

# Effects of induced-hypo and hyper-prolactinemia in male rabbit's liver

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**Abstract**— Prolactin is a polypeptide hormone secreted from of the anterior pituitary gland. The hormone levels can result from physiological causes, such as pregnancy and stress, or pharmacological causes, including the use of neuroleptics and opiates. Prolactin influences a large spectrum of mammalian tissues, including the mammary glands, gonads, immune cells, and liver's function and growth.

This study was performed on New Zealand male rabbits with access *ad libitum* to food and water until the end of the experiment (30 days). The animals divided into three groups were daily injected with sulpiride (7, 5 mg/ml/kg), bromocriptine (400 µg/ml/kg), or saline solution (control group). Animals were decapitated and liver homogenates prepared. The level of total prolactin was determined in serum and liver tissue homogenates of normal, bromocriptine and sulpiride- treated male rabbits by an Immunoradiometric assay (IRMA). The characteristics of the binding of  $I^{125}$  labeled anti-prolactin antibody with prolactin in liver homogenates were investigated using the technical basis of radio receptor assay (RRA) and different factors were studied (antigen concentration, antibody concentration, pH, time, temperature). The activity of two enzymes, glutamic pyruvic transaminase (GPT) and alkaline phosphatase (AP) was determined in liver homogenates of the studied groups with appropriate kits.

This study shows the presence of the prolactin in male rabbit's liver with a significant increase ( $P < 0.01$ ) of hormone levels in serum and liver homogenate of the group treated with sulpiride compared to control group. However, in bromocriptine group, there was a significant decrease ( $P < 0.01$ ) in prolactin levels after the day 15. The study of  $I^{125}$  labeled anti-prolactin antibody with prolactin complex confirmed that it is unstable and temperature-dependant. The protein concentration and relative liver weights increases significantly ( $P < 0.05$ ) in the sulpiride group. Our results indicated an alteration of enzyme activities following prolactin levels in serum and liver homogenates especially in the case of GPT.

We conclude that the administration of sulpiride affects metabolic prolactin functions on rabbit's liver. In addition, the ability to measure free hormone by the IRMA method in the liver could help to investigate prolactin in other healthy tissues or in pathological cases such as breast cancer.

**Keywords**— Prolactin; liver; rabbit; metabolic effects; IRMA; enzymes.

## I. INTRODUCTION

Prolactin is a polypeptide hormone that is synthesized and secreted from specialized cells of the anterior pituitary gland, the lactotrophs [1]. Its major action is lactogenesis (milk

production), but it seems to play an important role in implantation and subsequent placentation in the human endometrium, [2] and it is recognized as a crucial signal for the initiation and maintenance of decidualization [3]. It can either be categorized as reproductive, metabolic, osmoregulatory and immunoregulatory factor [6].

Prolactin levels can result from physiological causes, such as pregnancy and stress, or pharmacological causes, including the use of neuroleptics, estrogens, opiates, antihypertensive drugs or calcium channel blockers [4].

Dopamine, transported from the hypothalamus to the anterior pituitary by hypophyseal portal vessels, inhibits prolactin secretion via D2 receptors expressed by lactotrophs, and its disrupted secretion or transport can lead to hyperprolactinaemia [5].

PRL receptors (PRLRs) are expressed in nearly all organs, in lactiferous gland, gonads, uterus, prostate, adrenal glands, kidneys, pancreas, lymphatic tissue, thymus, heart, and liver. [11, 12].

The liver cells have low capacity for rapid proliferation but there is evidence that prolactin play a role in hepatic growth and turnover by its association with a high affinity receptors in hepatic cells in rat [7, 8] and mammals [9] and seems to stimulate the growth of liver cells *in vitro* [10].

In the liver, prolactin also intensifies glycogen phosphorylase activity, bile secretion, and DNA synthesis [13], and it plays the role of natural mitogen to stimulate the liver regeneration process [14].

The aim of the present study is to determine the influence of bromocriptine and sulpiride on prolactin hormone concentrations in serum and livers of male rabbits, through the evaluation of the content of liver tissues to detect the influences of these dopamine's agonist and antagonist on some physiological parameters represented by weight, protein and liver enzyme activities.

## II. MATERIAL AND METHODS

### A. Animals

This study was performed on New Zealand male rabbits (n=24) weighing between 1.5 and 2.2 Kg, housed in closed

controlled temperature cages with access *ad libitum* to food and water until the end of the experiment (30 days). The animals divided into three groups, were daily injected subcutaneously, with sulpiride (7, 5 mg/ml/kg), bromocriptine (400 µg/ml/kg), or saline solution (control group).

Every seven days, blood samples were taken by a small wound in the marginal ear vein, after sterilization by alcohol, blood was centrifuged at 3500 x g for 10 minutes. The serum was saved at - 20 °C until use.

#### B. Preparation of Liver tissues

At the end of experimentation, the animals were decapitated and the liver tissues were extracted, weighted and saved at - 20 °C until use.

Liver tissues were sliced and homogenized with manual homogenizer using cooling saccharose solution (300 mmol/L), filtered through layers of nylon gauze, then centrifuged at 4000 rpm for 30 min in a cooling centrifuge. The supernatant was diluted with Tris-HCl-MgCl<sub>2</sub> buffer and freeze until the time of experiments.

#### C. Buffers

All buffer solutions were prepared by dissolving the appropriate amount of salt in distilled water and the required pH was adjusted. While, the experimental solutions were prepared by dissolving 20mg of bromocriptine (Amon pharm, Egypt) or 375mg of sulpiride (biopharm, Algeria) in 10% acetic acid and completed till 50 cm<sup>3</sup> with saline solution.

#### D. Prolactin and protein concentrations

Prolactin concentration was measured in serum using Immunoradio metric assay (I.R.M.A) using the standard curve according to [17], in accordance with the steps mentioned in the assay kit Immunotech Inc. French. (Beckman coulter company) .The radioactivity emitted from the I<sup>125</sup> was measured in gamma counter (1270 – Rack gamma II).

Prolactin concentration was also estimated in liver homogenates of all experiment animals by IRMA method. While, the total homogenate protein content was determined by the method of Lowry using bovine serum albumin as the internal standard [18].

#### E. Factors affecting the binding of prolactin with I<sup>125</sup> labeled anti-prolactin antibody in liver homogenates

The study of the binding of prolactin to its labeled antibody in liver homogenates of the experimental groups is quite necessary to establish the most appropriate conditions that lead to maximum specific binding, so the quantity of prolactin, and the labeled anti-prolactin were determined .Therefore, the effect of pH and temperature on the extent of this binding were also investigated .

#### F. Enzyme activities

The glutamic pyruvic transaminase (GPT) activity was determined with the commercial Kit protocol provided by (Randox laboratories LTD, England) after incubation of 0.1cm<sup>3</sup> of liver homogenates of all experimental groups with appropriate reagent and measurement of absorbance at 546 nm after 5min.

The alkaline phosphatase (AP) activity was assessed using the protocol of the commercial Kit provided by (Biomerieux Sa Company, France) after incubation of 50µL of liver homogenates with appropriate reagent and measurement of absorbance at 510 nm.

#### G. Calculations

The values of the ratio B/F for the incubation of different amounts of prolactin, anti-prolactin, pH and temperatures were calculated for liver homogenates as follow:

B: The bound radioactivity in counts per minute (CPM), which represents the I<sup>125</sup> labeled anti-prolactin antibody.

T: Total count radioactivity in CPM.

The comparison of mean between treatment groups and control group was assessed by Student's t-test. The data were expressed as mean ± standard deviation (Mean ±SD).

### III. RESULTS

#### A. prolactin concentrations

The daily treatment of rabbits with bromocriptine (400 µg/ml/kg) resulted in a significant decrease ( $P \leq 0.05$ ) in the concentration of prolactin in serum after the seventh day of injection, and highly significant decrease ( $P \leq 0.01$ ) after the 15th day, compared to the control group. However, the administration of (7.5 mg/ml/kg) of sulpiride in male adult rabbits caused a significant increase ( $P < 0.01$ ) in the concentration of serum prolactin after the first week of treatment (Fig. 1).

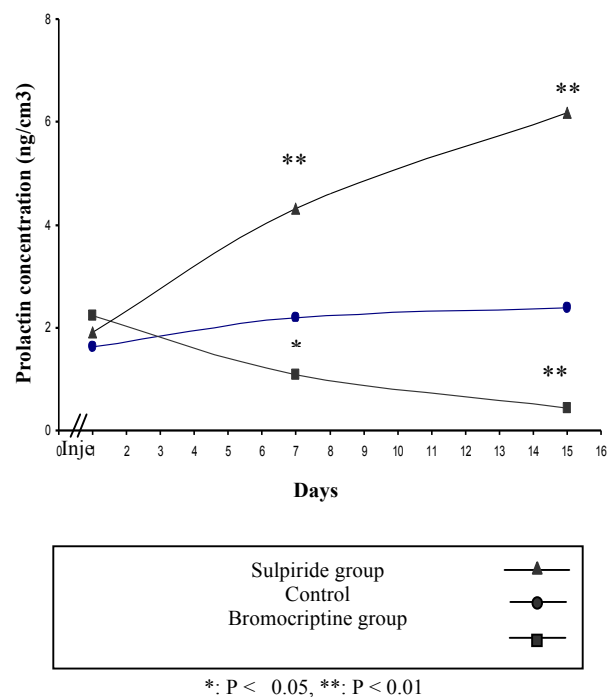
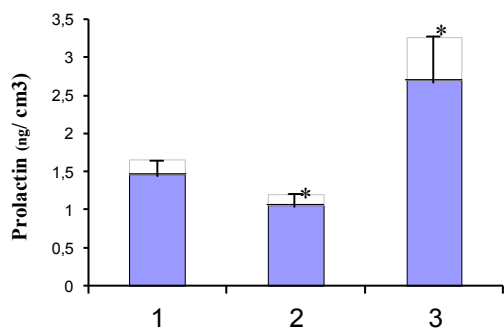


Fig. 1 . Prolactin concentrations in male rabbit's serum



\*:  $P \leq 0.05$ , \*\*:  $P \leq 0.01$   
 1: control group, 2: bromocriptine, 3: sulpiride group

Fig. 2. Prolactin concentration in male rabbit's liver homogenates

Fig. 2, shows a significant decrease ( $P \leq 0.05$ ) of prolactin concentrations in liver homogenates of the group treated by bromocriptine compared to control. While, the injection of sulpiride resulted in a significant increase in hormone concentrations in liver homogenates male rabbits.

*B. Factors affecting the binding of  $I^{125}$  labeled anti-prolactin antibody with prolactin in liver homogenates*

- To determine whether the specific binding was proportional to the amount of prolactin concentration, increasing amounts of the hormone in liver homogenates were incubated with a specific concentration of labeled anti-prolactin for one hour. Fig. 3, shows the increasing values of the specific bindings with increasing amount of the homogenate added to the incubation medium especially for the control and bromocriptine groups. 25 microgram's of liver of sulpiride treated animals and control group, and 37.5 microgram's was shown to give maximum value of specific bindings.

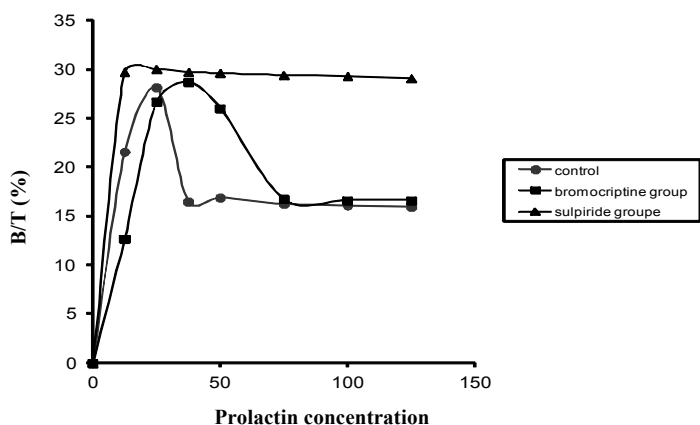


Fig. 3 . Effect of protein concentration on the binding with  $I^{125}$  labeled anti-prolactin in liver homogenates

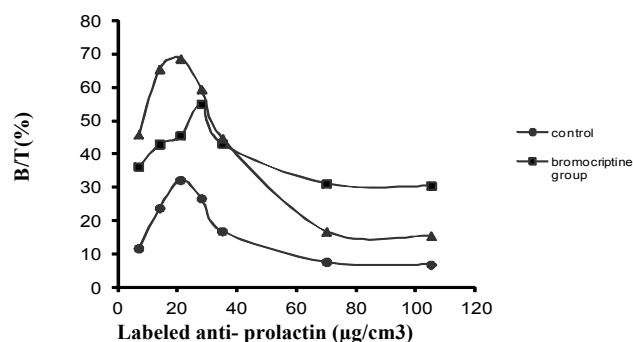


Fig. 4. Effect of different concentrations of anti-prolactin on the binding with prolactin in liver homogenates

- To estimate the suitable concentration of anti-prolactin antibody, the experiment was carried out in the presence of 25 µg of liver homogenates for control ad sulpiride group, and 37.5 µg for bromocriptine group. Our results show that the specific binding of prolactin to labeled anti-prolactin is a saturable process. Moreover, the specific binding of the sulpiride group was higher than the others (Fig. 4).
- The analysis of the effect of pH on labeled anti-prolactin with the hormone in liver homogenates shows that the optimum pH was found to be 7.6 in both control and sulpiride treated groups, while the optimum pH for the bromocriptine group was 7.4
- To determine the role of temperature in the binding of prolactin with  $I^{125}$  labeled anti-prolactin antibody in liver homogenates, optimal concentrations of prolactin and anti-prolactin were incubated for an hour with different temperatures (4° to 45 °C). Our results shows that the maximum value of the specific binding occurs at 4°C for the sulpiride group, however the optimum bindings for the control and bromocriptine groups, was observed at 45° and 37°C (Fig. 5).

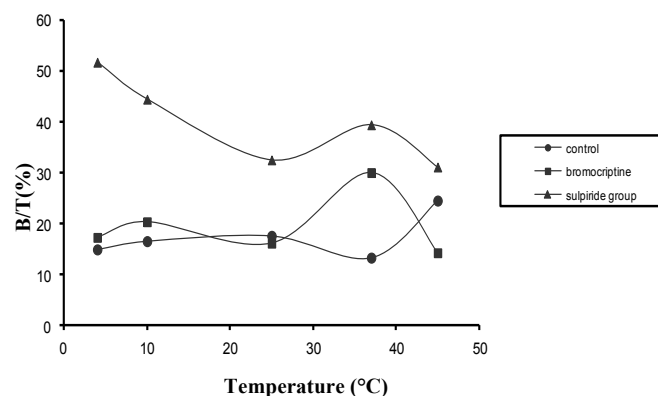


Fig. 5. Effect of temperature on the binding with  $I^{125}$  labeled anti-prolactin in liver homogenates

*C. Effect of prolactin on the functional activity of liver tissue*

The present data show the absence of a significant difference in the relative weight of the liver (g/Kg body weight) and protein concentration in animals treated with bromocriptine, despite there was a weight loss, however a significant increase ( $P \leq 0.05$ ) in the liver's weight and protein concentration was observed in animals injected with sulpiride (Table 1).

In addition, our results show a great change in liver's enzyme activities of GPT and alkaline phosphatase after administration of either bromocriptine or sulpiride to male rabbits. The GPT activity was higher in sulpiride-treated animals (126 U/L) despite there was no significant difference with the control group. At the contrast, the AP activity was the lowest in liver's animals injected with sulpiride (Table 1).

TABLE I. EFFECTS OF PROLACTIN ON LIVER ACTIVITIES OF MALE RABBITS

	Animals		
	Control group	Bromocriptine group	Sulpiride group
Liver's relative weight	2.56 ± 0.36	1.95 ± 0.38	3.39 ± 0.52
Protein concentration (mg/cm <sup>3</sup> )	2.038 ± 0.42	0.52 ± 1.53	3.55 ± 0.8
GPT activity (U/L)	97.33 ± 2.46	60.33 ± 3.51	126 ± 2.5
GPT specific activity	47.75	39.35	35.49
AP activity (King/100 cm <sup>3</sup> )	38.62 ± 1.95	8.91 ± 1.32	7.01 ± 0.41
AP specific activity	18.95	5.81	1.97

IV. DISCUSSION

*A. prolactin concentrations in serum and liver homogenates*

The effect of bromocriptine on prolactin serum concentration is consistent with many previous studies that used bromocriptine as a strong inhibitor of prolactin secretion in many animals as female rabbits [21], male rats [22], and even in human [23,24].

Bromocriptine is an ergotamine derivative, a dopamine receptor agonist that prevents the release of prolactin from the anterior pituitary gland [20], by inhibition of prolactin gene transcription process by influencing the level of cyclic AMP or by increasing the cellular degradation of this hormone [26].

As expected from a dopamine receptor antagonist, sulpiride-treated rabbits displayed hyperprolactinemia, this is in agreement with the results of other investigations on female rabbits [27], male rabbits [17,23], and even postmenopausal women [28].

Sulpiride is a substituted benzamide which selectively blocks postsynaptic dopaminergic neurons. It has been shown to be a potent stimulator of prolactin release whether by oral or

intramuscular administration, even at the low doses (3mg) in healthy volunteer's women [29].

*B. Factors affecting the binding of I<sup>125</sup> labeled anti-prolactin antibody with prolactin in liver homogenates*

The specificity of the interaction between antigen and antibody show close relationship between the two compounds, which depends on many factors that may affect their strong binding. One of the most important criteria of the true complex binding is saturability, but complete saturation however is theoretically never reached unless the amount of prolactin used reached infinity [38].

Our data show that the specific binding of the prolactin with labeled anti-prolactin antibody in liver homogenates of the sulpiride group is high compared to control group, and are in agreement with those obtained by other authors who reported that anti prolactin titers are directly proportional with prolactin levels in hyperprolactinemic idiopathic patients. [39]

The analysis of the effect of pH on the prolactin binding with I<sup>125</sup> labeled anti-prolactin antibody indicates that the shift in the pH of the environment may affect the properties of the macromolecules involved in the binding. Our results are inconsistent with those obtained by [36], who reported that the administration of either bromocriptine or sulpiride does not affect the optimum pH for prolactin binding with its receptors in prostate rabbit homogenates.

The present report show a difference in optimum temperature among the different groups, depending on the nature and concentration of reactive molecules including prolactin that may be changed as a result of treatment with bromocriptine or sulpiride. This result doesn't agree with other experiments which reported that the optimum temperature for the binding of the labeled prolactin was performed at 25°C [40].

*C. Effect of prolactin on the functional activity of liver tissue*

The present report is the first showing the assessment of prolactin level in liver homogenates of male rabbit's by the I.R.M.A method especially after induced-hypo and hyperprolactinemia, confirming the presence of prolactin in male rabbit's liver cells and that indicates that this organ is the goal of prolactin and is important in the regulation of physiological processes, such as growth [19]. Therefore, a liver histological study in male rabbits showed that there was a significant increase in the number of dinucleated parenchyma cells, significant increase in the number of Kupffer's cell and in the number of liver parenchyma diameters [17].

According to our data, the significant increase in the liver's relative weight in induced- hyperprolactinemic male rabbits, agree with previous study which reported that prolactin increase the number and size of prostate gland cells [34], as well as spleen weight in mammals [35].

Previous studies reported that chronic elevation of prolactin, induced by dopamine antagonists, was associated with increased food intake and body weight, and the following effects of sulpiride have been proposed to induce weight gain in female rats: the blockade of dopamine D<sub>2</sub> receptors in the

perifornical lateral hypothalamus [30]; a decrease in serum estradiol levels due to hyperprolactinemia or to a direct drug effect in the hypothalamus [31]; and sulpiride-induced changes in insulin sensitivity [32]. Controversially, the chronic sulpiride administration does not affect body weight, food intake and water intake in adult males rats [33].

In addition, the administration of sulpiride significantly increases the protein concentration in liver homogenates. These results are in agreement with those obtained by other investigators [36], who reported that the injection of sulpiride increased ( $P < 0.01$ ) the protein concentration in prostate rabbit homogenates, when compared to control group.

Both aminotransferase, are liver enzymes indicator whose activities increases in serum as a result of cell structure damage and changes in membrane permeability [15].

From the review of the literature, we confer that no studies have been conducted on enzyme activities in liver homogenates and despite the non significant rise of GPT activity in liver of sulpiride-treated male rabbits observed in the present report, our results may indicate that hepatic alterations are prominent after administration of sulpiride. Furthermore, it is well established that increased prolactin levels after sulpiride administration exerts down regulating control on several P450 isoforms in liver and may cause adverse effects, especially in the modification of the function of major signal transduction pathways involved in the regulation of P450 with crucial importance in the metabolism of numerous prescribed drugs, toxicants, and carcinogens [19].

Controversially, previous experiments indicated that prolactin significantly slows aminotransferase release from the liver into the preservation solution, implying hepatoprotective properties. The passage of these enzymes from hepatocytes into the preservation solution shows the cytolysis of hepatocytes, which is a consequence of liver destruction. Prolactin slows this process [15]. And it directly or indirectly accelerates the regeneration of rat liver while maintaining its function [16].

In addition, our data show that the alkaline phosphatase activity was the lowest in liver's animals injected with sulpiride, and are inconsistent with those obtained by others who reported that a dose of 500 microgram/kg of bromocriptine reduce prolactin plasma levels and lead to raise of alkaline phosphatase activity in the pituitary homogenates of lactating or post-lactating rats by 4 times [37], this is probably due to the species and sex difference.

In conclusion, the present report describes a useful experimental model for the assessment of free prolactin to investigate hormone effects in the liver, or in other healthy tissues or in pathological cases. Furthermore, the administration of sulpiride affects metabolic prolactin functions on male rabbit's liver, but the mechanisms by which prolactin levels impaired liver enzyme activities need further studies to define the exact role of dopamine -agonists and antagonist at the molecular level in hyperprolactinemic states and their connection with enzymes.

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