INTRODUCTION

Endocrine secretions in vertebrates represent highly potent and specialized organic molecules that serve as regulators and coordinators of biological functions. They exert their influence over the animal either directly or indirectly. Newly developed methods have provided detailed insight into the chemical nature of hormones and their receptors; their sites of biosynthesis, activation and degradation, and their mechanisms of interaction at the molecular level. These advances have changed our understanding of the endocrine system. The classical concepts are not worth examining since they have been widely disseminated.

The thyroid is unique among vertebrate endocrine glands in that it stores its secretory products (thyroid hormones) extracellularly. Two separate hormones are synthesized by thyroid cells from the amino acid tyrosine: 1. triiodothyronine (T_3) and 2. tetraiodothyronine or thyroxine (T_4). These hormones contain iodide ions (I^-) bound to the phenolic rings of the tyrosine.

Thyroid hormones influence reproduction, growth, differentiation and metabolism. These actions often occur cooperatively with other hormones, and the thyroid hormones enhance the effectiveness of the other hormones. This cooperative role of the thyroid hormones is referred to as a 'Permissive action' whereby thyroid hormones produce changes in target tissues that

allow these tissues to be more responsive to another hormone, to neural stimulation or possibly to certain environmental stimuli such as light. The major role for thyroid hormones in adult organisms may be to maintain the state of well being in many types of tissues so that maximum sensitivity to other regulating agents is retained. Thyroid hormones are also essential for normal development. A great variety of factors may influence thyroid state in mammals and thus influence many processes controlled by other hormones as well. The endocrine factors that affect thyroid functions are hypothalamic and hypophysial hormones.

Thyroid hormones affect many diverse tissues and influence major processes such as metabolism, differentiation and reproduction. The effects produced by thyroid hormones on mammalian metabolism include a so-called calorigenic or thermogenic action and specific effects related to carbohydrate, lipid and protein metabolism. Many of these metabolic actions are possibly permissive actions occurring in co-operation with other hormones such as epinephrine and growth hormone. They are known to accelerate the rate at which the glucose is oxidized and thus increase the amount of other metabolites. They are also known to uncouple oxidative phosphorylation, which decreases the efficiency of ATP synthesis in the mitochondria. This type of uncoupling has been observed in a hyperthyroid pathological state known as Thyroid Storm. They also induce increased synthesis of mitochondrial respiratory proteins, especially cytochrome c, cytochrome oxidase and succinoxidase. In addition to increasing glucose oxidation, thyroid hormones cause hyperglycemia and may secondarily stimulate lipid oxidation (oxidative hydrolysis of fats or oxidative lipolysis). These actions may in part be associated with potentiation of the hyperglycemic and lipolytic actions of epinephrine. Thyroid hormones alter nitrogen balance and are either protein anabolic or catabolic, depending on the tissue being examined and under what experimental conditions it is examined. These actions are probably related to enhancement of effects normally produced by other hormones.

Thyroid hormones are essential for normal growth and differentiation in mammals as evidenced in cretinism and juvenile myxedema. These growth promoting actions of thyroid hormones are closely related to the role of pituitary growth hormone [GH] (Martin, 1985), and they probably represent a permissive action on GH sensitive target cells. Thyroid hormones may also stimulate somatomedin production and hence augment the action of GH. The major tissue affected by the lack of thyroid hormones during differentiation is the nervous system. Normal development of the nervous system as well as attainment of normal mental capacities are strongly influenced by thyroid hormones. Hypothyroidism (HPOT), during early development, seriously impairs differentiation and functioning of the nervous system. Replacement of hair in adult mammals is stimulated by thyroid hormones (Martin, 1985). This is correlated with increased T_4 and return of cortisol to basal levels. Thyroid hormone activity is also related to moulting of hair in mammals.

The hypothalamo-hypophysial-gonad axis is the principal neuroendocrine axis controlling reproductive functions in mammals. The pituitary gonadotropic hormones form the classical hormonal principles involved in the control of gonadal functions. But many recent investigations have provided enough evidences for the involvement of non-classical hormones like insulin (Grizard *et al.*, 1991), gonadotropin releasing hormone [GnRH] (Limonta *et al.*, 1988), growth hormone (Bartlett *et al.*, 1990), corticosteroids (Kalimi *et al..*, 1983), thyroid hormones (Palmero *et al.*, 1988; Hardy *et al.*, 1993), and pineal hormones (Lang *et al.*, 1984; Reiter *et al.*, 1985), emanating from other endocrine glands in modulating reproductive functions. Of these, the three non-classical endocrine glands of importance are: **1**. the thyroid, **2**. the pineal and **3**. the adrenals. However, markedly varied effects have been observed regarding the modulatory influences of the non-classical hormones of reproduction on development, structure and functions of testes in mammals.

There are some reports that show the effect of thyroidectomy (Tx) on specific enzymes involved in glycolysis and the HMP shunt in testis of prepubertal, pubertal and adult rats showing that hexokinase (HK) and phosphofructokinase (PFK) did not show any significant change in activity after Tx. However, pyruvate kinase (PK) activity was reduced after Tx in the testis of prepubertal animals although in pubertal and adult rats it was not affected. Both G-6-PDH and 6-PGDH activities were reduced after Tx and, T_4 replacement brought both enzyme activities back towards normal (Aruldhas *et al.*, 1982). On the other hand, thyroxine induced hyperthyroidism (HPRT) showed that there is no change in either hexokinase or 6-phosphofructokinase activity in the testis and PK activity was reduced significantly. However, G-6-PDH and 6-PGDH activities were markedly increased. This shows that T_4 has an age dependent, specific effect over testicular PK activity (Aruldhas *et al.*, 1982).

Hyperthyroidism increased the specific activity of isocitrate dehydrogenase but, the specific activities of ATP-citrate lyase, malate dehydrogenase and malic enzyme were inhibited. Withdrawal of T_4 treatment, from HPRT rats, brought back all enzyme activities to normal. This shows a direct, specific influence of T_4 on different testicular enzymes of the pyruvate/malate cycle (Aruldhas *et al.*, 1984). Aruldhas *et al.*. (1986) also reported that T_4 treatment decreases testicular total lipids, total glyceride glycerols, total cholesterol and total phospholipids, while in HPOT, there is accumulation of testicular total lipids, cholesterol, glyceride glycerols and phospholipids. Tx rats replaced with T_4, reverted all lipid classes to normal (Aruldhas *et al.*, 1986). It is well established that optimal thyroid hormone stimulation is essential to sustain normal growth and maturation of the testis (Longcope, 1986; Fugassa *et al.*, 1987, Palmero *et al.*, 1989, 1992, 1993; Ruiz *et al.*, 1989; Francavilla *et al.*, 1991), and spermatogenesis (Amin and El-Sheikh, 1977; Chowdhury and Arora, 1984; Matsushima *et al.*, 1986). Prepubertal onset of HPOT may cause delayed or precocious puberty and atrophy in human and experimental animals (Gilberg and Walfish, 1983; Longcope, 1986; Boyages *et al.*, 1989).

Although a number of existing studies on HPOT men (Cavaliere *et al.*, 1988; Kumar *et al.*, 1990; Samuels *et al.*, 1990), rats (Bruni *et al.*, 1975; Aruldhas *et al.*, 1982a; Valle *et al.*, 1985; Ruiz *et al.*, 1989; Hardy *et al.*, 1993; Biswas *et al.*, 1994), and rams (Chandrasekhar *et al.*, 1985) point towards decreased serum testosterone levels, a few reports suggest a normal pattern of testosterone (Kalland *et al.*, 1978; Weiss and Burns, 1988). Increased metabolic clearance rate of testosterone due to diminished concentration of sex hormone-binding globulin (Cavaliere *et al.*, 1988; Leger *et al.*, 1990) or reduced gonadotropin titer/action (Bruni *et al.*, 1975; Valle *et al.*, 1985; Aruldhas *et al.*, 1986 a; Ruiz *et al.*, 1989) has been attributed to low androgen titer in HPOT. In general, Leydig cell activity in rats and rams suggested subnormal testosterone production by the testis (Chandrasekhar *et al.*, 1985; Valle *et al.*, 1985; Ando *et al.*, 1990). Leydig, sertoli and germ cell hyperplasia with reduced steroidogenic potential has also been reported in adult rats subjected to transient neonatal HPOT (Cooke and Meisami, 1991; Cooke *et al.*, 1991; Hardy *et al.*, 1993; Hess *et al.*, 1993). There are some reports which suggest that HPOT in female rats results in the disruption or elimination of estrous or menstrual cycles (Roges, 1958; Bray and Jacobs, 1974), with the resultant decreased fertility (Hagin, 1971; Pepper *et al.*, 1975). In male rats, although fertility is not characteristically diminished (Smelser, 1939; Jones *et al.*, 1946; Karkin and Mukherjee, 1952; Maqsood, 1952), reproductive system organ weights are affected (Baksi, 1973; Vilche-Martinez, 1973 Bruni *et al.*, 1975). There are other reports showing an increase in testis size and the number of germ and sertoli cells after PTU treatment. This is as a result of changes in endocrine or paracrine factors (Kirby *et al.*, 1992), and there is a direct effect of thyroid hormone on testis as specific receptors are present in sertoli cell nuclei, only during the critical window of thyroid hormone effectiveness on testis (Jannini *et al.*, 1990).

Experimental studies also support the notion that thyroid hormones influence gonadotropin release through an effect at the level of the hypothalamus. In spite of reports on modifications in the hypothalamo-hypophysial-testis axis, under hypo and hyperthyroid

conditions, no consensus could be arrived at regarding the exact role or action of thyroid hormones in modulating testicular functions.

Till 1973, the pineal was referred to as a rudimentary gland whose function in the adult is not fully known (Truex and Carpenter, 1973). Investigations of Kitay (1967) and Turner and Bagnara (1976) had contributed to the present knowledge about the pineal gland to a great extent. The pineal is a small but prominent secretary organ which synthesizes and secretes a number of exocrine and endocrine substances. Its secretary function is well established in birds and mammals (Takahashi *et al.*, 1980; Sugden, 1989; Reiter *et al.*, 1990, 1993; Binkley, 1993). The secretions are indoles, peptides, various enzymes, amino acids and their derivatives, lipids, carbohydrates and inorganic constituents. Among the indoles, serotonin is the primary hormone secreted by the pinealocytes. Most of it is converted to melatonin (MLT), tryptohols and other derivatives. These hormones are generally known to provide signals concerning photoperiod to the animal for synchronization with the atmospheric changes. It is also seen that light can exert pineal-independent influences and that reproductive functions are affected by nutritional status, non-photic sensory stimuli, social interactions and other factors.

Influence of pineal on reproduction is well documented in various vertebrate species (Reiter, 1981, 1982; Underwood, 1981; Blask *et al.*, 1982; Reiter *et al.*, 1988; Pevet, 1988; Patel and Ramachandran, 1988; Joy and Agha, 1989, 1991; Lewinski, 1993; Cavallo, 1993; Weaver *et al.*, 1993). Reiter (1987) has documented a relationship between pineal and reproduction in a number of mammalian species. It is shown that in adult males experiencing short photo period, the testes regress, spermatogenesis ceases, testosterone and prolactin (PRL) levels fall and the accessory reproductive organs undergo atrophy. Pituitary gonadotropin content and hypothalamic LRH also decline in short light period. Similarly, in female hamsters, estrous cycles are arrested and circulating PRL levels decline, but FSH and LH levels do not (Reiter, 1980). It is well known that MLT is the primary mediator of the responses to short photoperiods (Reiter, 1980). When

intact animals receiving long photoperiod are injected with MLT every afternoon for several weeks, the reproductive organs involute. The hormone is reported to exert inhibitory influences on the hypothalamus, the pituitary gland, the gonads and other target organs. In the white-footed mouse, similar responses to photoperiod were demonstrated (Glass and Lynch, 1983). In hamsters, the responses to exogenous MLT are markedly affected by the conditions under which the hormone is administered. Daily afternoon injections of effective dosage which promote gonadal regression in hamsters, fail to promote gonadal regression with morning injections. Melatonin serves as an anti-gonadotropic agent in various mammalian species (Reiter *et al.*, 1976, 1979; Johnston *et al.*, 1980; Health and Lynch, 1981; Blask *et al.*, 1982; Reiter, 1982; Richardson, 1982; Vaughan *et al.*, 1983). Implants that continuously release MLT are said to be counter anti-gonadotropic or pro-gonadotropic, since they block the effects of short photo periods in intact animals and of afternoon MLT injections in animals exposed to long days (Reiter *et al.*, 1974, 1975, 1978). Small amounts of exogenous testosterone blocks FSH and LH secretions in castrated hamsters exposed to short days, but relatively large doses are required when the days are long. Castrated animals pretreated with MLT become as sensitive as uninjected controls that are light-deprived (Sisk and Turek, 1982). It is also shown that some of the dark-involved changes in gonadotropin secretion that can be reversed by Px are steroid independent (Ellis *et al.*, 1981), and they may be linked with other mediators.

In rats it is reported that the duration of oestrous cycle is affected by exposure to long photoperiod during the neonatal period (Hoffman, 1973). Pinealectomy attenuates and reverses the effects of food and sensory deprivation; and it can accelerate pubertal maturation in neonatally healthy rats. Pinealectomy is also known to accelerate PRL secretion in some mammals (Clarke, 1980). Rats also show pineal gland MLT rhythms like hamsters and undergo diurnal variations in response to the hormone (Reiter, 1981). Plasma MLT concentrations fall to undetectable levels after Px. Exogenous MLT acts at several sites to decrease the functions of the reproductive system. In Syrian hamsters, MLT has been implicated in causing reversal of the

effect of Px (Turek, 1977; Carter *et al.*, 1982; Grosse *et al.*, 1993). It has also been suggested that the pineal may also modulate reproductive physiology in humans. Subcutaneous injections of MLT do not substantially affect the gonadotropin levels, but they bring about reduction in ovarian and uterine weights and also retard testicular and accessory reproductive organ development (Ariens, 1980). There are other reports which show that the adult male rat is insensitive to MLT under normal laboratory conditions, and chronic treatment with MLT in the afternoon inhibits development of testes and accessory organs in immature rats and also delays sexual maturation when given between 20 to 40 days of age (Lang *et al.*, 1984). Melatonin administration in pre-pubertal rats reduced testes and ovarian weights, more pronounced with evening rather than morning treatment (Patel and Ramachandran, 1992). Similarly, the effects of para-chlorophenylalanine (pCPA), a specific depletor of 5-hydroxy tryptamine (5-HT), showed decreased body weight and increased pineal, testes, adrenal and spleen weights. It is suggested that the pCPA induced gonadal enlargement is mainly due to the reduced MLT synthesis caused by the reduced availability of 5-HT and that the action of 5-HT on the hypothalamo- pituitary-adrenal axis is more dominant than that of MLT (Patel and Ramachandran, 1993).

The daily profile of MLT synthesis and its release is a species specific pattern in mammals. Reiter (1993) has shown that, in the domestic rat (*Rattus norvegicus*) and the human, MLT synthesis begins along with the onset of darkness. The information about the photoperiod is converted as an internal signal represented by the levels of MLT (Binkly, 1986). In addition, the pineal and other elements of the circadian system interact with each other through MLT as the chemical messenger (Underwood, 1979). Induction of gonadal regression by prolonged elevation of MLT, in animals kept under short days (long nights), is known as duration hypothesis of MLT action. Pineal gland transduces photoperiodic information into a usable hormonal signal and regulates the timings of reproductive activity in seasonal breeders. Melatonin protects animals from overheating and has a crucial role in circadian and seasonal thermoregulatory adjustments. It induces sleep and hypo-metabolism (Haim and Zisapal, 1992; Saarela and Reiter,

1994). It also modulates the effects of other hormones that are indirectly linked to thermoregulation. Melatonin has also been linked to aging specially because it is a nontoxic and highly endogenous radical scavenger. It provides protection against neuro-degeneration and against mutagenic and carcinogenic actions of hydroxyl radicals (Reiter, 1992, 1993; Hardeland *et al.*, 1993; Poeggeler *et al.*, 1993). The rate of aging and time of onset of age related diseases in rodents have been shown to be retarded by MLT administration (Grand and Rozencwaig, 1993; Poeggeler *et al.*, 1993). On the other hand, peptides, pterdines and tryptohols are among the products shown to possess anti-gonadotropic potencies, and it seems reasonable to believe that these molecules have physiological roles.

The effects of functional manipulations of either thyroid or pineal individually have not been uniform, and even more confusing and ill defined are the interactions between these endocrine glands and their role on reproductive functions or even the possible existence of an axis/axes linking these non- classical endocrine *vis-à-vis* gonadal structure and functions. The pineal gland is known to modulate the functions of several other endocrine glands. Apart from the gonads, thyroid, adrenal, and hypothalamo-hypophysial axis have also been shown to be linked to pineal. There are various reports on the interactions between pineal and thyroid (Johnson, 1981; Virend, 1983), where they have reviewed the disparate results on pineal-thyroid-interactions and have concluded that there are as yet unknown mechanisms or pathways which form a pineal-neuroendocrine-thyroid-gonad axis. Some reports show that in mammals, pineal exerts inhibitory influence on neuro-endocrine-thyroid axis (Virend *et al.*, 1979; Vaughan *et al.*, 1982; Virend, 1983, 1984). Other reports show that both MLT injections and Px affect thyroid gland directly, to influence TSH secretion via TRH-independent mechanisms, and to modulate the functions of neurons involved in regulating TRH release. In humans, a positive correlation between MLT concentration and TSH levels in HPOT and negative correlation of MLT with T_3 in HPRT provide indirect evidence for pineal-thyroid inter-relationship (Soszynski *et al.*, 1988). The physiological implications are not clear, since the pineal makes several other

regulators and its activity changes in responses to various environmental signals in different animals. The hamsters treated with moderate amounts of MLT display parallel inhibition of reproductive system and TRH functions. It is also shown that the pineal gland may function as both a regulator and a target organ, since it receives projections from the magno-cellular nuclei. It also affects steroid metabolism and the secretion of GH (Goldstein, 1984). Involvement of pineal in the regulation of intermediary metabolism in various species of vertebrates has also been suggested. The influence of pineal on metabolites may vary depending upon the season and day length (de Vlaming *et al.*, 1974; de Lahumty *et al.*, 1978). Melatonin administration decreases the blood glucose levels in rats and monkeys (Burns, 1972, 1973). It can be suggested from various reports that the pineal is involved as a part of the photoperiodic mechanism in regulating a variety of physiological events and that some of the actions of the pineal are independent of its extensively described actions on the reproductive axis (Vitale *et al.*, 1985). Variable effects of pineal on adrenal activity in several mammalian species have been documented (Motta *et al.*, 1971; Vaughan *et al.*, 1972; Ogle and Kitay, 1976; Nir, 1978; Volrath, 1981; Jimnez *et al.*, 1993). The pineal has an inhibitory influence on the adrenal gland in rats and mice (Weidenfeld *et al.*, 1993).

The disparate and often contradictory reports on the functional ability of these two glands and their mechanism of action in combination with each other have provided the necessary impetus for the present work which has been designed to involve experimental manipulations of both pineal and thyroid. The experimental manipulation was restricted to the preweanling period and the effects of these glands on growth and functions of male reproductive system in rats have been evaluated at prepubertal, pubertal and adult stages.