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Steroid Hormones and Antihormones can Reverse the Castration Induced Stimulation of the Pineal and Adrenal Karyomorphology and Cell Proliferation in Mice (*Mus musculus*)

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ABSTRACT

In the present investigation, influence of castration and castrated animals supplemented with steroid hormones and antihormones on pineal-adrenal karyomorphology and dynamics were studied in post pubertal male mice. A group of thirty five mice were orchidectomized and (N = 7) sham operated, were kept in laboratory condition for 30 days. Such castrated were separately supplemented with estradiol at a dose of 5 μg, testosterone at a dose of 100 μg and antihormones, tamoxifen at a dose of 500 μg and flutamide at 2 μg daily (all at doses per 100 g.b.w.) for ten consecutive days following thirty days of post castration. Present data reveal that both pineal and adrenal gland nuclear size and cell proliferation were significantly increased in thirty days post orchidectomized mice compared to control animals. The values are control pinealocyte nuclear diameter (dim): 4.75±0.06; castrated pinealocyte nuclear diameter (μm): 5.34±0.04 ($p<0.001$). Control pineal M% 1.25±0.07; castrated pineal M% 2.02±0.11 ($p<0.001$). In control adrenal, representative of zones was Z. fasciculata nuclear diameter (μm) (5.11±0.04); castrated Z. fasciculata nuclear diameter (μm) 5.41±0.03 ($p<0.001$). Control adrenal M% (1.03±0.06) castrated adrenal M% (1.63±0.09) $p<0.001$. It was further observed that such pineal and adrenal stimulation in orchidectomized mice were significantly decreased when orchidectomized mice were administered with steroid hormones (estradiol and testosterone) and antihormones (tamoxifen and flutamide) compared to orchidectomized mice. Our study indicates that there exists a mutual stimulatory relationship between pineal and adrenal under conditions of steroid deprivation. However, exogenous administration of steroid hormones and antihormones to those castrated mice caused inhibition of these two peripheral endocrine glands.

Key words: Pineal, adrenal, karyomorphology, mitotic incidence, castration, steroid hormone, antihormones

INTRODUCTION

Researchers have indicated the occurrences of sex steroid receptors in pineal (Cardinali *et al*., 1975; Luboshitzky *et al*., 1997; Bhatnagar *et al*., 2002; Itoh *et al*., 2006) and in adrenal (Calandra *et al*., 1980; Lüthy and Calendra, 1984; Hirst *et al*., 1992; Hedberg *et al*., 2009). Consequently, it appears that the mammalian pineal (Cardinali *et al*., 1987; Alonso-Sollis *et al*., 1996; Ishizuka *et al*., 2000; Bandyopadhyay *et al*., 2010) shows the ability to be influenced by altered concentration of steroid hormones and additionally with antihormones

(Bandyopadhyay *et al*., 2010). Modulatory roles of steroid hormones on adrenocortical function has also been indicated (Kitay, 1963; Nussdorfer, 1986; Bandyopadhyay *et al*., 2010).

It is also known that castration causes immediate increase in serum gonadotropin (Moguilevsky *et al*., 1979; Peek and Watkins, 1980; Balasinor *et al*., 1992) and such gonadotropin level shows a decrease following the administration of gonadal steroids (Eldrige *et al*., 1974; Moguilevsky *et al*., 1979; Peek and Watkins, 1980). Presence of gonadotropin receptors in pineal (Bhatnagar *et al*., 2002; Itoh *et al*., 2006) and in adrenal (Pabon *et al*., 1996; Rao *et al*., 2004; Rao, 2010) shows responsiveness to altered level of gonadotropin induced by castration. There are reports which indicates that gonadotropins have been effective in influencing adrenal status although any such evidence in pineal remain to be investigated (Weiss and Carson, 1987; Kerr and Weiss, 1991; Ray and Maiti, 2004; Gay *et al*., 2008).

An extensive study of pineal and adrenal responsiveness to steroid hormones and antihormones has recently been reported (Bandyopadhyay *et al*., 2010). In view of such, it needs to be mentioned that a complete understanding of pineal and adrenal responsiveness towards steroid modulation is incomplete without an in-depth study of pineal and adrenal relationship following steroid deprivation and increased endogenous gonadotropin through castration. Examination of such a profile of pineal adrenal relationship needs additional study following exogenous supplement of steroid hormones and antihormones in any animal species for a complete understanding of the nature of pineal-adrenal gland towards steroid modulation. It appears that the effect of gonadectomy on pineal gland are conflicting, as some investigators failed to observe any alterations, while others found atrophy or claimed hypertrophy in the pineal gland after gonadectomy (Ito and Matsushima, 1968).

Gonadectomy in male rats reportedly lowers the activity of melatonin forming enzyme, hydroxyindole-O-methyl transferase (HIOMT) in the pineal gland (Reiter, 1977) accompanied by a significant rise in serotonin content of the pineal gland (Reiter, 1981). However, other investigation in mammals following castration failed to show any change in the adenylate cyclase activity, required for melatonin synthesis by the pineal gland (Weiss and Crayton, 1970).

On the reverse, results ascribe that orchidectomy has a stimulatory effect on the morphology of the pineal gland. Removal of both the testes in rats induces hyperactivity of the pineal gland cell structure (Das Gupta, 1968; Gusek, 1976; Reiter, 1977, 1978; Kus *et al*., 2000). Further, studies reveal that the stimulatory effect of orchidectomy could be reversed by therapeutic administration of testosterone (Das Gupta, 1968). However, biochemical evidences indicate that castration induces an increase in the acid phosphatase activity within the pineal gland which can not be suppressed by androgen replacement therapy (Vaughan *et al*., 1994).

All earlier reports in male animals are in agreement that orchidectomy is followed by adrenal hypertrophy. The investigators explained that the adrenocortical hypertrophy due to castration in male mice is because of enhanced corticosteroid yield, indicating adrenocortical activation (Nandi *et al*., 1967).

Additionally, the effect of castration on adrenal cortex, suggests that gonadectomy influences adrenal 5 α-reductase activity (Kitay *et al*., 1971; Colby and Kitay, 1972). In male or female gonadectomized rats, the 5 α-reductase activity increases, enhancing the intra-adrenal conversion of corticosterone to the 5 α-dihydro-corticosterone and 3β, 5 α-tetrahydrocorticosterone, thereby increasing the total steroid content of the adrenal cortex. Thus, orchidectomy resulted in adrenal hypertrophy (Colby and Kitay, 1972). However, administration of testosterone or estradiol to castrated rats restores the activity of 5α-reductase and secretion of corticosterone to control levels. Recent findings also reveal that gonadectomy increase the adrenocortical enzyme

5α-reductase activity, regardless of the sex, whereas testosterone replacement in male rats and estradiol replacement in female rats reduced the 5α-reductase activity (Yokoi *et al*., 1998). These findings are corroborated from a recent study which shows that orchidectomy accounts for a profound hypertrophy of the adrenal cortex of Saharan gerbil (Nawel *et al*., 2009). Other findings, on the other hand, indicate that gonadectomy of male or female rats results in a decline in the secretion of corticosterone by the adrenal glands, an effect which is reversed by replacement with appropriate gonadal hormones, namely testosterone or estradiol (Kitay *et al*., 1966).

Besides steroid hormones used to study, endocrinological aspects, a number of non steroidal hormones that antagonize the action of a particular hormone have been developed. Studies have revealed that non steroidal antihormones like tamoxifen (an antiestrogen) and flutamide (an antiandrogen) have the capability to bind with sex steroid receptors present in the tissue. Tamoxifen acts as a pure estrogen antagonist at a low dose to full estrogen agonist at higher dose (Rastogi and Chieffi, 1975). Unlike in rats, tamoxifen administered to mice acts as a full estrogen agonist (Furr *et al*., 1979) and also causes atrophy of steroid sensitive glands (Harper and Walpole, 1967).

In view of such, it was predicated that a study of castration induced changes in pineal and adrenal would provide new evidence for such a relationship following *in vivo* modulation of gonadotrophin and steroidal hormonal milieu with respect to castration. It has been seen that these steroids and non steroidal antihormones have the capability to bind with sex steroid receptors of the target organ and alter their activity. It was also apprehended that it would be interesting to further investigate, the effect of changes induced by administration of steroid hormone and antihormones on any such responsiveness of the pineal and adrenal to castrated mice. Undoubtedly this will effectively provide first time document of their influence on cell morphology, activity and proliferation nature of pineal and adrenal cortex in castrated male postpubertal mice.

MATERIALS AND METHODS

The relationship between pineal and adrenal cortex was evaluated following alteration in the internal steroidal milieu, namely through castration and with or without steroid and antihormone supplementation in postpubertal male mice. Following acclimatization to the laboratory conditions for three days. The post pubertal male mice (Charles Foster Strain) weighing between 15-18 g were used for the current study. A total of thirty-five postpubertal male mice were used for the experiment. The year of experimentation was June-July, 2008 and the materials were processed later, during August, 2009. The manuscript was prepared in May, 2010.

Sham operated control: Laparotomy was performed on postpubertal male mice (N = 7). The mice were anaesthesized using ketamine anaesthesia (Ketajet, Sterfil Laboratories, India) at a dose of 5 mg/100 g body weight. A small incision of 1 cm was made in the lower abdomen and the incision was sutured without removing the testis. The mice were then kept under the warmth of lamp till they regained consciousness. This group was kept in normal laboratory condition for the next thirty days.

Castration: Postpubertal male mice (N = 38) were castrated under ketamine anaesthesia (Ketamine hydrochloride, Ketajet, Sterfil Laboratories, India) after acclimatization to laboratory condition at a dose of 5 mg/100 g body weight. First the left testis was externalized through a 1 cm lower abdominal incision and the blood vessels and vas deferens were ligated with a silk thread. The testis was excised and the epididymis alongwith the blood vessels and the vas deferens were

returned to the abdominal cavity. Similarly, the right testis was also excised following the above mentioned technique. Finally, the incision was sutured and the mice were kept under the warmth of the lamp till they regained consciousness. The mice (N = 35) were housed for thirty days in normal laboratory conditions.

Castration followed by estradiol treatment: Postpubertal male mice (N = 7), castrated and kept for thirty days post surgery, were administered daily with estradiol valerate (Progynon Depot, Schering AG, Germany) diluted with peanut oil vehicle daily at a dose of 5 μg/100 g body weight (Chakraborty *et al.*, 1981) for further ten consecutive days.

Castration followed by tamoxifen treatment: After thirty days of castration, the postpubertal male mice (N = 7) were treated with tamoxifen (Tamoxifen cirtrate, Lyka Labs Ltd., India) in peanut oil vehicle daily at a dosage of 500 μg/100 g body weight (Rastogi and Chieffi, 1975) for further ten consecutive days.

Castration followed by testosterone treatment: Thirty days post-castration, the postpubertal male (N = 7) were administered with testosterone enanthate (Testosterone Depot, Schering AG, Germany) diluted with peanut oil vehicle, daily at a dose of 100 μg 100 g^{-1} body weight (Chakraborty *et al.*, 1981) during the entire.

Castration followed by flutamide treatment: Flutide (Flutide, Samarth Pharmaceuticals, India) in peanut oil vehicle was injected daily to postpubertal male mice (N = 7) that were castrated and kept for thirty days. The dose of flutamide was 2 mg/100 g body weight (Gromoll *et al.*, 1993) for ten consecutive days.

All the injections were given intramuscularly in alternate thigh muscle daily for ten consecutive days. The mice were housed in photoperiodic chambers fitted with fluorescent light and exhaust fan. The daily photoperiod 12L: 12D lights on at 6 h and off at 18 h were controlled by timer switches (Surrey, U.K.). The animals were supplied with mice pellets and water *ad libitum*.

The experimental animals were maintained and used as per guidelines of Institutional Animal Ethics Committee, University of Calcutta acredited by the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment of Forest, Govt. of India.

On the day of autopsy, i.e., on the eleventh day of experiment, after thirty days post orchidectomy, the animals were injected with colchicine (0.1 mg/100 g body weight) to obtain metaphase arrested mitotic cells. The animals were killed by an overdose of ether six hours after colchicine treatment (Quay and Levine, 1957; Sahu and Chakraborty, 1986; Bandyopadhyay and Chakraborty, 2010; Bandyopadhyay *et al.*, 2010) approximately twenty four hours after the administration of last dose of the drug. Both the pineal and adrenal glands were excised out, fixed in Bouin's fixative and routine processes required for histological study were performed.

Extensive studies have shown that in pineal and adrenal an active phase is characterized by increased nuclear size indicating synthesis activity whereas inhibition of pineal and adrenal is characterized by decreased nuclear size. In all cases pineal (Quay, 1976; Chakraborty, 1981, 1993, 1994; Sahu and Chakraborty, 1986; Sinha *et al.*, 2009; Bandyopadhyay and Chakraborty, 2010; Bandyopadhyay *et al.*, 2010) and adrenal (Miller, 1954; Maitra and Chakraborty, 1983; Chakraborty, 1994). Cytological studies were made from midsaggital paraffin section of 5 μm thickness stained following haematoxylin-eosin and observed under oil immersion (15 ocular x100 objective). Only the right adrenal glands were used for histological studies.

Karyomorphology: Morphometric evaluation of at least 150 oval to round nuclei were made from each of the five randomly selected mid-sagittal sections per specimen. Furthermore nuclear diameters of the adrenal cortex were randomly measured zone wise. In all cases, nuclei measured under oil immersion using 15 ocular x100 objective lenses along with ocular micrometer scale. All the ocular diameter values were then converted to μm values. Individual value of the specimen was the mean values of the five sectional measurements. The final mean values of the experimental group were computed from these individual measurements.

Karyodynamics: The number of colchicine arrested metaphase cells or the mitotic figures have been considered as an index for cell proliferative activity (Quay and Levine, 1957; Sahu and Chakraborty, 1986; Bandyopadhyay and Chakraborty, 2010; Bandyopadhyay *et al*., 2010). In the present work, the numbers of mitotic figures found in the pineal and adrenal cortex were recorded from each section under oil immersion (15 ocular x100 objective). The ratio of number of metaphase cells per hundred cells were counted from the same, five different randomly selected mid-sagittal sections per animal were considered for morphometric studies and expressed as mitotic percentage (M%).

Statistical analysis: Values were presented as the means of the observations following experimental manipulation. All the karyomorphological and karyodynamic values for the control and treated mice were compared and the level of significance were statistically evaluated by Student's 't' test (Winer, 1971) and one-way ANOVA (Microcal Origin, Version 4.00).

RESULTS

Sham operated control: The pineal gland in mice is a compact, homogenous mass of parenchymatous tissue. It is comprised of pinealocytes with prominent round or oval nuclei, enclosing single or more centric nucleolus and dense chromatin material. The pinealocytes lack any specific orientation (Fig. 1).

Castration: The pineal gland in castrated mice exhibited a homogenous parenchymatous structure, with large pinealocytes (Fig. 2). Castration was found to induce hyperactivity of the pineal cells as evident from significantly increased pinealocyte nuclear diameter ($p<0.001$, Fig. 3). The nucleus had a single nucleolus and granulated chromatin material. It was further noted that gonadectomy induced significant augmentation of cell proliferative activity ($p<0.001$, Fig. 4).

Results from one way ANOVA of pinealocyte nuclear diameter [$F(1,12) = 58.22$; $p<0.001$] and mitotic incidence [$F(1,12) = 30.29$; $p<0.001$] in sham operated controls and castrated groups indicate significant differences of the mean values in between the two groups.

Castration followed by estradiol treatment: Estradiol administration to castrated postpubertal mice, reversed the stimulatory effect of castration on the pineal gland when compared with castrated mice pineal. The pinealocytes appeared small having round nuclei associated with low karyometric values compared to castrated animals ($p<0.001$, Fig. 3). This change was accompanied with the reduction in cell proliferative activity ($p<0.001$, Fig. 4). Thus, the overall histological picture of pineal gland was that of hypoactivity in castrated mice treated with estradiol.

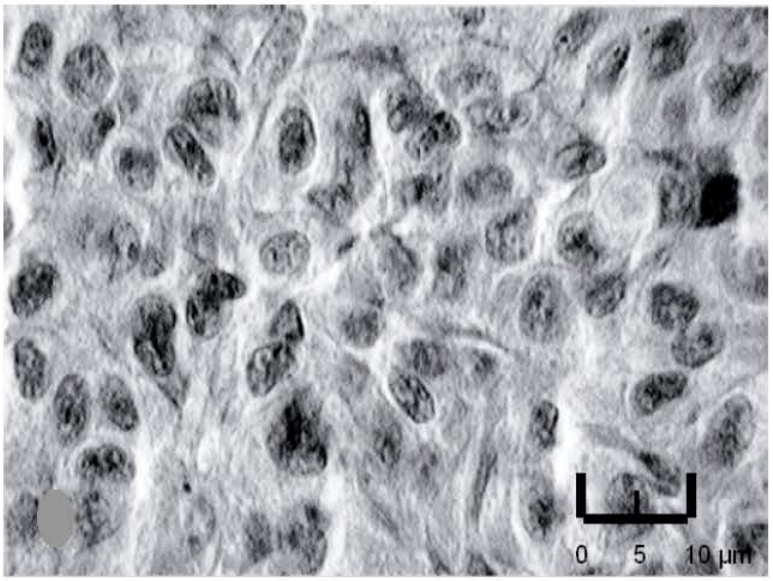

Fig. 1: Microphotograph of pineal gland section from control male mice showing nuclei with nucleolar presence

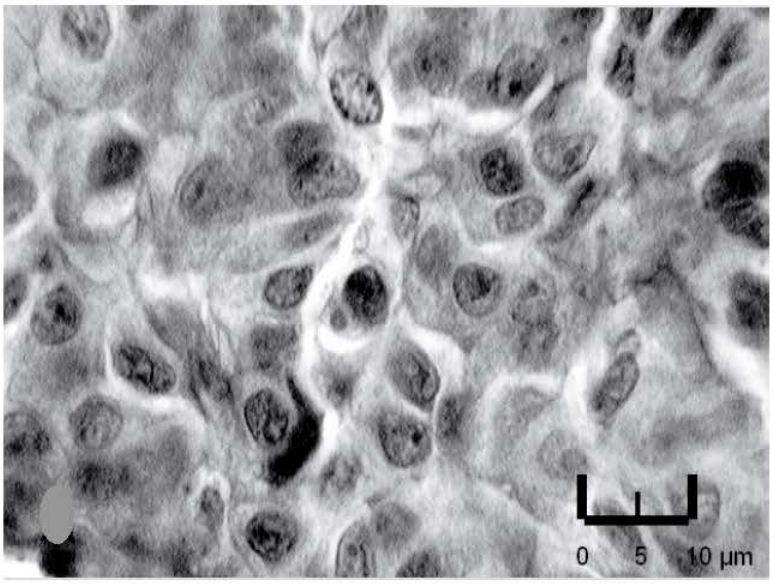

Fig. 2: Microphotograph of pineal gland section from castrated male mice showing increased nuclear diameter of the pinealocytes

Castration followed by tamoxifen treatment: Antiestrogen, tamoxifen evoked similar changes in castrated mice as observed in the case of estradiol treated castrated mice. Pineal parenchyma

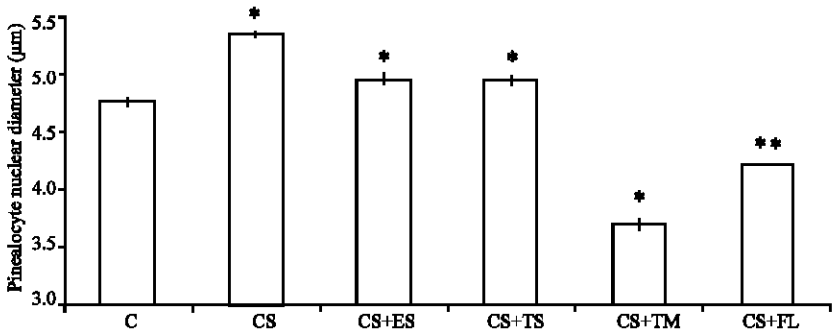

Fig. 3: Histograms showing effect of castration [CS] induced increased pinealocyte nuclear diameter (µm) values compared to sham control group [C] (*p<0.001) and the effect of castration with steroid hormones (estradiol [CS+ES], testosterone [CS+TS]) and antihormones (tamoxifen [CS+TM], flutamide [CS+FL]) supplement as compared to castrate values. Castrates with steroid hormone and antihormone supplement showed significantly reduced pineolocyte nuclear diameter (µm) as compared to castrated group (*p<0.001, **p<0.01). The vertical lines signify the SEM

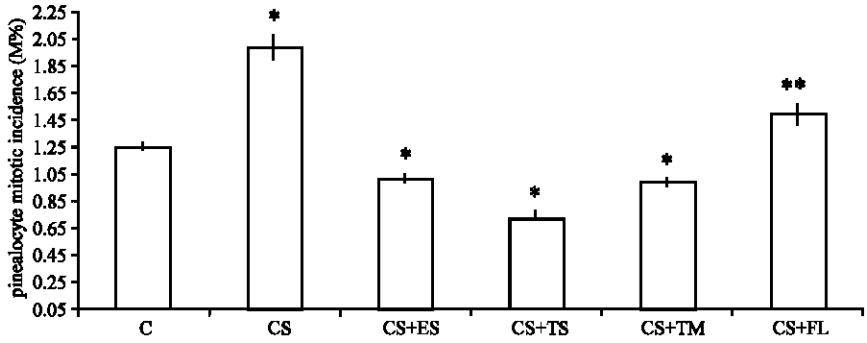

Fig. 4: Histogram showing the effect of castration [CS] induced increased pinealocyte mitotic incidence (M%) as compared to sham control group [C] (*p<0.001) and comparing effects of castration with sex steroids (estradiol [CS+ES], testosterone [CS+TS]) and antihormones (tamoxifen [CS+TM], flutamide [CS+FL]) on pinealocyte mitotic incidence (M%). Castrates with steroid hormones and antihormone supplement showed significantly reduced mitotic incidence in comparison to castrate values (*p<0.001, **p<0.01). The vertical lines signify the SEM

exhibited small pinealocytes lacking definite differentiating membrane. There was a significant decrease in nuclear diameter (p<0.001, Fig. 3). Single small nucleolus and granular chromatin materials were observed within the nuclei. Such karyometric alterations was associated with significant suppression of mitotic incidence (p<0.001, Fig. 4) compared to castrated mice.

Castration followed by testosterone treatment: In castrated mice, testosterone administration provokes hypoactivity of the pineal gland when compared to the castrated mice. Pineal parenchyma appears almost homogenous in structure and the pinealocyte lack specific orientation and the cell

membrane not very distinct. They exhibited round or oval nuclei with low karyometric values ($p<0.001$, Fig. 3). Further, testosterone treatment of castrated mice reduced the mitotic percentage of the pineal ($p<0.001$, Fig. 4).

Castration followed by flutamide treatment: In male mice, gonadectomy followed by flutamide administration induced notable atrophy of the pineal gland when compared to the castrated group. The homogenous pineal parenchyma is comprised of small pinealocytes with numerous round nuclei possessing low karyometric values ($p<0.01$, Fig. 3). The nuclei contained not so distinct nucleolus and granular chromatin material. There was significant reduction in the mitotic incidence of the pineal gland ($p<0.01$, Fig. 4).

Result of one way ANOVA of mean values of pinealocyte nuclear diameter [$F(4,30) = 131.62$; $p<0.001$] and mitotic percentage [$F(4,30) = 37.49$; $p<0.001$] in castrated animals and in groups of castrated animals administered with either estradiol, tamoxifen, testosterone or flutamide reveal significant variations in the mean values between the different experimental groups.

Adrenal cortex

Sham operated control: The cytological structure of the adrenal cortex in sham castrated postpubertal male mice showed that the cortical region is differentiated into three distinct zones, namely zona glomerulosa, zona fasciculara and zona reticularis. All the three zones show definite arrangement pattern and the nuclei possess distinct nucleolus and dense chromatin material (Fig. 5).

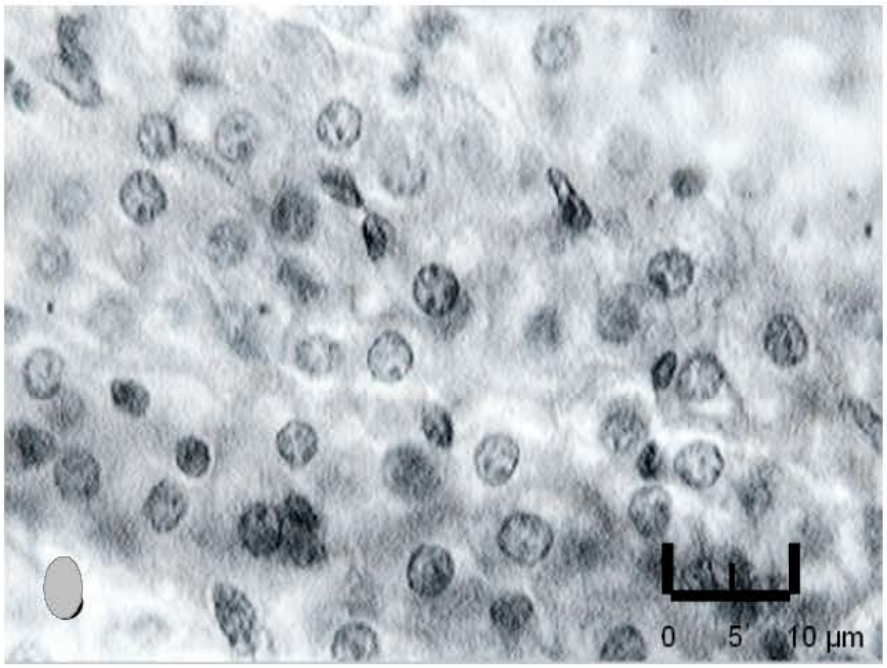

Fig. 5: Microphotograph of adrenal cortex section from control male mice showing normal nuclear diameter

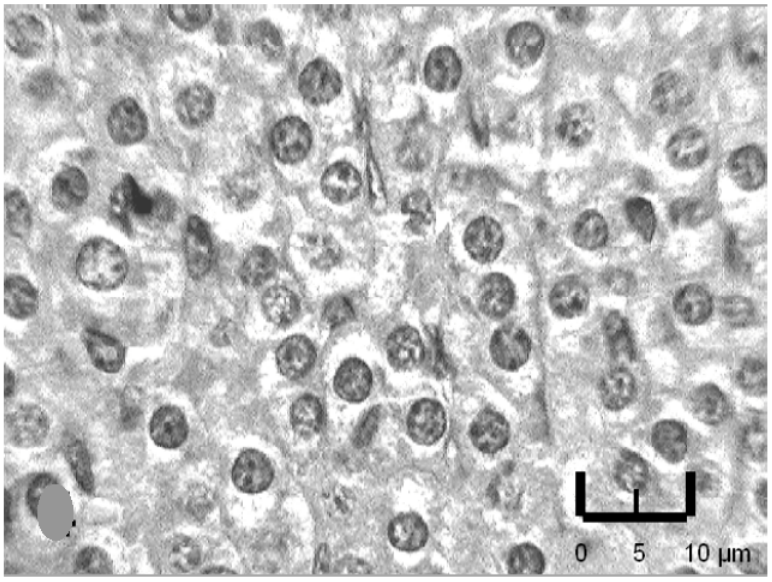

Fig. 6: Microphotograph of adrenal cortex section from hemicastrated male mice showing increased nuclear diameter

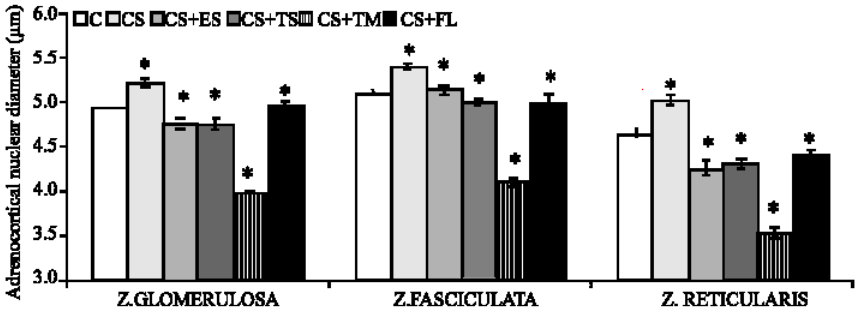

Fig. 7: Histograms showing effect of castration [CS] induced increased adrenocortical nuclear diameter (µm) of zona glomerulosa, zona fasciculata and zona reticularis values compared to sham control group [C] (*p<0.001) and in castration with exogenous steroid hormones (estradiol [CS+ES], testosterone [CS+TS]) and antihormones (tamoxifen [CS+TM], flutamide [CS+FL]) supplement. Castration with steroid hormones and antihormones supplement showed significantly reduced nuclear diameter compared to castrate values (*p<0.001). The vertical lines signify the SEM

Castration: In male mice, gonadectomy produced a notable hyperactivity of the adrenal cortical cells (Fig. 6). This was evident from the increased nuclear size in all the three zones, zona glomerulosa, zona fasciculata and zona reticularis (p<0.001, Fig. 7) of the adrenal cortex. This was accompanied with significant increase in mitotic incidence (p<0.001, Fig. 8). It was noted that all

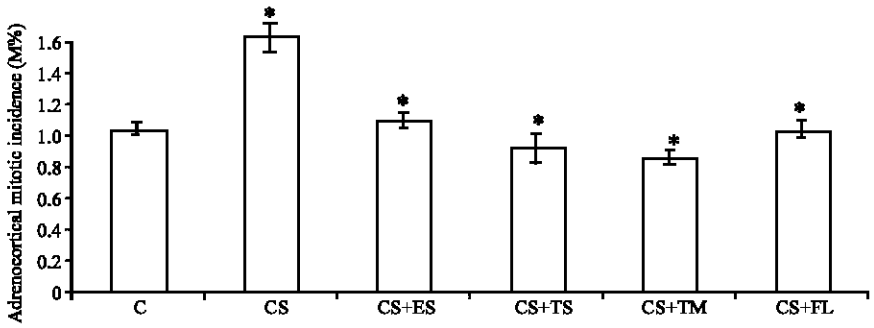

Fig. 8: Histograms showing effect of castration induced higher adrenocortical mitotic incidence (M%) compared to that in sham control [C] group (*p<0.001) and values following castration [CS] with exogenous steroid hormones (estradiol [CS+ES], testosterone [CS+TS]) and antihormones (tamoxifen [CS+TM], flutamide [CS+FL]) supplement. Castration with steroid hormones and antihormones supplement showed significantly decreased mitotic percentage compared to castrate values (*p<0.001). The vertical lines signify the SEM

the three zones had specific structural arrangement and nuclei of each zone contained prominent large nucleolus and loose chromatin material.

Results of one way ANOVA of mean values of adrenocortical nuclear diameter [$F(1,12) = 11.89$; $p<0.001$ for zona glomerulosa; $F(1,12) – 35.31$; $p<0.001$ for zona fasciculata and $F(1,12) = 14.76$; $p<0.001$ for zona reticularis] and mitotic incidence [$F(1,12) = 28.31$; $p<0.001$] in sham operated controls and castrated animals reveal significant differences in the mean values between various experimental groups.

Castration followed by estradiol treatment: Estradiol administration to gonadectomised mice, reversed the stimulatory action of gonadectomy on adrenal cortex. The cortical cells of all the three zones of adrenal cortex lost their definite arrangement, with the fasciculata and reticularis zone cells becoming less compact. The adrenocortical nuclei failed to show distinct nucleolus and have granulated chromatic material. This was accompanied with a notable reduction in nuclear size in zona glomerulosa, zona fasciculata and zona reticularis ($p<0.001$, Fig. 7) and significant hypoplasia ($p<0.001$, Fig. 8) in comparison to castrated ones.

Castration followed by tamoxifen treatment: Tamoxifen, an antiestrogen produced similar changes in the adrenal cortex of castrated mice as those observed in estradiol treated castrates. The atrophic alteration in the adrenocortex was indicated by significant reduction in nuclear size of zona glomerulosa, zona fasciculata and zona reticularis ($p<0.001$, Fig. 7). The vacuolated adrenocortical cells exhibited indistinct nucleolus and granular chromatin material. It was also noted that the cellular arrangement was lost and cords of cells of fasciculata and reticularis appeared as loose strands. Such an alteration was followed by significant hypoplasia ($p<0.001$, Fig. 8).

Castration followed by testosterone treatment: Testosterone administration in castrates evoked hypoactivity of the adrenal cortex. The definite cellular arrangement was lost and the adrenocortical nuclei enclosed distorted nucleolus and granular chromatin material. In comparison

to the castrated mice; the nuclear size in testosterone treated castrates show significant reduction as observed in zona glomerulosa, zona fasciculata and zona reticularis ($p<0.001$, Fig. 7) of adrenal cortex, alongwith perceptible hypoplasia ($p<0.001$, Fig. 8).

Castration followed by flutamide treatment: Androgen antagonist, flutamide administration in gonadectomised male mice decreased the adrenocortical activity. The adrenocortical cells became clear and vacuolated with the nuclei enclosing indistinct nucleolus and granular chromatin material. The reduced size of nuclei of each zone of adrenal cortex, namely zona glomerulosa, zona fasciculata and zona reticualris ($p<0.001$, Fig. 7) was simultaneously accompanied with a decrease in the cell proliferative activity ($p<0.001$, Fig. 8).

Results from one way ANOVA of mean values of adrenocortical nuclear size [$F(4,30) = 71.71$, $p<0.001$ for zona glomerulosa; $F(4,30) = 24.87$, $p<0.001$ for zona fasciculata and $F(4,30) = 67.88$, $p< 0.001$ for zona reticularis] and mitotic incidence [$F(4,30) = 16.88$, $p<0.001$] in animals castrated and castrated animals treated either with estradiol, tamoxifen, testosterone or flutamide reveal significant variations in mean values between the different experimental groups.

DISCUSSION

Current quantitative morphology as evident from karyometric and cell proliferative values and associated histological studies indicate that both pineal and adrenocortical cytology show response to altered gonadotrophin and steroid milieu, induced by castration and castration followed by exogenous administration of sex steroids and non-steroidal antihormones.

It is interesting to note that the cytophysiological changes indicated by karyomorphological alterations in the pineal gland (Quay, 1976; Chakraborty *et al*., 1982; Diehl *et al*., 1984; Sahu and Chakraborty, 1986; Hira *et al*., 1989; Sinha *et al*., 2009; Bandyopadhyay and Chakraborty, 2010; Bandyopadhyay *et al*., 2010) and adrenal cortex (Miller, 1954; Maitra and Chakraborty, 1983; Chakraborty *et al*., 1994) provide valuable indices of the nature of activities of these glands.

Current experimental results shows that castration in postpubertal male mice causes pineal and adrenocortical stimulation at cytological level as indicated by increased karyometric values compared to control group of mice. Castration was found to increase the nuclear diameter of pinealocytes and cells of all the three zones of adrenal cortex, namely zona glomerulosa, fasciculata and reticularis. This alteration is accompanied with a significant increase in mitotic incidence in both the glands. However, administration of peripheral sex hormones to castrated animals reverses the increase in nuclear size and cell proliferative activity of both pineal and adrenal cortex. Furthermore, non-steroidal antihormones evoke similar responses in the pineal and adrenocortical tissues of castrated mice, indicating diminution of the glandular activities. It was also noted that tamoxifen acts as estrogen agonist while flutamide as an antiandrogen in the castrates when compared to the respective estradiol or testosterone treated castrated animals.

It may be emphasized from the current experimental observations that the morphofunctional aspects of both pineal gland and adrenal cortex are susceptible to endogenous gonadotrophin and sex-steroid manipulation through castration and subsequently followed by sex-steroid or antihormone supplementation influence the activities of both pineal gland and adrenal cortex.

The current data corroborated with earlier morphological observations where orchidectomy in male rats induces hyperactivity of pinealocytes with the resultant increase in synthesis and secretion of melatonin (Das Gupta, 1968; Gusek, 1976; Reiter, 1977, 1978) and this stimulatory effect is reversed by testosterone treatment in castrates (Das Gupta, 1968).

In addition to the morphological evidences of an enhanced pineal activity following gonadectomy, biochemical studies also demonstrate an increase in HIOMT activity in pineals of castrated immature rats (Alexander *et al*., 1970). Furthermore, increase in FSH and LH following castration are found to promote melatonin synthesis in castrated rats (Cardinali *et al*., 1976a, b). Thus the current investigation imply a physiological role of the testis in the alleged pineal-gonadal interactions in the postpuberal male mice as evident from the enhanced pineal activity following castration.

The relationship between the adrenal cortex and the gonads has been the subject of controversy and is quite perplexing. Evidences reveal that removal of gonads leading to significant rise in both FSH and LH levels, influences the normal functioning of adrenal cortex (Schwartz and Justo, 1977). Experimental results indicate that testosterone propionate, 5α-dihydrotestosterone propionate and oestradiol benzoate act as a suppressor of the serum gonadotrophins levels in castrated adult male rats (Swerdloff and Walsh, 1973; Verjans *et al*., 1974) thereby influencing the functional capacity of adrenal cortex.

Microscopic examination of the adrenal cortex following castration revealed significant stimulatory changes in the functional morphology of this gland in postpuberal male mice. A notable increase in nuclear size in the entire cortical region of adrenal gland serves as a tentative indicator of heightened adrenocortical activity (Miller, 1954; Bandyopadhyay and Chakraborty, 2010; Bandyopadhyay *et al*., 2010). This alteration is accompanied with enhancement of cell proliferative activity of adrenal cortex. However, the reduced quantitative nuclear morphology and associated cytological observations in the adrenocortex of castrated animals treated with peripheral sex steroids-estradiol or testosterone and their antihormones-tamoxifen or flutamide respectively indicate hypoactivity of the adrenal cortex. Thus, the stimulatory action of castration on adrenal cortex could be reversed by the administration of either sex steroids or their non-steroidal antihormones.

The current observations are in agreement with previous reports showing that orchidectomy induced adrenal hypertrophy (Troop and Possanza, 1962). Furthermore, castration results in hypertrophy of both zona fasciculata and zona reticularis in rats (Dorfman and Shipley, 1956) and only reticularis zone in adult sheep which could be reversed by testosterone propionate injection (Stokoe, 1959).

In addition, morphological studies conducted in the castrated rat adrenal gland show that the mitotic activity increases in the zona reticularis, which in turn increases androgen biosynthesis, an effect reversed by administration of testosterone propionate (Kasprazak *et al*., 1986). Such findings are conducive with the present experimental results where castration enhances the cell proliferative activity in the adrenal cortex and that this effect is reversed by steroid or non-steroidal antihormone supplement.

However, it needs to be mentioned that similar to adrenocortical stimulation following castration in postpubertal male mice was also observed by previous investigators who found that gonadectomy in male mice enhance corticosteroid yield, indicating adrenocortical activation (Nandi *et al*., 1967). Prepubertal castration in male rats resulted in an increased plasma ACTH concentration leading to an activation of the adrenal gland and this effect was reversed by testosterone replacement in the castrated rats (Coyne and Kitay, 1971). Furthermore, gonadectomy results in an increase in adrenal 5α-reductase activity (Kitay *et al*., 1971; Colby and Kitay, 1972; Yokoi *et al*., 1998) inducing intra-adrenal conversion of corticosterone to 5α-dehydrocorticosterone and 3β,5α-tetrahydrocorticosterone, accompanied with an increase in the total adrenocortical steroid content

leading to adrenal hypertrophy (Colby and Kitay, 1972). However, estradiol or testosterone replacement in castrated animals restores the activity of 5α-reductase and secretion of corticosterone to control levels (Colby, 1978). It is hence inferred from our observation that castration induces adrenocortical metabolic activity while replacement of gonadal activity through estradiol or testosterone administration reverses the stimulatory effects.

Thus, it is conjectured that increase in plasma gonadotrophin level and reduced testosterone following castration may directly influence the normal functioning of both pineal and adrenal cortex as evidenced from the current experimental investigation in postpubertal male mice. However, it remains to be known whether pineal and adrenal stimulation due to castration is because of steroid deprivation following orchidectomy or supposedly due to increased gonadotrophin which is an accompaniment of orichidectomy.

It is know that testosterone causes inhibition of pineal adrenal function in animals (Nagle *et al*., 1974). Cardinali *et al*. (1987) and Bandyopadhyay *et al*. (2010) have shown exquisitely that testosterone-induced inhibition of pineal and adrenal karyomorphology and dynamics. Consequently, it can be argued that testosterone deprivation may have been instrumental in causing pineal and adrenal stimulation of karyomorphology values and heightened mitotic activity (M%) in these post pubertal mice.

In addition, reports present indicate that FSH or LH can augment adrenocortical activity by increasing the corticosteroids in mammals (Vinson *et al*., 1975). *In vitro* experiments in rats show that gonadotropins, namely LH and FSH stimulate production of testosterone and corticosteroids by the adrenal gland (Vinson *et al*., 1976). Thus, it could be interpreted that increased gonadotrophin and reduced steroid levels may influence the functioning of the adrenal cortex that possess both gonadotrophin and steroid receptors. These findings confirm the current observations showing castration leading to an increase in gonadotrophin levels influence the adrenal cortex by inducing its hyperactivity.

On the other hand, it can be suggested that orichidectomy induced increased titre of gonadotrophin (Moguilevsky *et al*., 1979; Peek and Watkins, 1980; Balasinor *et al*., 1992) may have stimulated the pineal and adrenal cytomorphology and function because of the fact that gonadotrophin receptors are evident in both pineal (Bhatnagar *et al*., 2002; Itoh *et al*., 2006) and adrenal (Pabon *et al*., 1996; Rao *et al*., 2004; Rao, 2010).

Our investigations suggest that in castrated mice exogenous administration of gonadal steroids, estradiol and testosterone and antihormones tamoxifen and flutamide reversed the pineal-adrenal stimulatory changes. Such an observation is natural as it has been reported earlier that castration causes an immediate increase in serum gonadotrophin (Moguilevsky *et al*., 1979; Peek and Watkins, 1980; Balasinor *et al*., 1992) and such gonadotrophin levels show a decrease following administration of gonadal steroids (Eldrige *et al*., 1974; Moguilevsky *et al*., 1979; Peek and Watkins, 1980).

The current investigative treatise thus culminates to the idea that the mutual stimulatory relationship between the pineal and the adrenal cortex in castrated mice could be influenced by diversely altered steroid miheu. It can be argued that such disparate changes in pineal and adrenal function in castrates and castrates supplemented with diverse array of steroids may have affected the pineal-adrenal functioning either directly through audiogon receptor in these two glands or following alteration in hypothalamo-hypophyseal axis the gonadotrophin receptors in these two prolific gonadotrophin and steroid sensitive endocrine glands.

Thus, it appears that the current revelation is useful in projecting integrated information about the pineal-adrenocortical interrelationship and also instrumental in providing some much needed new insights about the modulation of this relationship following alteration in the gonadotrophrin and steroidal milieu following castration and castrated mice supplemented with steroid hormones and antihormones.

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