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Original Article

Influence of cimetidine and bromocriptine on prolactin levels in rat fertility

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Abstract: The present study was designed to see the effects of parenterally administered drugs cimetidine and bromocriptine affecting serum prolactin upon the fertility of adult male albino rats. Ninety adult young male albino rats between the ages of 60 to 120 days were selected. The animals were divided into three groups. Cimetidine was administered in a dose of 200 mg/kg body weight to group B intramuscularly and in addition to cimetidine, bromocriptine in a dose of 2.5 mg/day intramuscularly was given to group C. Normal saline was administered intramuscularly to control group A. Plasma prolactin was measured by Enzyme Immunoassays. Spermatogonia, spermatocytes, and spermatids were studied under oil immersion. The final plasma prolactin level instead of being elevated was found slightly depressed though insignificant in case of group B while remained slightly elevated instead of being suppressed/depressed though insignificant in group C. In group B spermatogenesis was normal in almost all of the tubules but a few of them were seen lined with only Sertoli cells and all the other germ cells like spermatogonia, primary spermatocytes, spermatids early and late, and spermatozoa were absent indicating total atrophy with both Sertoli cells and Leydig cells hyperplasia. While in the moderately affected tubules different types of spermatogonia A/B or intermediate were seen near the basement membrane. In group C both normal and abnormal germinal epithelium was seen in same/different tubules but a few of them were seen lined with only Sertoli cells and all the other germ cells like spermatogonia, primary spermatocytes, spermatids early and late, and spermatozoa were absent. The process of spermatogenesis was variable and appeared to be normal in most but in some it was found to be suppressed. This study revealed that the toxic effect of the drugs contributes to the infertility. It has not shown to be mediated through hormones in present study for which further research work is needed using low dose and longer duration to see the role of prolactin in causing infertility.

Key Words: Fertility, cimetidine, bromocriptine, prolactin

Introduction

Cimetidine a histamine H_2 receptor antagonist (H_2 blocker) has been widely prescribed for about 20 years worldwide [1]. Histamine H_2 receptor antagonist medications are used to treat heartburn, gastroesophageal reflux disease and ulcers [2]. It is currently sold "over-the-counter" to reduce gastric acid secretion and the resulting discomfort. It increases survival after gastric cancer [3] in general and colorectal cancer in particular [4, 5]. As recently reported in literature, it blocks cancer metastasis by inhibiting cancer cell adhesion to endothelial cells [6]. Cimetidine is also a known reproductive toxicant as indicated by significantly reduced weight of accessory sex organs [7] it causes gynecomastia in males and galactorrhea in females [8].

As the side effects mentioned above could

Table 1. Experimental Schedule

S.No	Groups	No. Of Animals	Treatment	Dose	Duration	Sacrificed
1	A (Control)	20	Inj. Normal Saline (I/M)	1 ml (in 2 equal divided doses)	2 weeks	15th day
2	B (Treated)	30	Inj.Cimetidine (I/M)	200mg/Kg in 2 equal divided doses	2 weeks	15th day
3	C (Treated)	30	Inj.Cimetidine (I/M) + Inj. Bromocripte (I/M)	200 mg/kg +2.5mg/day (in 2 equal divided doses)	2 weeks	15th day

supposedly be due to raised levels of prolactin which could be blocked by a prolactin depressing drug such as bromocriptine. Bromocriptine (PARLODEL) is the dopamine receptor agonist most frequently used to treat hyperprolactinemia [9]. Bromocriptine lowers the plasma prolactin levels and normalizes the prolactin level in 70% to 80% of patients with prolactinomas [10, 11]. The prolactin response in men was swift and only occurred in association with high circulating concentrations of cimetidine and was blocked by bromocriptine and ergot alkaloid derivative that possessed both dopamine agonist and antagonist properties [12]. These observations have led to the proposal that cimetidine is acting directly or indirectly as a dopamine antagonist at the dopamine receptor sites in the anterior pituitary causing increased secretion of prolactin hormone (hyperprolactinemia) and since it has already been noted in male rats that there is inhibition of gonadotrophins by induced hyperprolactinemia, which is associated with hypogonadism in general and testicular atrophy/degeneration, in particular [13]. The aim of this study was to evaluate the effects of parenterally administered drugs cimetidine and bromocriptine affecting serum prolactin upon the fertility of adult male albino rats.

Research Design and Methods

Subjects and sample collection

This study was conducted at the Department of Anatomy, Army Medical College (AMC), and Rawalpindi in collaboration with National Veterinary Laboratories (NVL), Chak Shahzad, Islamabad (**Table 1**). Ninety adult young male albino rats between the ages of 60 to 120 days were selected. They were bred in the animal house of the National Institute of Health (NIH), Islamabad and were supplied with diet pellets supplemented with vitamins and water ad libitum. The animals were divided into three groups. Out of them thirty male rats in group A were given injection of one ml of normal saline intra- muscularly daily for two weeks. This group served as control for group "B" and "C". Thirty male rats were in group B given the injection of cimetidine intra muscularly in a dose of 200 mg/kg body weight daily for two weeks. Thirty male rats were in group C given injection of cimetidine intra muscularly daily in a dose of 200 mg/kg and in addition an injection of bromocriptine 2.5 mg was also given intramuscularly to each animal of this group for two weeks. All the animals were killed on the next day after the last injection. Two drugs cimetidine and bromocriptine were administered mainly the ulcerex brand (SAMI PHARMACEUTICALS) cimetidine ampoules containing 200mg in 2ml solution were used undiluted in these experiments. Two ml/kg body weight of the drug was injected intra muscularly twice a day at an interval of six hours, one injection at 9.00 a.m. and another at 3.00 p.m. for a period of two weeks to group "B" and "C". Bromocriptine is available in the form of tablets of 5mg with the trade name of 'Parlodel', manufactured by Sandoz Pakistan. Fifteen 5mg tablets of bromocriptine were crushed, powdered and mixed with thirty ml of distilled water to make a suspension. Out of this solution 0.5ml which contained 1.25 mg of bromocriptine was injected intra muscularly twice a day at an interval of six hours immediately after injecting cimetidine as

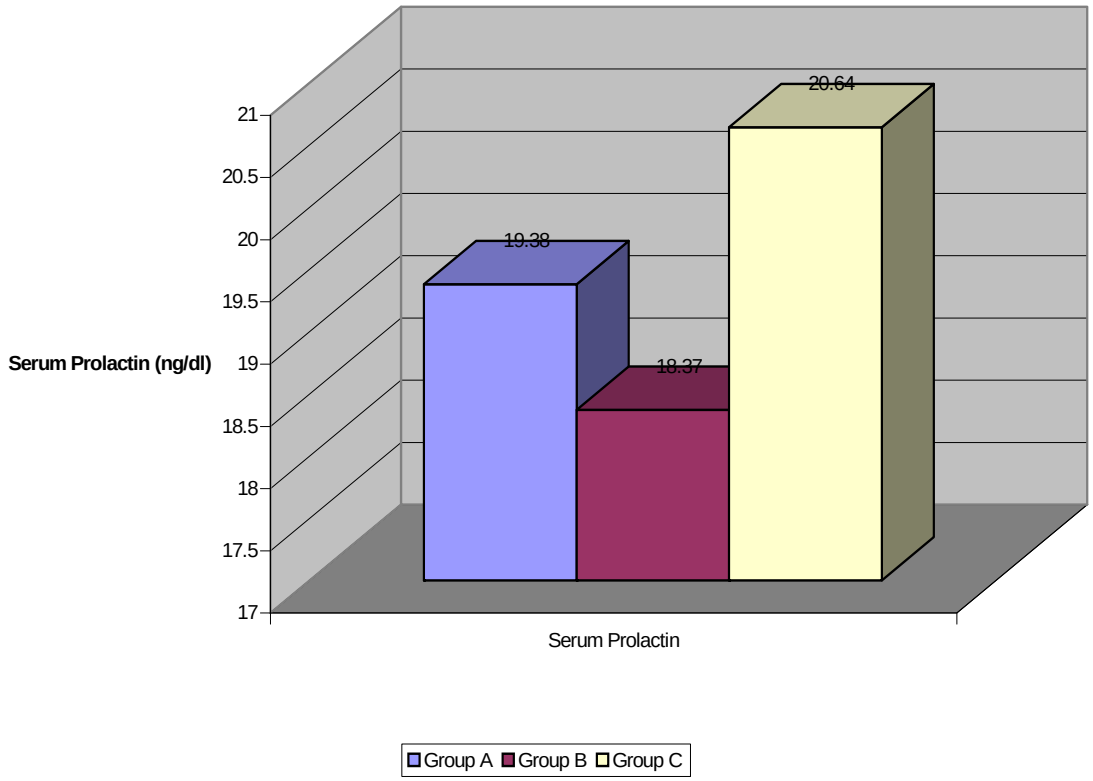

Figure 1. The mean serum prolactin levels in different groups of experimental animals.

mentioned above to rats of group "C" only for a period of two weeks. The site of injection had to be changed frequently because of local swelling and induration caused by repeated injections with one ml tuberculin plastic syringes having 0.01ml graduations for cimetidine and 3 ml plastic syringes for injecting bromocriptine suspension as it was quite thick as compared to cimetidine solution. The animals used in the present study were numbered and weighed initially before starting the experiment and again finally at the end of the experiment. The animals to be sacrificed were killed by an over dose of ether anaesthesia, cotton was soaked in ether and placed into the jar. The animal to be sacrificed was lifted by its tail and dropped into the jar and when it became unconscious it was taken out of the jar, placed on a clean sheet of paper on a dissecting board and while still keeping continuously anesthetized by a bottle covering the head of the rat containing swabs soaked in ether. The chest was palpated for locating the heart. Once it was located, it was penetrated by the needle of a 5-10 ml plastic aseptic syringe to draw 2-10 ml blood directly from the heart. These collected blood samples were then immediately transferred into labeled plastic pipettes kept in a rack refrigerated at 2-8°C for a few days till they were centrifuged later on to separate serum which was kept frozen at -20°C in labeled vancure bottles for Enzyme Immunoassays for the quantitative measurement of rat prolactin by an EIA kit (catalog number 12-MKVRP1, size 96 Tests, version 2003-09-09 – ALPCO 10-02-03). Spermatogonia, spermatocytes, and spermatids were studied under oil immersion.

Statistical analysis

All calculations were done utilizing computer software, Microsoft Excel in windows 2000 XP and SPSS version 10 (using one way ANOVA)

Table 2. Final Serum Prolactin (Ng/Dl) of Animals of Different Groups

GROUPS	SERUM PROLACTIN (NG/DL)*
A (n=20)	19.38 ± 0.8512
B (n=30)	18.37 ±0.8877
C (n=30)	20.64 ± 0.5160

*Mean±SEM; Statistic analyses show that A *vs.* B: *P*>0.3; A *vs.* C: *P*>0.2; C *vs.* B: *P*<0.02

followed by a post hoc test like LSD. The results are presented as mean ± SEM. The statistical significance of the difference of various quantitative changes between the experimental and control groups was evaluated by "Student *t*-test". The difference was regarded statistically significant if the "*P*" value was equal to or less than 0.05. "P" value was found by means of "*t*" distribution table with the help of which each "*P*" value was read against the degree of freedom (d.f.).

Results

Initially we started with thirty rats but since ten of them died on the very same date due to change of place and bad weather conditions prevailing in our laboratory as there was some fault in the cooling system. Therefore we were forced to proceed with only twenty animals as controls in the present study as compared to thirty animals in both experimental groups B and C and also since ten extra adult male rats of the same strain were not available at the present center. The mean serum prolactin was 19.38 ± 0.85 ng/dl in the control group (**Figure 1**). Different types of spermatogonia A/B or intermediate were seen near the basement membrane/germinal epithelium /both in group A. In group B the mean serum prolactin level was 18.37 ± 0.88 ng/dl which was lower than that of group C (**Table 2**). It was found significant (P < 0.02) when compared with group C. In group B all tubules did not show disorganization but a few of them displayed disruption and disorganization of germinal epithelium or both. The spermatogenesis was normal in almost all of the tubules but a few of them were seen lined with only Sertoli cells and all the other germ cells like

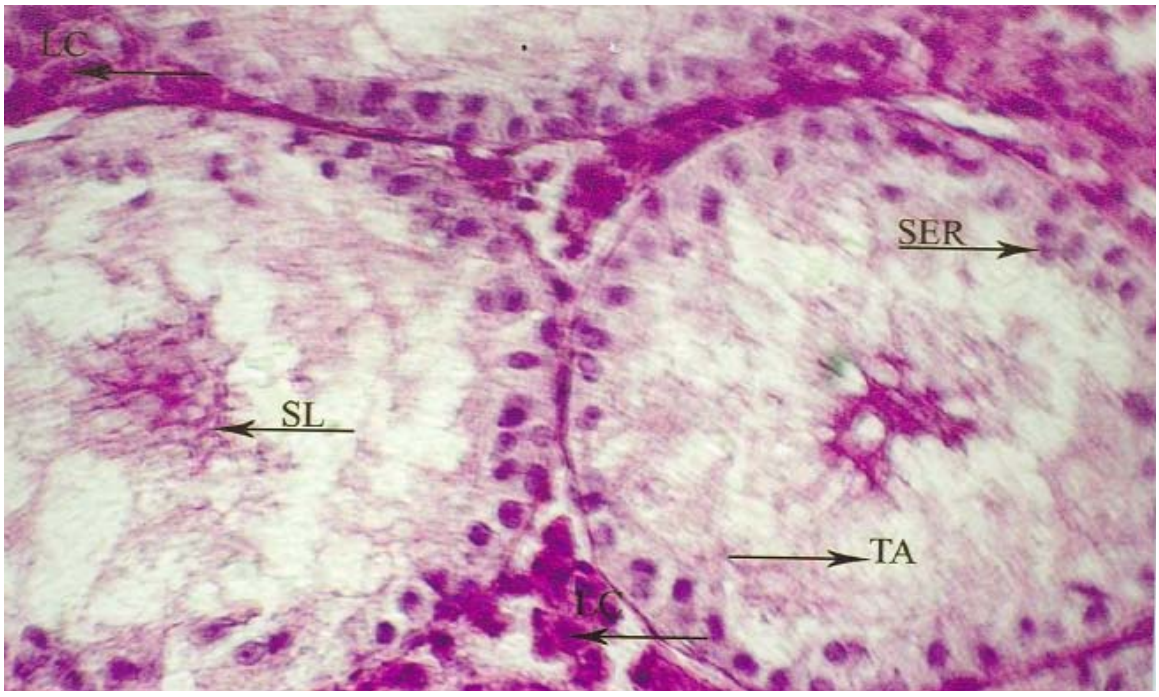

Figure 2. Section of cimetidine treated testis, (group B), showing total atrophy(T.A.) sertoli (SER) cells hyperplasia, and slough (SL) within the lumen of seminiferous tubule, there is Hyperplasia of Leydig cells (LC) outside tubule in the interstitial stroma. PAS & Harris Haematoxylin stain (Photomicrograph x 400).

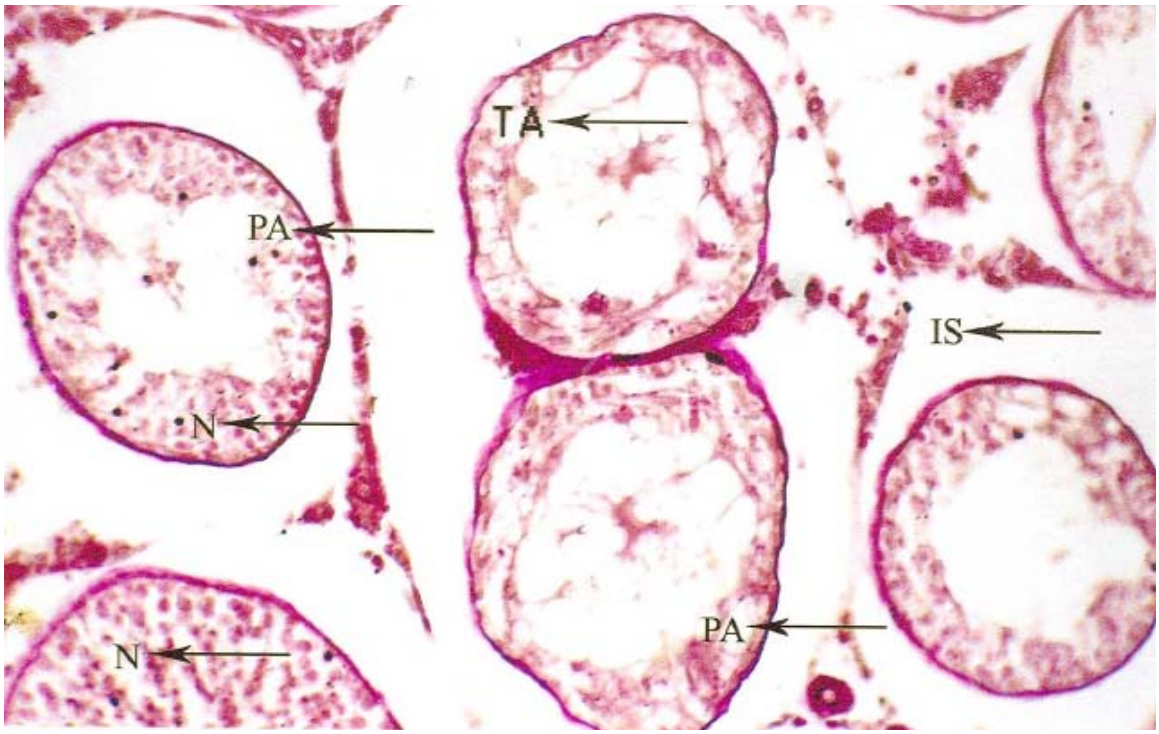

Figure 3. Section of cimetidine & bromocriptine treated testis, (group C), showing normal (N) & abnormal (PA) germinal epithelium in same or different seminiferous tubules. In the middle of the section one of the upper seminiferous tubule is showing total atrophy (TA) and a lower seminiferous tubule is showing partial atrophy (PA), There is a sparse tissue seen in the interstitial space (IS), PAS & Harris Haematoxylin stain (Photomicrograph x 400).

spermatogonia, primary spermatocytes, spermatids early and late, and spermatozoa were absent indicating total atrophy with both Sertoli cells and Leydig cells hyperplasia (**Figure 2**).While in the moderately affected tubules different types of spermatogonia A/B or intermediate were seen near the basement membrane.

In group C the mean serum prolactin level was shown to be 20.64 + 0.51 ng/dl which was found to be highest amongst the three groups i.e. higher than the control as well as group B. The difference between the group C and control was not found significant while it was found statistically significant ($P<0.02$) when compared with group B. Both normal and abnormal germinal epithelium (**Figure 3**) was seen in same/different tubules but a few of them were seen lined with only Sertoli cells and all the other germ cells like spermatogonia, primary spermatocytes, spermatids early and late, and spermatozoa were absent. The process of spermatogenesis was variable and appeared to be normal in most but in some it was found to be suppressed.

Discussion

Cimetidine is a known testicular toxicant, but its mechanism of action remains uncertain. In the present study rats were treated with cimetidine in a dose of 200 mg/kg body weight to group B intramuscularly and in addition to cimetidine, bromocriptine in a dose of 2.5 mg/day intramuscularly was given to group C. The prolactin response in man is swift and only occurs in association with high circulating concentration of cimetidine and is blocked by bromocriptine. In acute dose studies cimetidine does not enter rat brain but is localized in pituitary gland. It has been proposed that cimetidine is acting directly or indirectly at the dopamine receptor in the anterior pituitary to cause hyperprolactinemia [15]. High levels of prolactin are associated with hypogonadism in men [16]. Stress also

stimulates the ACTH, adreno-cortical system and inhibits secretion of other pituitary hormones particularly gonadotrophin since the latter are not essential during the stressful state. It has already been noted in male rats that there is inhibition of gonadotrophin by induced hyperprolactinemia [17].

This inhibitory influence could be at the target organ or pituitary gland or mediated through hypothalamus. Regarding cimetidine apart from raised serum prolactin other untoward effects have been recorded such as gynecomastia [8], increase in weights of liver, and necrosis in livers of dogs which probably suggest accumulation of oestrogens in the body which fails to be inactivated by liver damaged by cirrhosis [18]. It has also been conclusively demonstrated that administration of oestrogen in human and in laboratory animals results in atrophy of testis because of its influence in arresting the output of gonadotrophin from pituitary [19]. In the present study we see a degree of hypogonadism as indicated by decreased morphologic and morphometric parameters but when we try to co-relate this finding with the expected increase in the level of serum prolactin in case of group B on the contrary instead of increased level of serum prolactin we come across unexpected decreased levels of serum prolactin though not significant as compared to control group. Perhaps this could be due to: 1. different species/strain of rats; 2. low dose of the drug cimetidine; 3. Different brand of cimetidine (ulcerex instead of Tagamet used in previous study); or 4. Shorter duration of study. Since both drugs cimetidine as well as bromocriptine have been given in case of group C and since cimetidine elevates serum prolactin which is blocked/neutralized by bromocriptine which reduces serum prolactin, thus does not allow cimetidine to affect the gonads adversely, by preventing hypogonadism, which in male rats, indicate sexual dysfunction and loss of libido, by a direct stimulatory effect on follicle stimulating hormone, leading to increase in the weight/ size of the testes [20]. On the basis of the results of group C it is proposed that since both drugs cimetidine as well as bromocriptine has acted synergistically/agonistically to raise the level of serum prolactin though insignificant which is not blocked by a meager 2.5mg/day dose of bromocriptine.

In a report of number of previous studies "oral route" was employed while in case of present experiment "parenteral route" was opted for which is partly consistent with previous studies reported earlier and also in agreement with the low dose of our previous study where 150mg/kg body weight of cimetidine was given intra muscularly for three weeks, also in agreement with the high dose of our previous study where 950mg/kg body weight of cimetidine was given intramuscularly for ten days only [19,21]. Since both the routes have their peculiar merits and demerits, parenteral (intra-muscular) route was preferred in case of both group B as well as group C of present study as well as in the high and low dose of our previous study because it ensures "better absorption" and "lesser dependency" on gastrointestinal tract which is not predictable in case of oral route employed by previous workers [22]. While considering intra-muscular route of administration, one of the factors responsible for producing physical stress in case of present study and as acute release of both pituitary prolactin and ACTH occurs following a variety of stressful situations [23].

In the case of germ cells, many developing ones in both vertebrates and invertebrates are lost as a result of apoptosis [24]. Roosen-Runge reported that cell death is a common feature in spermatogenesis and occurs exclusively or preferentially in certain developmental stages though species specific in quality and quantity [25]. For example in some vertebrates, spermatogonia are the most common observed dead cells in testes [26]. Degeneration of spermatogenic cells at the transition from spermatogonia to spermatocytes is frequently observed in seasonal breeding animals. In the crested newt, administration of ovine prolactin induces spermatogonial cell death, but co-injection of follicle stimulating hormone prevents it in Japanese red-bellied newt, this degeneration occurs following the elevated titer of plasma prolactin which occurs after animals are transferred to low temperature, suggesting that this cell death causes the cessation of spermatocytogenesis from late autumn to early spring. The urocele testis displays well-marked zones of spermatogenic cell types because lobules formed at the cephalic regions gradually acquire more caudal positions as the cells mature [27]. When longitudinal sections of newt testis which is ideal as a seasonal breeder are made all spermatogenic stages for the season can be

observed such as apoptosis by prolactin occurs only in penultimate mitotic generation of spermatogonia, prolactin acts directly on testis and follicle stimulating hormone counter acts the action of prolactin in vitro. In newt spermatogonia spermatogenesis at a specific time, namely in secondary spermatogonia after their sixth mitotic division, the penultimate one before spermatogonia normally enters meiosis [28]. In a report of a recent study they directly showed in vitro that the decision for the life or death of spermatogonia is regulated by the follicle stimulating hormone and prolactin ratio, consistent with a previous in vivo observation. In spring when temperature rises the follicle stimulating hormone /prolactin concentration ratio in the plasma increases because the pituitary prolactin reduces preventing spermatogonial death and permitting them to proliferate into primary spermatocytes. On the other hand in late fall when the ambient temperature lowers the follicle stimulating hormone /prolactin ratio also decreases because pituitary prolactin increases & causing sperm death and cessation of spermatogenesis [29] which is yet to be explained.

The final plasma prolactin level instead of being elevated was found slightly depressed though insignificant in case of group B while remained slightly elevated instead of being suppressed/depressed though insignificant in group C. On the basis of the results of present experiment it is concluded that the adverse effects on the qualitative changes such as cellular proliferation/ spermatogenesis could be due to the toxic effect of the drugs on the testes. In the present study it has not been shown to be mediated through hormones which needs further research work.

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