Role of Prolactin on Leydig, Sertoli and Germ Cellular
Neutral Lipids in Bonnet Monkeys, *Macaca radiata*

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Abstract. To elucidate the specific influence of prolactin on neutral lipids in Leydig, Sertoli and germ cell compartments of the testis in immature and mature monkeys, the present study was carried out by injecting ovine prolactin (oPRL) (1 mg/kg body weight/twice daily for 10 days ip), to both age groups. Similarly, bromocryptine (an ergot alkaloid which inhibits prolactin secretion) was given to other sets of immature and mature monkeys (1 mg/kg body weight/twice daily for 10 days ip) to induce hypoprolactinemia. It was observed that after oPRL administration the total lipid accumulated in the germ cells of immature and mature monkeys. Total lipid was markedly decreased in the Leydig cells of mature monkeys only. But no such influence of PRL was evident in the Leydig cells of immature monkeys. The increase in total lipid in the germ cells following PRL treatment was contributed by mono, di- and triacyl glycerols and free cholesterol. However, an opposite effect of PRL was evident in the Leydig cells of mature monkeys, where the cholesterols and gylceride fractions registered a decrease. The reduced cholesterol fractions in the Leydig cells following PRL treatment suggests the utilization of cholesterol for steroidogenesis. Sertoli cells were found to be comparatively resistant to change in PRL status. Bromocryptine treatment brought about the opposite effect of PRL in almost all parameters studied in both immature and mature monkeys. In general, these findings with prolactin suggests that PRL has a specific and definite influence on testicular neutral lipids and the response of different cellular compartments was found to vary.

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LIPIDS, being a major biochemical component of the testis, play a vital role in spermatogenic and steroidogenic processes [1] and also maintain the structural integrity and fertilizing capacity of the spermatozoa [2, 3]. Any change in testicular lipid metabolism will be reflected in testicular functions. Several hormones such as LH, FSH [4–6], dexamethasone [7] and thyroid hormones [8] have been shown to influence the testicular lipid metabolism in rats. Bartke [9] reported that similarly to LH, prolactin also mobilizes cholesterol for steroidogenesis. An earlier study at our laboratory has also revealed that prolactin decreased the testicular free cholesterol and at the same time increased the concentration of esterified cholesterol in intact rats [10].

Both clinical and experimental studies showed that hyper- and hypoprolactinemia are often associated with fertility disorders like hypogonadism, loss of libido, impotence, oligosperma and testicular atrophy [11–13]. However, the mechanism by which prolactin induces such disturbances is far from clear due to a lack of information pertaining to each compartment of the testis under altered prolactin status. The present investigation was therefore carried out as a preliminary step in ascertaining the specific effect of prolactin on Leydig, Sertoli and germ cellular neutral lipids in both immature and mature monkeys.
Materials and Methods

Animals and treatment

Healthy immature (2–2.5 kg body weight) and mature (6–10 kg body weight) bonnet monkeys, Macaca radiata species were acclimatized for 30 days in a well ventilated temperature controlled (28±2°C) animal house with a 1400 h light and 1000 h dark schedule. Standard monkey pellet diet (Gold Mohur, Hindustan Lever, India) and water were provided ad libitum. Immature and mature animals were further divided into 4 groups each consisting of 10 monkeys.

Group 1. Hyperprolactinemic monkeys—the animals were treated with 1 mg/kg body weight of ovine prolactin in 10% polyvinyl pyrrolidone (PVP) in 0.9% saline.

Group 2. Control for hyperprolactinemia—the animals were injected with 10% PVP in saline vehicle alone.

Group 3. Hypoprolactinemic monkeys—the animals were administered with 1 mg/kg body weight of bromocryptine mesylate in 0.9% saline.

Group 4. Control for hypoprolactinemia—the animals were treated with 0.9% saline vehicle alone.

All injections were given twice daily, for 10 days ip, at 0900 and 2100 h, respectively. This was mainly to sustain hyper- and hypoprolactinemic states throughout the experimental period. 1200 h after the last injection, blood samples were collected from the anticubital vein. The blood was allowed to clot and the serum was used for the assay of PRL. Hypo- and hyperprolactinemia were assessed on the basis of serum prolactin titres by radio-immunoassay [14]. Animals were sedated with sodium pentobarbitone (30 mg/kg body weight) and perfused with physiological saline till the testes were completely blanched. The testes were then removed quickly, cleaned from adhering connective tissues, weighed and used for the isolation of Leydig, Sertoli and germ cells as described below.

Isolation of Leydig, Sertoli and germ cells

Leydig cells were isolated from the decapsulated testes by incubating a known amount of tissue with collagenase (Sigma type 1) in culture media 199, following the method of Dufau and Catt [15]. 1.0 g of decapsulated tissue was incubated in a 45 ml sterile polypropylene tube containing 7 ml culture medium 199 plus Hepes (0.1 mg/ml), collagenase (1 mg/ml) and bovine serum albumin (1 mg/ml). The tubes were gassed with 95% O₂ and 5% CO₂ and capped. After incubation was performed by shaking the tubes in their long axis in a thermostated shaker water bath at 34°C for 10 min, 25 ml saline was added to the tube which was inverted gently several times and allowed to stand for 5 min at room temperature. Then the supernatants were transferred to another sterile polypropylene tube. This procedure for isolation of cells was repeated once more with the addition of 20 ml saline to the sedimented fraction to remove additional Leydig cells. The combined supernatants were centrifuged at 1700×g for 10 min. The contamination of germ cells in the Leydig cell sediment was less than 10% as assessed by haemocytometer counts. The separated Leydig cells were identified by histochemical localization in the 3β-hydroxysteroid dehydrogenase staining process described by Mendelson et al., [16].

From the remaining seminiferous tubules, both Sertoli and germ cells were separated by the methods of Steinberger et al. [17]. Seminiferous tubules thus obtained were resuspended in Hank's balanced salt medium containing 0.5 mg/ml of trypsin and 5 µg/ml of DNAse and they were cut into small pieces with a razor blade. Incubation was performed in capped tubes for 10 min in an incubator at 37°C. The suspension was shaken gently. Subsequently, tubular aggregates were broken with a loose fitting Dounce glass homogenizer (less than 10 strokes). Then it was filtered and thoroughly rinsed with Hank's salt medium through 100 µm wire mesh grids. The clusters of Sertoli cells on the top of the wire mesh were collected in a separate tube. After separating Sertoli cells, germ cell pellet was obtained from the suspension by centrifugation for 10 min at 80×g; this fraction was particularly devoid of Sertoli cell contamination or cellular debris as judged by microscopic examination. The viability of the cells was assessed by the trypan blue exclusion method.

Analytical methods

The lipids were extracted in chloroform/methanol (2:1 V/V) containing 0.01% butylated hydroxytoluene as an antioxidant [18] from all the three cellular fractions of the testis, and the lipid content was estimated by the classical gravimetric method [19]. Standard spectrophotometric methods were
used for determining total cholesterol [20] and glyceride glycerol [21]. Thin layer chromatography was used for the isolation of neutral lipid fractions. Suitable standards obtained from Cabbiochem-Behring Corp., Switzerland, were run along with samples. Neutral lipid fractions were separated by means of three solvent systems containing n-hexan, diethylether and glacial acetic acid in different proportions as proposed by Mangold [22] and eluted in chloroform.

All the data were statistically analysed by Student's t-test. P values of 0.05 or less were considered significant.

Results

The distribution pattern of different lipid classes in the testicular compartments of control animals reveal that most of the neutral lipid classes are comparatively higher in the Leydig cells, followed by Sertoli and germ cells. The Administration of PRL favoured the accumulation of lipids in the germ cells of immature and mature monkeys (p<0.01) as shown in Fig. 1. Whereas in Leydig cells of mature monkeys, it decreased the lipid content (p<0.05). But no such decrease was evident in the Leydig cells of immature monkeys. However, bromocryptine treatment registered an opposite trend with PRL.

Fig. 2 shows that PRL did not bring about any appreciable change in the cholesterol concentration of Leydig and Sertoli cells, but it enhanced it in the germ cells (p<0.05) of immature monkeys. However, the cholesterol concentration was significantly diminished (p<0.001) in the Leydig cells and stimulated (p<0.001) in the germ cells by PRL in mature monkeys. Neither PRL nor bromocryptine influenced the cholesterol and its fractions in the Sertoli cells of either maturity group. But in germ cells, PRL increased the free cholesterol in immature (p<0.01) and mature monkeys (p<0.001) and decreased (p<0.001) the esterified cholesterol in mature monkeys only. However, in Leydig cells, both free and esterified cholesterol were reduced significantly (p<0.001) due to PRL, whereas bromocryptine brought about an almost
The concentration of glyceride glycerol in the Leydig cells of immature (p<0.05) and mature (p<0.001) monkeys was decreased by PRL, following significant reduction (p<0.001) of its fractions, mono, di- and triacyl glycerols. Such an effect of prolactin on the glyceride pattern was also evident in the Sertoli cells of immature (p<0.05) and mature monkeys (p<0.01), whereas, in germ cells PRL increased the glyceride glycerol concentration (p<0.001) significantly in both maturity favoured the utilization of cholesterol for steroidogenesis as suggested by Bartke [9] concerning the mouse. This could be evidenced from the increased serum testosterone and steroidogenic enzymes in the same animals [14]. The enhanced concentration of total cholesterol in the germ cells of both maturity groups was contributed to by the stimulatory effect of PRL on free cholesterol in germ cells with a corresponding decrease in esterified cholesterol in PRL treated mature monkeys suggests the possible PRL mediated formation.

![Fig. 3. Effects of prolactin and bromocryptine on Leydig, Sertoli and germ cellular free and esterified cholesterol in immature bonnet monkeys. Each value is mean±SEM for 10 animals. (b) P<0.01; (c) P<0.001; Control ■ Vs. Experimental □ 1. Free cholesterol, 2. Esterified cholesterol](image)

![Fig. 4. Effects of prolactin and bromocryptine on Leydig, Sertoli and germ cellular free and esterified cholesterol in mature bonnet monkeys. Each value is mean±SEM for 10 animals. (b) P<0.01; (c) P<0.001; Control ■ Vs. Experimental □ 1. Free cholesterol, 2. Esterified cholesterol](image)

![Fig. 5. Effects of prolactin and bromocryptine on Leydig, Sertoli and germ cellular total glyceride glycerol in immature and mature bonnet monkeys. Each value is mean±SEM for 10 animals. (a) P<0.05; (b) P<0.01; (c) P<0.001; Control ■ Vs. Experimental □ L—Leydig cell; S—Sertoli cell; G—Germ cell](image)
of free cholesterol at the expense of esterified cholesterol. Besides, the increased cholesterol may partly be due to decreased utilization. Sertoli cell cholesterol was found to be resistant to change in PRL status compared to that of Leydig and germ cells.

Glycerides in testicular cells act as a reserve of energy which can be utilized by the germ cells as fuel for their energy requirements [29, 30]. The data on glyceride glycerol reveal a different picture from that of cholesterol. PRL has influenced the glyceride glycerol in almost all the cellular compartments of the testis. Since PRL favoured the accumulation of glyceride glycerol in the germ cells of both immature and mature monkeys, and an opposite effect to that of PRL was observed in PRL-deficient monkeys, it is suggested that PRL plays a direct and definite role in glyceride glycerol turnover in the germ cells. The increased glycerides in the germ cells was contributed to by increased amounts of mono, di and triacyl glycerols due to PRL. White et al. [30] have shown that the synthesis of phospholipid may occur at the expenses of glyceride glycerols. The increase in glyceride glycerol with a corresponding decrease in phospholipid content [31] of the germ cells suggest that the phospholipid synthesis might have been decreased by PRL and therefore resulted in the accumulation of glyceride glycerols. In addition to this, the accumulation of glyceride glycerols may also be due to the enhanced lipid synthesis. This could be supported by the finding that PRL favoured the NADPH supply, an essential co-factor in lipogenesis [31].

The glyceride glycerol pattern in the Leydig and Sertoli cells differ from that of germ cells in their response to PRL, as they exhibited an opposite trend. This may be due to the differential functional significance of glyceride glycerols in these compartments. It is known that lipid and lipid monkeys. This could be due to the significant increase in the number (p<0.001) of its fractions after PRL treatment. However, when bromocryptine was administered, an opposite action of PRL was observed (Fig. 5, 6 & 7).
Discussion

The data obtained in the present study reveal that PRL plays a definite role in the regulation of testicular lipid metabolism. However, the impact of PRL on lipid was different in various cell compartments of the testis. While it favours the accumulation of lipids in the germ cells, it has an opposite effect on the Leydig cells. Unlike germ cells, the lipid content of the Leydig cells showed an age dependent response to PRL as it was evident only in mature monkeys. This may be attributed to the specific interaction of PRL with their own receptors which have been shown to increase during testicular maturation [23]. Besides this, it may also be due to increased gonadotrophin receptors [24] and the enhanced Leydig cell response towards LH, since PRL has been shown to increase the sensitivity of LH receptors to LH action [25–27]. However, there was an opposite effect of PRL after bromocryptine treatment, further strengthening the possible direct action of PRL on different lipid classes.

In testicular cells, the synthesis of androgenic hormones is associated with the mobilization of cholesterol esters, and conversely the inhibition of androgenic production causes the accumulation of esterified cholesterol [28]. LH administration caused an increase in androgen production, partial restoration of spermatogenesis and a reduction in free and esterified cholesterols in the testis of mouse [28]. PRL administration to hypophysectomized and hereditary dwarf mice increased the availability of esterified cholesterol in the testis for steroidogenesis [9, 28]. In the present study the unaltered cholesterol concentration in the Leydig and Sertoli cells of immature monkeys implied that PRL has no obvious impact on cholesterol turnover in these cell types. This was further supported by the unaltered levels of Leydig cellular steroidogenic enzymes and serum testosterone following PRL and bromocryptine administration in immature monkeys [14]. Probably the unaltered steroidogenesis could account for the unaltered levels of cholesterol in the Leydig cells.

On the other hand, in mature monkeys, PRL favoured the utilization of cholesterol for steroidogenesis as suggested by Bartke [9] concerning the mouse. This could be evidenced from the increased serum testosterone and steroidogenic enzymes in the same animals [14]. The enhanced concentration of total cholesterol in the germ cells of both maturity groups was contributed to by the stimulatory effect of PRL on free cholesterol in germ cells with a corresponding decrease in esterified cholesterol in PRL treated mature monkeys suggests the possible PRL mediated formation of free cholesterol at the expense of esterified cholesterol. Besides, the increased cholesterol may partly be due to decreased utilization. Sertoli cell cholesterol was found to be resistant to change in PRL status compared to that of Leydig and germ cells.

Glycerides in testicular cells act as a reserve of energy which can be utilized by the germ cells as fuel for their energy requirements [29, 30]. The data on glyceride glycerol reveal a different picture from that of cholesterol. PRL has influenced the glyceride glycerol in almost all the cellular compartments of the testis. Since PRL favoured the accumulation of glyceride glycerols in the germ cells of both immature and mature monkeys, and an opposite effect to that of PRL was observed in PRL-deficient monkeys, it is suggested that PRL plays a direct and definite role in glyceride glycerol turnover in the germ cells. The increased glycerides in the germ cells was contributed to by increased amounts of mono, di and triacyl glycerols due to PRL. White et al. [30] have shown that the synthesis of phospholipid may occur at the expenses of glyceride glycerols. The increase in glyceride glycerols with a corresponding decrease in phospholipid content [31] of the germ cells suggest that the phospholipid synthesis might have been decreased by PRL and therefore resulted in the accumulation of glyceride glycerols. In addition to this, the accumulation of glyceride glycerols may also be due to the enhanced lipid synthesis. This could be supported by the finding that PRL favoured the NADPH supply, an essential co-factor in lipogenesis [31].

The glyceride glycerol pattern in the Leydig and Sertoli cells differ from that of germ cells in their response to PRL, as they exhibited an opposite trend. This may be due to the differential functional significance of glyceride glycerols in these compartments. It is known that lipid and lipid classes in the testis are regulated by hypophysical hormones such a FSH and LH [5, 6, 32]. However, the data obtained in the present investigation suggest that PRL may have a direct inhibitory effect on glycerides in the Leydig and Sertoli cells.
This is indicated by the fact that PRL decreased these lipids in immature and mature monkeys, while bromocyrptine increased them, irrespective of changes in serum gonadotrophins and testosterone [14].

The close association between Sertoli and germ cells is well known [33]. It is also clearly established that Sertoli cells supply nutrient for germ cells [34]. Probably PRL facilitates the transport of glycerides from Sertoli cells to germ cells to meet the metabolic needs. This may result in the decrease in the amount of glycerides in Sertoli cells and their accumulation in germ cells under the influence of PRL. However, further confirmative studies on this are required.

In Leydig cells of immature and mature monkeys, the phospholipid levels were increased due to PRL administration [31]. The increased phospholipid content in the Leydig cells with a decrease in glyceride indicate that PRL might have facilitated the conversion of glycerides into phospholipid [30]. Early reports on rabbits and monkeys suggest that PRL favours the accumulation of triacyl glycerol in the liver [35, 36]. The data obtained in the present study clearly show that the influence of PRL on testicular cells is unique in exhibiting both stimulatory and inhibitory effects on triacyl glycerol, depending upon the cell type on which it acts. This may perhaps be due to the difference in the metabolic pattern of the testicular compartments to suit their functional needs.

In general, it is concluded that PRL has specific differential role to play in the regulation of neutral lipids in different testicular compartments of the monkeys. It favoured the mobilization of cholesterol for testosterone biosynthesis and glyceride glycerol for phospholipid synthesis in the Leydig cells. At the same time, PRL appeared to play a facilitatory role in neutral lipid accumulation in germ cells.

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