INFLUENCE OF PROLACTIN ON LIPOLYSIS IN RABBITS

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Abstract - Two experiments were conducted to assess the influence of prolactin on lipolysis in rabbits. *In vivo*, a subcutaneous injection of 1 mg of ovine prolactin (dissolved in 1 ml of phosphate buffered saline) induced an increased plasma glycerol and non esterified fatty acids concentrations within 30 min. Plasma concentrations of the two metabolites remained high 2 hours after the injection (basal level x2 and x7 for glycerol and non esterified fatty acids, respectively; $P < 0.01$). *In vitro*, ovine prolactin was able to stimulate glycerol release in isolated adipocytes only at supraphysiological concentrations (over 10-8 M). These results suggested that in rabbits prolactin promotes lipid mobilization. However, a direct activation of adipocyte lipolysis seems unlikely.

INTRODUCTION

In a variety of mammals, lactation is associated with mobilization of adipose tissue lipids accumulated during pregnancy. Hormones and mechanisms involved in these adaptations are not yet fully understood. Growth hormone and insulin seem to be involved in rats and cows, and a metabolic role for prolactin in lactating animals has also been suggested (VERNON, 1989).
The depletion of lipid stores in the lactating rabbit is well established (LEBAS, 1973; PARIGI-BINI *et al.*, 1990) and is accompanied by an elevated plasma concentration of non-esterified fatty acids in early lactation (FORTUN, 1994). However, in this species, little is known about the endocrine control of metabolic adaptation during lactation. In the rabbit, prolactin is the major lactogenic and galactopoietic hormone. Nevertheless, influence of prolactin on lipolysis in the rabbit has not yet been explored. The aim of this experiment was to study the influence of prolactin on *in vivo* and *in vitro* lipolysis in the rabbit.

MATERIALS AND METHODS

Animals

The rabbits were New Zealand x Californian and were caged individually with a controlled light / dark cycle (16 h / 8 h). They were given *ad libitum* access to water and to a commercial diet (17.5% crude protein and 2,330 kcal DE/kg).

Experiment 1

In this experiment we studied the effect of prolactin on *in vivo* lipolysis. In the treated group (PRL group, n = 8), the rabbits received subcutaneously 1 mg of ovine prolactin (oPRL, Sigma Chemical) dissolved in 1 ml of phosphate buffered saline (PBS), pH 7.4 (time 0). In the control group (CTL group, n = 8), the rabbits received subcutaneously 1 ml of vehicle (PBS) instead of prolactin. Blood samples (2 ml) were collected in heparinized tubes 30 min before the injection, and 30 min, 1 h, and 2 h after the injection, by puncture of an auricular artery. Samples were immediately centrifuged and the plasmas were stored at -20°C until they were assayed for glycerol and non esterified fatty acids (NEFA) concentrations. Glycerol and NEFA were measured by enzymatic methods (Boehringer Mannheim and Unipath S.A., respectively).

Experiment 2

Six rabbits were slaughtered in order to remove perirenal adipose tissue. Isolated adipocytes were obtained by collagenase digestion of adipose tissue fragments in Krebs-Ringer bicarbonate buffer containing 3.5 g/100 ml albumin (KRBA) and glucose (6 mM), pH 7.4, at 37°C under shaking at , 120 cycles/min. At the end of the incubation, the fat cells were filtered through a silk screen and washed three times with KRBA buffer to eliminate collagenase. Packed cells were brought to a suitable dilution in KRBA buffer (,50 000 cells/ml). Isolated fat cells were incubated in 0.5 ml of KRBA containing glucose (6 mM) at 37°C in polyethylene tubes under a 95% O2-5% CO2 gas phase, with gentle shaking (60 cycles/min) in a water bath. Ovine prolactin (10-12 to 10-6 M) was added to the cell suspension just before the beginning of the assay. All determinations were in duplicat. After 90 min of incubation, the tubes were placed in an ice bath, and 200-µl aliquots of the infranatant were taken for enzymatic determination of glycerol, which was used as the index of lipolysis (WIELAND, 1957). The lipid content of the incubation vials was determined gravimetrically (DOLE and MEINERTZ, 1960).

Statistics

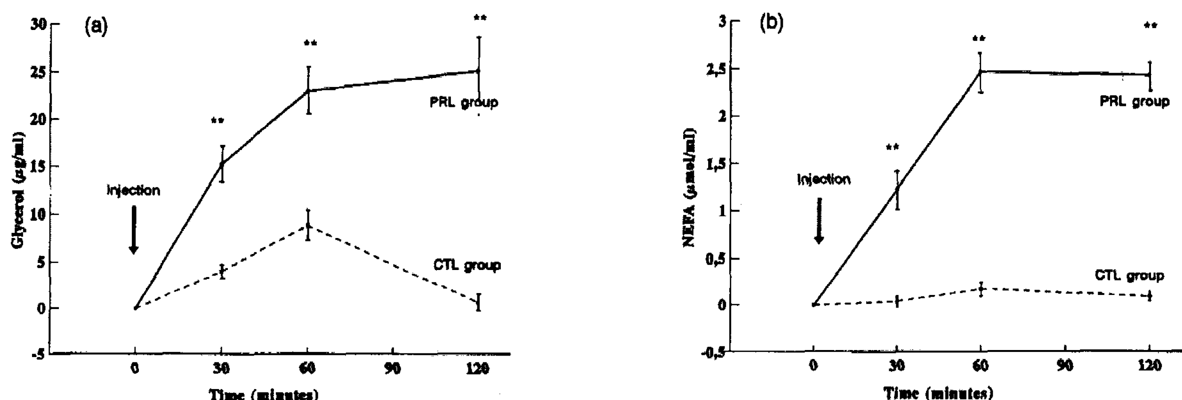
The data from each experiment were analyzed separately, using the Student-Newmann-Keuls procedure (SAS, 1987). In the first experiment, glycerol and NEFA concentrations were firstly analyzed according to a split-plot design including the effect of treatment, the effect of rabbit doe within treatment (error to test the treatment effect), the effect of time of sampling and the treatment x time of sampling interaction. However, the treatment x time of sampling interaction was significant, therefore comparisons between treatments were made for each time of sampling.

RESULTS

Experiment 1

Before injection (-30 min), the plasma concentrations of glycerol (21.2 ± 2.5 and 25.5 ± 3.3 µg/ml) and NEFA (0.43 ± 0.1 and 0.39 ± 0.1 µmol/ml) were similar in CTL and PRL groups ($P > 0.1$). In the CTL group, the concentrations of glycerol and NEFA remained similar before and after the injection ($P > 0.05$). Conversely, in the PRL group the concentrations of glycerol and NEFA were higher 30 min, 1 h and 2 h after the injection of oPRL than before ($P < 0.01$; Figure 1a,b).

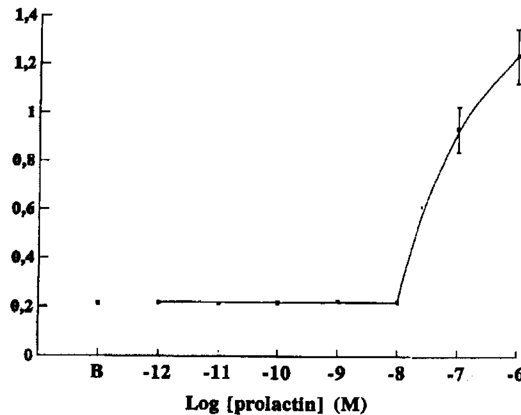
Figure 1 : Experiment 1. Variations of glycerol (a) and NEFA (b) concentrations, 30 min, 1 h and 2 h after subcutaneous injection (time 0). In the PRL group rabbits received 1 mg of oPRL dissolved in 1 ml of PBS (pH 7.4), while in the CTL group rabbits received 1 ml of PBS (pH 7.4). ** Groups PRL and CTL differ at $P < 0.01$.



Experiment 2

In vitro, oPRL was effective in stimulating glycerol release from isolated adipocytes at concentrations >10-8 M (Figure 2).

Figure 2 : Experiment 2. The effect of oPRL on glycerol release in isolated rabbit adipocytes. B, basal lipolysis without oPRL



DISCUSSION

In the rabbit, suckling occurs once a day (ZARROW *et al.*, 1965) and induces a release of prolactin within 15 min. The concentration of prolactin after nursing is 2- to 10-fold higher than the levels observed during pregnancy or after weaning (Mc NEILLY and FRIESEN, 1978). The biological activity of ovine prolactin in rabbits has previously been described (COWIE, 1969), and former experiments have shown that subcutaneous injection of 1 mg of ovine prolactin results in a level of prolactin similar to that found in lactating rabbits after suckling (FORTUN *et al.*, 1994). Therefore, the injection of ovine prolactin should have mimicked the release of prolactin following the nursing. The injection resulted in an immediate elevation in plasma glycerol and NEFA concentrations, as previously described in humans (BERLE *et al.*, 1974) and dogs (WINKLER *et al.*, 1971). Moreover, we have previously shown that chronic supplementation of PRL (1 mg of oPRL during 14 days) led to a lower weight of adipose tissue than in control rabbits (FORTUN *et al.*, 1994). These results suggest that, in rabbits, prolactin promotes lipid mobilization.

In vitro, the minimum concentration of prolactin required to stimulate lipolysis is much greater than the concentration of prolactin present under physiological conditions (Mc NEILLY and FRIESEN, 1978). Similarly, FIELDER and TALAMANTES (1987) and Ng (1990) have shown, in the mouse and the guinea pig, that prolactin was effective in stimulating lipolysis only at supraphysiological concentrations. The hypothesis of a contamination of the PRL preparation with growth hormone proposed to explain the *in vitro* lipolytic activity of PRL (FIELDER and TALAMENTES, 1987) is highly questionable due to purity of recent preparations. If prolactin has a direct effect on adipose tissue, then there must be prolactin receptors on the adipocyte. The existence of such receptors remains unclear (VERNON, 1989). Therefore, *in vivo*, the action of prolactin on adipose tissue could be indirect. Indeed, in rat and pigeon several studies suggested direct and indirect effects of prolactin on target tissues (mammary gland or crop-sac) by stimulation of the secretion of an hepatic factor (synlactin) that acts synergistically with prolactin (ANDERSON *et al.*, 1984; ENGLISH *et al.*, 1990). However, existence of such factor in rabbit and its action on adipose tissue remain to be demonstrated.

CONCLUSION

The mobilization of the fat stores during lactation is of importance for the release of free fatty acids used as energy substrates in various tissues. Our results show that, *in vivo*, prolactin, at concentrations similar to that found in lactating animals, promotes lipid mobilization in the rabbit. However, *in vitro* results suggest that a direct activation of adipocyte lipolysis by prolactin is unlikely. Therefore, the mechanims involved in this effect remain to be elucidated.

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