Note

Prolactin and Bromocryptine Induced Changes in Liver, Adipose Tissue and Blood Lipids of Mature Male Bonnet Monkeys, *Macaca radiata* (Geoffroy)

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Abstract. Effects of prolactin and bromocryptine on neutral and phospholipids of liver, adipose tissue and serum were studied in mature male bonnet monkeys. Hyperprolactinemia (ovine prolactin, 250 µg/kg body weight/day, i.p. for 30 days) elevated hepatic total lipids and phospholipids and decreased total and free cholesterol. While triacyl glycerol accumulated, mono- and diacyl glycerols diminished in liver and adipose tissues of prolactin treated monkeys. Concentrations of all phospholipid fractions, except sphingomyelin and cardiolipin in adipose tissue accrued in both tissues. Serum triacylglycerol, phosphatidyl ethanolamine, phosphatidyl choline and phosphatidyl inositol showed a significant increase in hyperprolactinaemic monkeys. Bromocryptine (1 mg/kg body weight/day for 30 days) treatment reduced serum phospholipids without altering hepatic or adipose tissue lipids. The present study indicates that hyperprolactinaemia leads to hyperlipidemia due to accretion of hepatic and adipose tissue triacyl glycerol and certain phospholipid fractions. Bromocryptine has a specific inhibitory effect on serum phospholipids.

Key words: Adipose tissue, Bromocryptine, Cholesterol, Glycerides, Liver, Prolactin, Phospholipids, Serum, Lipids.

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HYPERPROLACTINAEMIA is one of the major endocrine disorders, often associated with gonadal dysfunctions in men and women [1–4]. The physiological changes in reproductive and other endocrine organs in hyperprolactinaemia have been reviewed extensively [5–6]. Although prolactin is known to influence lipid metabolism in a number of vertebrates [7–10], the impact of hyperprolactinaemia on lipid metabolism, particularly in higher primates is not well understood.

Earlier, we reported the impact of hyperprolactinaemia on lung [11], testicular [12] and epididymal

lipids [13] of monkeys. In the present paper, we report the impact of experimentally induced hyper- and hypoprolactinaemia on various classes of lipids in liver, adipose tissue, the important sites of lipid metabolisms [14], and in blood, to obtain a comprehensive idea of the impact of altered prolactin status on lipid metabolism in primates. The study was carried out in bonnet monkeys, a close animal (primate) model for simulation of human diseases.

Materials and Methods

Chemicals

Ovine prolactin for biological studies was a gift from the National Institute of Diabetes, Digestive

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and Kidney Diseases (NIDDK), National Hormone and Pituitary Program, Baltimore, Maryland, USA. Bromocriptine mesylate was a gift from Sandoz Pharmaceuticals, Switzerland. Polyvinylpyrrolidone (PVP) and lipid standards were from Sigma Chemical Company, USA. All other chemicals were of analytical grade and purchased from Glaxo and BDH (India). Radioimmunoassay kit for prolactin was from the World Health Organisation.

Animals

Mature male bonnet monkeys, *Macaca radiata* (Geoffroy) weighing 8–10 kg were housed in a well ventilated animal house with a 12 h dark and 12 h light schedule and provided with clean water and standard monkey pellet diet (Gold Mohur, Hindustan Lever Ltd., India) *ad libitum*. The monkeys were divided into three groups of 5 each.

Group I: Monkeys belonging to this group were given a single dose of 250 μ g ovine prolactin/kg body weight/day, intraperitoneally, for 30 days to induce hyperprolactinaemia. 10% PVP in saline was used as the vehicle as it enhances hepatic prolactin binding [15].

Group II: Bromocriptine mesylate, an ergot alkaloid which inhibits pituitary prolactin release through β adrenergic receptors was injected at a dose of 1 mg/kg body weight/day, intraperitoneally, for 30 days to induce hypoprolactinaemia. Bromocriptine was dissolved in 0.9% alkaline saline.

Group III: This group served as control and was injected with 0.9% alkaline saline or PVP/day, for 30 days. As there was no appreciable difference between the lipid values obtained in the saline and PVP treated controls, a common control (PVP) was used for statistical analysis of data.

Blood sampling

Blood was drawn periodically from the femoral vein to assay prolactin in order to ascertain whether the animals had sustained hypo- and hyperprolactinaemia.

Serum prolactin, assessed on RIA double antibody technique with reagents supplied by the WHO, rose from 329 ± 62.4 mU/l in the control animals to 2640 ± 107.9 in the prolactin treated monkeys and fell to 26.4 ± 1.90 mU/l in the bromocriptine treated monkeys, confirming

hyper- and hypoprolactinaemia, respectively.

Twenty-four hours after the last injection, the animals were fasted overnight, blood was drawn from the femoral vein, the serum separated and used for the estimation of lipids. After this, the animals were anaesthetized with sodium pentobarbitone (30 mg/kg body wt.) and transcardially perfused with 0.9% saline till the liver was completely blanched.

Liver and adipose tissues were excised, cleared of adhering tissues and blood clots and stored at -20°C till used subsequently for biochemical analysis. All assays were completed within 1–2 wks of sacrifice.

Extraction and quantification of lipids

Lipids were extracted from known amounts of serum, liver and adipose tissue in a mixture of chloroform: methanol (2 : 1) [16], containing 0.01% butylated hydroxy toluene as an antioxidant was centrifuged thrice at $3000 \times g$ for 10 min, supernatants were concentrated *in vacuo* in a flash rotatory vacuum evaporator (Buchi, Switzerland) at $40-45^{\circ}\text{C}$ [16] and proteolipids were broken by repeating the above process. Total lipids were quantified with vanillin in a medium of sulphuric and phosphoric acids to form chromogen [17], and cholesterol quantification was based on Tachugaeff's color reaction [18]. Estimation of glyceride glycerol involved saponification of glycerides, oxidation of glycerol to formaldehyde and formic acid and estimation of the formaldehyde chromotropic acid color complex [19]. Phospholipids [20] were estimated by following the reduction of phosphomolybdic acid with amino naphthosulphonic acid. Neutral and Phospholipid fractions were separated by thin layer chromatography on silica gel G plates, using n-hexane: diethyl ether: glacial acetic acid [21] and chloroform: methanol: 7N ammonia solvent systems [22], respectively.

The recovery percentages for the individual fractions of neutral and phospholipids were: Monoacyl glycerol 88%, diacyl glycerol 93%, triacyl glycerol 97%, free cholesterol 97%, esterified cholesterol 98%, phosphatidyl inositol 96%, phosphatidyl serine 96%, phosphatidyl choline 95%, phosphatidyl ethanolamine 97%, phosphatidic acid 87%, sphingomyelin 95% and cardiolipin 96%.

Statistical analysis

The data were statistically analysed by Student's *t* test.

Results

Hyperprolactinaemia caused a significant increase in hepatic total lipids ($P < 0.05$) without altering the same in serum and adipose tissue. Even though total glyceride glycerol remained unaltered, mono- and diacyl glycerol concentrations decreased and triacyl glycerol increased in liver and adipose tissue due to prolactin treatment. Hyperprolactinaemia increased serum triacylglycerol also. Total and free cholesterol diminished in the liver alone. Bromocryptine treatment did not produce any significant change in neutral lipid classes of liver, adipose tissue and serum (Table 1).

Hepatic and adipose tissue phospholipids (total) and its fractions, accumulated in response to prolactin treatment except sphingomyelin and cardiolipin in adipose tissue. Serum phosphatidylcholine, phosphatidyl ethanolamine and phosphatidyl inositol also exhibited increases in hyperprolactinaemic monkeys. Bromocryptine induced hypoprolactinaemia decreased these phospholipids in serum. Even though, serum phosphatidyl serine was low in hypo- and hyperprolactinaemic monkeys, the bromocryptine induced decrease was more obvious than hyperprolactinaemia (Table 2).

Discussion

The data obtained in the present study demonstrate that chronic hyperprolactinaemia modifies the tissue and serum lipid pattern in primates. Hypertriglyceridemia and accumulation of phospholipids in liver, adipose tissue and serum seems to be a major effect of chronic hyperprolactinaemia. The observed hypertriglyceridemic effect in hyperprolactinaemic monkeys is therefore parallel to that reported in hyperprolactinaemic human subjects [23].

Short term prolactin treatment (1 mg/kg body weight/day for 10 days) also revealed accumula-

tion of hepatic glycerol stores and phospholipids in immature male monkeys [24]. The above study also showed enhanced activities of hepatic lipogenic enzymes like ATP citrate lyase, malic enzyme, malate dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase indicating a lipogenic effect of prolactin on primate liver. Additional evidence of a lipogenic effect of prolactin can also be obtained from studies on the mammary glands and monkey brain [25–27]. Nevertheless, one has to be cautious in arriving at such conclusions as reduced triacylglycerides is reported in the lungs [11], testes [12] and epididymis [13] of hyperprolactinaemic monkeys, suggesting an organ specific effect.

Unlike glycerides, the cholesterol pool is adversely affected in hyperprolactinaemic monkeys. The observed reduction in total and free cholesterol in the liver is similar to earlier reports on the testes [12] and lungs [11] of hyperprolactinaemic monkeys. This may point out a differential effect of hyperprolactinaemia on the two classes of neutral lipids. The present study also reveals the specific stimulatory influence of hyperprolactinaemia on hepatic, adipose tissue and serum phospholipid classes. Phospholipids and cholesterol play a vital role in membrane function and are essential modulators of membrane bound enzymes [28]. Therefore, the observed reduction in cholesterol and accumulation of phospholipids in liver and adipose tissue of hyperprolactinaemic monkeys is suggested to modify membrane permeability and related metabolic activities by altering the cholesterol: phospholipid ratio. Latha, [24] also reported enhanced hepatic phospholipids and its fractions in prolactin treated immature male bonnet monkeys. This effect of prolactin is specific to hepatic and adipose tissues and serum as major classes of phospholipids have remained unchanged in the lungs [11] testes, [12] and decreased in the epididymis [13] of hyperprolactinaemic monkeys.

Liver contains a high concentration of prolactin receptor [29, 30] and prolactin has a positive effect on its own receptor [31, 32]. Therefore, the observed effect on neutral and phospholipids could be due to a direct effect of prolactin on liver. Nevertheless, the indirect effect mediated through insulin cannot be ruled out as prolactin acts like insulin in enhancing hepatic lipogenic enzyme activities although serum insulin titres were unaltered in hyperprolactinaemic monkeys [24, 33].

Table 1. Effects of hyper- and hypoprolactinaemia on serum, liver and adipose tissue total lipids, cholesterol and glyceride glycerol and their fractions in mature male bonnet monkeys, *Macaca radiata*

Tissue	Status	Total lipids	Total cholesterol	Free cholesterol	Esterified cholesterol	Total glyceride glycerol	Monoacyl glycerol	Diacyl glycerol	Triacyl glycerol
Liver	Control	7.1 ± 0.68	17.4 ± 1.25	5.8 ± 0.42	7.5 ± 0.62	25.0 ± 1.07	4.4 ± 0.22	7.85 ± 0.65	10.8 ± 0.58
	Prolactin	10.8 ± 0.90 ^A	9.0 ± 0.65 ^C	0.8 ± 0.08 ^C	8.2 ± 0.81	27.0 ± 0.89	2.8 ± 0.10 ^C	3.36 ± 0.48 ^C	20.6 ± 0.38 ^C
	Bromocryptine	6.8 ± 0.56	17.0 ± 0.65	5.3 ± 0.26	7.7 ± 0.65	23.0 ± 0.56	4.3 ± 0.30	7.80 ± 0.54	9.8 ± 0.45
Adipose	Control	41.8 ± 4.00	15.0 ± 1.20	5.3 ± 0.40	8.7 ± 1.20	324.0 ± 40.00	88.0 ± 5.00	104.0 ± 8.00	130.0 ± 5.00
	Prolactin	42.5 ± 3.90	11.0 ± 2.40	3.5 ± 1.80	7.5 ± 1.60	302.0 ± 55.00	35.0 ± 6.00 ^C	30.0 ± 6.00 ^C	244.0 ± 6.00 ^C
	Bromocryptine	37.8 ± 4.10	12.0 ± 2.10	4.3 ± 0.80	7.7 ± 1.20	312.0 ± 43.00	90.5 ± 5.00	102.0 ± 8.00	124.0 ± 4.00
Serum	Control	275.0 ± 18.00	0.76 ± 0.14	0.32 ± 0.05	0.48 ± 0.04	0.97 ± 0.13	0.17 ± 0.01	0.36 ± 0.03	0.48 ± 0.03
	Prolactin	300.0 ± 25.00	0.83 ± 0.10	0.34 ± 0.05	0.55 ± 0.05	1.18 ± 0.18	0.15 ± 0.02	0.32 ± 0.03	0.60 ± 0.04 ^A
	Bromocryptine	256.0 ± 15.00	0.63 ± 0.10	0.29 ± 0.04	0.43 ± 0.04	0.95 ± 0.15	0.16 ± 0.01	0.33 ± 0.03	0.41 ± 0.03

Each value is the mean ± SEM for 5 estimations. Total lipids are expressed as mg/100mg tissue and mg/100ml serum and other fractions of cholesterol and glycerol as mg/kg tissue and mg/ml serum. Control *vs.* experimental ^A, *P*<0.05; ^C, *P*<0.001.

Table 2. Effects of hyper- and hypoprolactinaemia on serum, liver and adipose tissue phospholipid and its fractions in mature male bonnet monkeys, *Macaca radiata*

Tissue	Status	Total phospholipid	Phosphatidyl inositol	Phosphatidyl serine	Sphingomyelin	Phosphatidyl choline	Phosphatidyl ethanolamine	Cardiolipin	Phosphatidic acid
Liver	Control	16.02 ± 0.98	3.70 ± 0.24	0.91 ± 0.09	4.04 ± 0.24	3.86 ± 0.23	1.69 ± 0.12	0.74 ± 0.06	2.11 ± 0.10
	Prolactin	24.00 ± 1.50 ^B	5.69 ± 0.28 ^C	1.86 ± 0.10 ^C	3.87 ± 0.18	5.71 ± 0.28 ^C	2.25 ± 0.09 ^B	0.98 ± 0.07 ^A	3.26 ± 0.20 ^C
	Bromocryptine	14.00 ± 0.94	3.65 ± 0.24	0.82 ± 0.08	3.59 ± 0.27	3.78 ± 0.25	1.46 ± 0.11	0.74 ± 0.04	1.92 ± 0.16
Adipose	Control	7.02 ± 0.65	1.02 ± 0.05	0.70 ± 0.06	1.20 ± 0.10	1.20 ± 0.07	2.10 ± 0.08	0.38 ± 0.05	0.91 ± 0.09
	Prolactin	12.44 ± 0.97 ^B	1.50 ± 0.09 ^B	1.10 ± 0.09 ^B	1.40 ± 0.11	2.60 ± 0.09 ^C	2.80 ± 0.12	0.50 ± 0.06	2.32 ± 0.18 ^C
	Bromocryptine	6.60 ± 0.64	0.81 ± 0.08	0.60 ± 0.08	1.00 ± 0.09	1.30 ± 0.07	1.90 ± 0.09 ^B	0.36 ± 0.03	0.90 ± 0.05
Serum	Control	0.85 ± 0.08	0.14 ± 0.003	0.12 ± 0.006	0.13 ± 0.009	0.13 ± 0.003	0.12 ± 0.006	0.082 ± 0.008	0.12 ± 0.01
	Prolactin	1.00 ± 0.08	0.15 ± 0.003 ^A	0.10 ± 0.005 ^A	0.12 ± 0.008	0.21 ± 0.001 ^C	0.23 ± 0.012 ^C	0.074 ± 0.010	0.09 ± 0.01
	Bromocryptine	0.78 ± 0.11	0.13 ± 0.003 ^A	0.02 ± 0.001 ^C	0.12 ± 0.008	0.12 ± 0.001 ^A	0.08 ± 0.002 ^C	0.080 ± 0.008	0.13 ± 0.01

Total phospholipids and its fractions are expressed as mg/g tissue and mg/ml serum. Each value is the mean ± SEM for 5 estimations. Control *vs.* experimental ^A, *P*<0.05; ^B, *P*<0.01; ^C, *P*<0.001.

The data on neutral and phospholipid fractions suggest that hyperprolactinaemia favours the shunting of diacyl glycerol for the synthesis of triacyl glycerol and phospholipids as the diacyl glycerol concentration decreased in prolactin treated monkeys. It is well-established that diacyl glycerol serves as a precursor for triacyl glycerol, phosphatidyl choline and ethanolamine [14]. Despite an increased concentration of phosphatidyl serine in the liver and adipose tissues of hyperprolactinaemic monkeys, serum fractions decreased, possibly indicating interference with the release or uptake of this phospholipid class in the hyperprolactinaemic condition. The observed unchanged sphingomyelin in hyper- and hypoprolactinaemic conditions suggest that this phospholipid fraction is resistant to altered prolactin status. Further, *in vitro* studies on the role of prolactin on lipogenesis in isolated cell types may throw more light on this aspect.

The effect of bromocriptine is felt mainly on serum phospholipids such as phosphatidyl inositol, choline and ethanolamine, which is opposite to hyperprolactinaemia. This might be a direct effect of

bromocriptine, specific to serum lipids, as hepatic and adipose tissue fractions were not altered. Additional supportive data on *in vitro* effects of bromocriptine on blood cells may clarify this.

The present study therefore, points out hypertriglyceridemia and accumulation of phospholipid stores in hyperprolactinaemic monkeys, leading to hyperlipidemia.

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