

## The effects of prolactin and corticosterone on insulin binding to rat Leydig cells

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### SUMMARY

The influence of prolactin (PRL) and corticosterone on insulin binding to purified rat Leydig cells was assessed *in vitro*. The lowest dose of PRL (50 ng/ml) increased ( $p < 0.05$ ) and the remaining PRL concentrations (100, 150, 200, 250 ng/ml) decreased ( $p < 0.05$ ) the insulin binding to Leydig cells. All doses of corticosterone (150, 200, 250, 300 ng/ml) except the lowest one (100 ng/ml) decreased the insulin binding. In conclusion, hyperprolactinemia or excess glucocorticoids associated with an impairment of testicular steroidogenesis may be mediated by a defective insulin binding to Leydig cells. *Reproductive Biology* 2009 9 2: 189-194.

**Key words:** prolactin, corticosterone, Leydig cells, insulin receptor

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## INTRODUCTION

Leydig cells, the main source of testosterone production in mammals, are regulated by various factors. Besides luteinizing hormone (LH), the function of Leydig cells is influenced by insulin [3, 4, 8, 10, 12]. Moreover, hyperprolactinemic men have shown a reduction in LH receptor number in Leydig cells [13]. An excess of glucocorticoids due to Cushing's syndrome in men was reported to be associated with a decreased number of Leydig cells and testicular steroidogenesis [7]. However, there is no data on insulin binding to Leydig cells under pathophysiological conditions. The present study was designed to test whether insulin binding by rat Leydig cells is affected by prolactin (PRL) and corticosterone.

## MATERIALS AND METHODS

The culture medium used in the present study was Dulbecco's Modified Eagle's Medium supplemented with F12+Nutrient mixture (1:1; DMEM). The reagents and chemicals used in the study included collagenase, nicotinamide adenine dinucleotide, bovine serum albumin, nitro blue tetrazolium sodium salt (NBT), dehydroepiandrosterone, corticosterone, fetal calf serum (FCS) and porcine insulin were purchased from Sigma (St. Louis, USA). Rat PRL was obtained as a gift from the National Institute of Diabetes and Digestive and Kidney Diseases, National Hormone and Pituitary Program, Baltimore, Maryland (USA). Percoll was obtained as a gift from Fluka (Switzerland). Sephadex G-25 and G-75 were purchased from Pharmacia Fine Chemicals (Sweden). Radioactive iodine was purchased from Bhaba Atomic Research Center (Bombay). Other chemicals were purchased from British Drug House (England and India), Merck (Germany) and Glaxo (India).

Adult male albino rats (120-150 days; Wistar strain; 200-250 g) were purchased from the Fredrick Institute of Plant Protection and Toxicology, Padappai, Tamilnadu, India. The animals were kept in 14 h light/10 h dark regime and pelleted rat feed (Gold Mohur, Lipton India Ltd., India) and clean

water were available *ad libitum*. The experiments were approved by the Local Ethics Committee and were in compliance with the national guidelines for care and use of animals. The animals were sacrificed after intra peritoneal general anaesthesia (sodium thiopental). Each pair of testes were aseptically decapsulated with fine forceps. Leydig cells were isolated and purified by Percoll density gradient [10]. Testes were incubated in 45 ml of polypropylene centrifuge tube with 7 ml of collagenase containing DMEM (0.25 mg/ml). The supernatant was aspirated and centrifuged (500×g, 20 min). The resulting pellet was resuspended in 1 ml of DMEM. The crude preparation was layered on discontinuous Percoll gradient (10-70%) and centrifuged (500×g, 10 min). The interface between 50-70% containing Leydig cell fraction was collected, washed twice and resuspended in DMEM. The cell viability was as assessed as described previously [2].

Porcine insulin was radioiodinated by chloramine-T method and purified (specific activity of purified  $^{125}\text{I}$ -insulin: 40-50  $\mu\text{Ci}/\mu\text{g}$ ) using Sephadex G-25 and 75 [5, 6]. Leydig cells were cultured according to the modified method described previously [1]. Briefly, Leydig cells ( $3 \times 10^5$ ) were cultured (24 hrs, 34°C, 95% air and 5%  $\text{CO}_2$ , humidified atmosphere) in sterile culture tubes in 1 ml of DMEM supplemented with FCS (1%), antibiotics and fungicide. At the end of the first 24-h culture period, DMEM was replaced with fresh FCS-free DMEM containing PRL (0, 50, 100, 150, 200, 250 ng/ml) or corticosterone (0, 100, 150, 200, 250, 300 ng/ml) and the cells were cultured for the subsequent 24 hrs (34°C, 5%  $\text{CO}_2$  in air). Following the second 24-h culture period, the medium was replaced with fresh FCS-free DMEM containing  $^{125}\text{I}$ -insulin and the cells were incubated (4°C) for the next 24 hrs. The cells were cultured either in the presence or absence of 1/  $^{125}\text{I}$ -insulin (10 000 cpm; total radioactivity) and 2/ unlabelled insulin (15  $\mu\text{g}$ ) to assess maximum binding (B) and non-specific binding (NSB), respectively.

The last culture period was ended by the addition of 300  $\mu\text{l}$  of ice cold binding buffer, and then the tubes were centrifuged (4°C, 10 000×g, 20 min) and supernatant was decanted. The pellet radioactivity was counted in a LKB gamma counter. Specific binding of  $^{125}\text{I}$ -insulin was expressed as % bound =  $\text{B}-\text{NSB}/\text{total radioactivity} \times 100$  [13]. All samples were run in duplicates. The Wilcoxon signed-rank test (SPSS 11 software) was used to

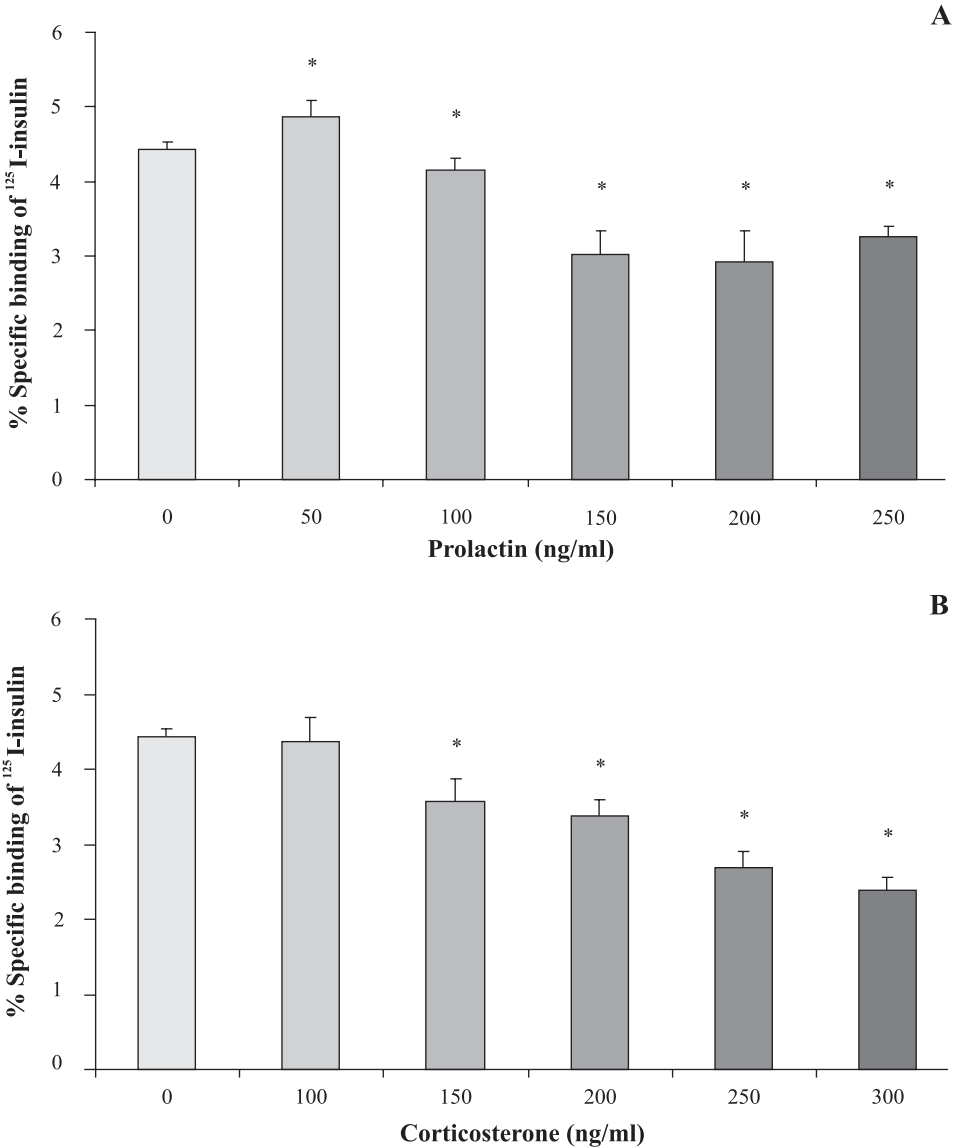


Figure 1. The effects of prolactin (A) and corticosterone (B) on <sup>125</sup>I-insulin binding (means  $\pm$ SD) to cultured rat Leydig cells (n=5-6 independent experiments). The details of the procedure are described in the Materials and Methods section. No treatment (0 ng/ml) in medium designates control group. \*p < 0.05 vs. control.

compare insulin specific binding between control cells (no treatment) and PRL or corticosterone-treated Leydig cells.

## RESULTS AND DISCUSSION

The rat Leydig cells treated (34°C, 24 hrs) with PRL or corticosterone demonstrated a significant ( $p < 0.05$ ) change in  $^{125}\text{I}$ -insulin binding. The lowest dose of PRL (50 ng/ml) increased ( $p < 0.05$ ) and the remaining PRL concentrations (100, 150, 200, 250 ng/ml) decreased ( $p < 0.05$ ) the insulin binding to Leydig cells (fig. 1A). All doses of corticosterone (150, 200, 250, 300 ng/ml) except the lowest one (100 ng/ml) decreased the insulin binding (fig. 1B). Insulin binding to Leydig cells enhanced by 50 ng/ml of PRL suggests the stimulatory PRL effect at doses slightly elevated above the physiological levels. Higher PRL concentrations (100, 150, 200, 250 ng/ml) caused an opposite effect implying the adverse effects of severe hyperprolactinemia on insulin binding. These results are supported by a previous report concerning decreased insulin binding to monocytes and erythrocytes under elevated prolactin levels [12]. An excess of corticosteroids was shown to reduce LH receptor number [11] in Leydig cells of rats in our laboratory. An increase in serum corticosterone level induced by 3- or 16-h lasting stress had no effect on LH receptor number in rat Leydig cells [9]. In the present study, 24 hours of pre-incubation with corticosterone, significantly reduced insulin binding to Leydig cells. This suggests that the effect of corticosterone may be time dependent. In conclusion, PRL and corticosterone affected insulin binding to Leydig cells. Further studies should elucidate their mechanism of action.

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