of labeled cells is seen in the septum and the hippocampus of the primate brain. In the amygdala, only the nucleus medialis shows positivity. The estrogen-target neurons are clustered in the same general periventricular regions of the phylogenetically old brain of the mammalian species examined (Warembourg, 1985). The progestagen-retaining cells are located in the regions of the brain corresponding with the sites of estrogen target neurons, but, compared to estrogen, their distribution in forebrain is more restricted. Similarly, in the hen, the preoptic area, medial basal hypothalamus, and the anterior lobe of pituitary show a greater concentration of $^{3}$H-estradiol-17β in vivo than in other tissues (Kawashima et al., 1987). The regulation of the progesterone receptors by estradiol in the hypothalamus and pituitary has been reported (Gasc and Baulieu, 1988). It appears that estradiol levels required for progesterone-receptor-induction deplete LH content in gonadotrophs, thus rendering colocalization difficult to demonstrate, although most progesterone receptors containing cells are actually LH producing cells (Gasc and Baulieu, 1988). The autoradiographic
localization of progestin concentrating cells was observed in the brain of Zebra Finch (Lubischer and Arnold, 1990). More recently the estrogen receptors were observed during fetal development in the Rhesus monkey brain (Sholl and Kim, 1989).

In fishes, brain mechanisms underlying reproductive behaviour and neuroendocrine processes are not clearly understood. Basic neuroendocrinological mechanisms regulate reproduction in nearly all groups of vertebrates that have been studied. The sensory systems and specific brain areas serve to integrate external and internal events to bring about changes in the reproductive system. In the brain specific nuclei in the limbic system that concentrate steroid hormones, receive input from these sensory areas and in turn project to the hypothalamus where both internal and external cues are integrated. The hypothalamus secretes gonadotropin-releasing hormone, that influences release of gonadotropin from the anterior pituitary. Gonadotropins in turn stimulate gonadal maturation and steroid hormone production. Steroid hormones feed back on the hypothalamus and the pituitary to modulate subsequent function of these organs (Whittier and Crews, 1987). Gonadal steroids are known to participate in the regulation of some aspects of reproduction in fishes (Hoar, 1969; Liley, 1969). A number of studies have examined long term seasonal changes in the neuroendocrine system in fishes (Billard et al., 1978; Peter, 1981). Brain areas that have been implicated in the control of seasonal reproduction in fishes include the nucleus lateralis tuberis. Lesions in this area induce regression of the gonad in early phases of gonadal growth. However, in mature female, lesions stimulate ovulation (Peter and Crim, 1979).
It has been demonstrated that the estrogen and androgen levels in gonad and other organs vary with the reproductive seasons (Hoar, 1969; Schreck and Hopwood, 1974). Sex steroids influence the development of external morphological secondary sex characteristics which occur at puberty and, in some species, prior to breeding seasons (Yamamoto, 1969; Liley, 1969). Androgen-dependent male positive secondary sex characteristics predominate in fishes, but estrogen-dependent female positive characteristics also occur. Investigations of the brain mechanisms of reproductive behaviour in fishes are increasing. Autoradiographic localization of sex steroid concentrating cells in the brain of the teleosts, Macropodus opercularis, has been demonstrated (Davis et al., 1977). Tritiated estradiol or testosterone was administered to gonadectomized male paradise fish, Macropodus opercularis, to investigate the neuroanatomical locations of sex-steroids retaining cells. Steroid-concentrating cells have been located in the ventral telencephalon, preoptic area, lateral tuberal nucleus, nucleus of the lateral recess of third ventricle and caudal portion of the posterior periventricular nucleus. In addition, the caudal pars distalis of the pituitary contains many labeled cells. No steroid retaining cells are seen in the mesencephalon, rhombencephalon or anterior spinal cord (Davis et al., 1977).

The tritiated 17ß-estradiol uptake by the brain and other tissues of the cichlid Jewel fish, Hemichromis bimaculatus, was observed (Myers and Avila, 1980). In platyfish, Xiphophorus sp., autoradiographic study for topographic distribution of estrogen target cells in forebrain has been made (Kim et al., 1979). The steroid binding sites in the brain have also been investigated by autoradiography in green sunfish, Lepomis
cyanellus (Morrell et al., 1975), platyfish and goldfish (Kim et al., 1978, 1979). In these species, labeled estradiol and testosterone are bound in a similar distribution in the brain to perikarya in the nucleus lateral tuberis, nucleus recessus lateralis, ventral nucleus preopticus, ventral nucleus preopticus periventricularis, the area ventralis pars ventralis in the telencephalon, and in the pituitary. In goldfish, labeled estradiol is also bound in the nucleus posterioris periventricularis, in the posterior hypothalamus and in the thalamic periventricular region dorsal to the nucleus posterioris periventricularis (Peter, 1983). Regarding the gonadotropin in the pituitary, immunofluorescent location of teleost gonadotropin was studied in flounder pituitary (Burton et al., 1981). The LH RH stimulated gonadotropin release from the rainbow trout pituitary gland was also discussed (Crim and Evans, 1980). In the brain of goldfish, Carassius auratus, immunocytochemical localization of LH Rh was observed (Kah et al., 1984; Goos and Murathanoglu, 1977). Even there were also found the muscarinic cholinergic components in the carp brain (Szabo et al., 1989).

There is good evidence, primarily from studies in rodents, that formation of estrogen from circulating androgen in the brain itself is obligatory for certain neuroendocrine and behavioral responses (Naftolin et al., 1975; McEwen et al., 1979). Aromatase, the enzyme complex, which regulates this transformation is not restricted to the mammalian brain but can be detected in the central nervous system of all major vertebrate groups from fishes onwards (Callard et al., 1978). Since androgens and estrogens also share common actions in a wide variety of non-mammals (Young, 1961), it seems likely that central aromatization
has an important role in regulating reproduction. In the earlier studies, high levels of aromatase activity were identified in sculpin forebrain and relatively little estrogen is synthesized by the inferior lobes of the hypothalamus, thalamus, optic lobes, basal midbrain, cerebellum and medulla. The marked sex and seasonal differences in brain regions in which aromatase is concentrated further suggest that estrogen synthesis in the central nervous system is somehow related to reproduction. The gonadal aromatization appears negligible in comparison to that of brain (Callard et al., 1981). It has been reported that the changes in brain aromatase and 5-reductase activity correlate significantly with seasonal reproductive cycles in goldfish, Carassius auratus (Pasmanik and Callard, 1988).

In Macroopus (Davis et al., 1977), Xiphophorus sp. (Kim et al., 1979) and Carassius sp. (Kim et al., 1978), following $^3$H-estradiol injections clusters of labeled neurons have been located by autoradiography in the ventromedial telencephalon, preoptic area, tuberal nuclei of the anterior hypothalamus and in more caudal regions of the hypothalamus adjacent to the ventricular system. The preoptic area and telencephalon of teleosts have been implicated in the control of sexual behaviour, nest building, spawning and sperm release (Demske et al., 1975; Macey, et al., 1974; Davis et al., 1976; Schwagmeyer et al., 1977). It has been found that in lower vertebrates, in which the brain retains the potential for growth throughout life (Bernstein, 1970), estrogens may have prolonged growth promoting actions that are not restricted to the reproductive brain. Finally, it is possible that estrogen produced in certain brain regions is not utilized locally (Callard et al., 1981). All these
These cells may transduce a systemic gonadotrophic stimulus into a local steroid signals accepted by the oocytes for the reinitiation of the arrested meiosis. In teleosts, a variety of \( C_{21} \) - steroids have been shown to be potent initiators of the breakdown of the germinals vesicle in vitro.

In the catfish, *Heteropneustes fossilis*, the terminal hormones that act on the oocytes to induce maturation appear to be corticosteroids (Sundararaj et al., 1979). Temporal changes in blood concentrations of steroid hormones during the final oocyte maturation and ovulation period have been observed in fishes (Goetz, 1983; Postei et al., 1983; Nagahama et al., 1985). It has been reported that the mechanism of action of steroids on the meiotic maturation may not involve a modulation of gene transcription as in somatic steroid target tissues (O'Malley et al., 1976). The steroid-induced maturation in teleosts is blocked by puromycin or cycloheramide but not by actionomycin-D or mitomycin-C, indicating that DNA-dependent RNA synthesis is not necessary although protein synthesis of obligatory (Sundararaj and Goswami, 1977). Certain steroid hormones also have been reported to block hormone-induced oocyte maturation in vitro. In rainbow trout, estradiol prevents maturational process to some extent (French, 1976). However, the meiotic response may be triggered by a variety of non-estrogenic steroids (Jalabert et al., 1973, Goswami and Sundararaj, 1974; Morril and Bloch, 1977).

Progesterone is an important factor in the control of reproductive functions in lower vertebrates, such as amphibians and birds. Progesterone exerts its effect on the differentiation and function of some
observations show that the fish brain also is a source of estrogen formation, has estrogen concentrating cells or areas and also respond to estrogen with respect to reproductive behaviour and prolonged growth.

**OBJECT OF THE THESIS**

The foregoing discussions amply reflects the physiological role of sex steroids, particularly of estrogen and progesterone, in the activity of brain of mammals as well as fish, leading to their regulation of the reproductive behaviour. However, some reports indicate that progesterone is ineffective in altering the brain activity in fish. Seasonal variations are always associated with the changes in the levels of circulating gonadotropins and estrogens in fish (Peter and Crim, 1979; Lamba et al., 1993; Dasmahapatra and Medda, 1980; Ghosh et al., 1989), which are likely to alter brain activity. In Singi fish, *Heteropneustes fossilis* Bloch, seasonal changes in cellular constituents in liver, muscle and ovary have been reported (Dasmahapatra, 1980; Dasmahapatra and Medda, 1982, 1989). But to our knowledge no comprehensive investigations on the effect of estrogen and progesterone and their interactions with and without tamoxifen, in relation to breeding and non-breeding seasons, on various metabolic parameters of brain of fresh water tropical fish, *Heteropneustes fossilis* Bloch, were made, before we undertook the present study in eighties. Moreover, cellular changes in the brain of Singi fish at different seasons were also not studied before we stepped into the present investigations. Also, we thought that the influence of estrogen and progesterone on different cellular components of brain of Singi fish deserved thorough investigations in order to provide more supportive data for better understanding of the physiological involvement of these two
hormones in the metabolism in fish brain. In view of these facts, the thesis embraces the following aspects of investigations.

PART - I

Morphometric and histological studies on the effect of estrogen and/or progesterone on the brain of Singi fish, *Heteropneustes fossilis* Bloch.

I. Changes in the cranio-somatic index and gonadosomatic index after administration of $17\beta$-estradiol and progesterone in male and female Singi fish in non-breeding and breeding seasons.

II. Effect of estrogen and progesterone on the weight of different substructures, viz., cerebrum, cerebellum, midbrain and medulla oblongata of brain of male and female Singi fish at different breeding seasons.

III. Histological study on the different parts of brain of male and female Singi fish in normal as well as after administration of $17\beta$-estradiol.

PART - II

Biochemical studies on the variations of cellular constituents of the central nervous system of male and female Singi fish in normal as well as after estrogen and progesterone treatments.

I. Seasonal variations of protein, RNA and DNA contents of different substructures (cerebrum, cerebellum, midbrain, medulla oblongata and spinal cord) of the central nervous system of male and female Singi fish.
II. Effect of 17β-estradiol on protein, RNA and DNA contents of cerebrum, 
cerebellum, midbrain, medulla oblongata and spinal cord of male 
and female Singh fish in non-breeding and breeding seasons.

III. Effect of progesterone on protein, RNA and DNA contents of cerebrum, 
cerebellum, midbrain, medulla oblongata and spinal cord of male 
and female Singh fish of non-breeding and breeding seasons.

IV. Effect of 17β-estradiol with and without tamoxifen on protein, RNA 
and DNA contents of whole brain of male and female Singh fish in 
non-breeding and breeding seasons.

V. Effect of 17β-estradiol with and without progesterone and tamoxifen 
on cholesterol and total lipid contents of whole brain of female 
Singh fish.

VI. Effet of 17β-estradiol with and without progesterone and tamoxifen 
on some ion-dependent (Na⁺K⁺ and Mg²⁺) ATPase activities in 
different fractions of whole brain of male and female Singh fish in 
non-breeding and breeding seasons.

VII. Effect of 17β-estradiol and progesterone on adenylate cyclase 
activity and cAMP production in brain of female Singh fish.
PART I  Morphometric and histological studies on the effect of estrogen and/or progesterone on the brain of Singi fish *Heteropneustes fossilis* Bloch.
Morphometric and histological studies on the effect of estradiol and or progesterone on the brain of Singi fish, *Heteropneustes fossilis* Bloch.

A good number of reports have been presented in the general Introduction (Review) to highlight the significant involvement of sex steroids in brain metabolism, development and functions in different animals. Considerable concentrations of sex steroids like estrogens, progesterone, and androgens occur in brain tissue as evidenced from the studies using labelled sex steroids (Davis et al., 1977; Morrel et al., 1975; Andersen and Greenwald, 1969; Pfaff and Keiner, 1973). Certain areas of brain in fish have been implicated in the control of seasonal reproduction (Hoar, 1969; Macey et al., 1974; Schreck and Hopwood, 1974; Liley, 1969; Demski et al., 1975; Peter, 1981). Although extensive investigations have been made on the effect of sex steroids on the brain of different animals including fish, it appears to us that the brain mechanism underlying reproductive behaviour, neuroendocrine processes are not clearly understood in different tropical fishes. Further, the temperature sensitivity or suitability of optimum temperature for optimum metabolic functions and reproductive behaviour is not identical in all tropical fishes (Vasal and Sundararaj, 1976; deVlaming, 1972; Peter and Crim, 1979 Dasmahapatra, 1980). Moreover, the behavioral differences between male and female are clear indications of the structural and functional differences at least in certain brain areas in all vertebrates including fish.

Thus the control of brain functions and the resultant overall physiological activities are obviously governed by the endogenous hormones
and naturally the sex steroids have a vital role in this processes. If the behavioral, structural and functional differences of the brain between male and female are resulted from the hormonal influences, it is likely that the different areas of the brain or at least the concerned areas will respond to the administration of exogenous sex steroids differently in male and female. To our knowledge, no studies have been made on the effects of estrogen and progesterone on the changes of morphometric and histological parameters of different substructures of brain in tropical fish, such as *Heteropneustes fossilis* Bloch. Moreover, no elucidation of the differences in the responsiveness of brain structures to estrogen and progesterone in male and female Singi fish has been made. Several examples of sexual differences of brain or sexual dimorphism in higher vertebrates are known (Gorski and Gordon, 1978; MacLusky and Naftolin, 1981; Brown et al., 1988; Rasmussen et al., 1990). But these were mostly unknown to us in case of tropical fishes before we ventured to undertake the present investigations.

In view of these facts, we first attempted to focus the changes in cranio-somatic index (CSI), gonado-somatic index (GSI), weight of different substructures (cerebrum, cerebellum, midbrain and medulla oblongata) of brain and histological differences in these different brain parts in male and female Singi fish, after administration of exogenous estrogen and progesterone. In some cases, we used tamoxifen, an estrogen inhibitor, to provide supportive evidence on the effect of estrogen on the morphometric and histological parameters of brain of male and female Singi fish.