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Effect of Experimentally-Induced Chronic Hyperprolactinemia on Insulin Binding and Antilipolytic Response in Adipocytes from Male Rats

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Male Wistar rats with chronic hyperprolactinemia induced by grafting an anterior pituitary gland under the right kidney capsule were studied as experimental model. In these animals basal plasma glucose and insulin levels were unaltered. Epididymal adipocytes from hyperprolactinemic rats showed a significant increase in insulin binding at low unlabeled insulin concentrations. This increase in insulin binding can be principally attributed to an increase in the high affinity-low capacity binding sites, as demonstrated when Scatchard analysis was interpreted in terms of two types of insulin receptors. The dissociation constants (K_{D1} and K_{D2}) were not different between the groups. The apparent insulin receptor affinity was also unchanged. Moreover, a decreased sensitivity to the antilipolytic effect of insulin was also obtained in adipocytes from hyperprolactinemic rats. These findings indicate that chronic hyperprolactinemia is able to increase high affinity insulin receptors in epididymal adipocytes, but tends to diminish the antilipolytic response, suggesting a lack of coupling between insulin binding and its biological activity in male adipose tissue. Several possible mechanisms involved in the process are suggested.

Key words: Hyperprolactinemia, Adipocyte, Insulin receptor, Antilipolysis.

In some physiological situations of hyperprolactinemia, such as pregnancy and lactation in humans and animals, insulin binding to different target tissues, including adipose tissue, has been studied rendering ambiguous results. Thus, in adipocytes from pregnant rats, an increase in insulin binding has been described (12, 36), whereas other authors found no changes in binding in this same situation (38). In adipocytes from pregnant women a decrease in insulin-binding activity has also been reported (31). However, in lac-

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tating rats, insulin receptor numbers on adipocytes are unchanged (12). In addition, the biological activities of insulin on adipose tissue were also altered in the aforementioned physiological situations of hyperprolactinemia. In this sense, in adipocytes from pregnant rats, decreased oxidation and glucose transport (36, 38), and decreased glucose incorporation into lipid (12) have been found. Moreover, the antilipolytic activity of insulin is also reduced in adipocytes from pregnant women (31). Similar findings have been reported in adipose tissue from lactating rats, where lipogenesis and CO_2 production from glucose is decreased (3, 6, 31).

Moreover, chronic hyperprolactinemia in humans has been associated with hyperinsulinemia and decreased glucose tolerance (16, 24), and this state is also accompanied by a decrease in insulin binding to monocytes and erythrocytes from these patients (34). Finally, evidence has also been found of a short-term *in vitro* effect of prolactin (PRL) on insulin binding to adipocytes from pregnant women (18).

The anterior pituitary-grafted rat has been reported to be a useful experimental model for the study of the physiological effects of increased PRL levels (2). Accordingly, in this study, we employed this experimental model to evaluate the possible effects of chronic hyperprolactinemia on insulin binding and antilipolytic activity in male rat adipocytes.

Materials and Methods

Induction of hyperprolactinemia and sampling. — Male Wistar rats were housed from birth in a room with a controlled photoperiod (08.00-20.00 h light) and temperature (23 ± 1 °C), and with free acces to standard rat chow (Sanders, Madrid, Spain) and water. At 30 days of age the rats received an anterior pituitary gland from a littermate donor. Rats of the same age were sham-operated to be used as controls (2). Forty-five days after implantation, no weight differences were found in the grafted rats as compared to the controls (250-260 g). The animals were decapitated without anaesthesia two hours after light onset. Trunk blood was collected into tubes containing 30 µl of 10 % (EDTA) and 1500 U aprotinin (Trasylol®) (Bayer, Leverkusen, FRG) for glucose, insulin and PRL measurements. Plasma was obtained by centrifugation of the samples at 1000 g for 10 min at 4 °C and stored at −70 °C until analysis.

Isolation of adipocytes. — Rat adipocytes were isolated by collagenase (Worthington Biochemicals Corp., Freehold, NJ, USA) digestion of epididymal adipose tissue, according to RODBELL (32). Adipocyte numbers were determined with a Neubauer-type haemocytometer and cell viability —assesed by the ability to exclude 0.2 % Tripan blue— was greater than 90 %. Cell diameter measurements were also performed as previously reported (10). In each experiment, cell size was determined as the mean cell diameter of 100 cells.

Iodination of insulin. — Porcine monocomponent insulin (Novo Industrias, Denmark) was iodinated with carrier-free $Na^{125}I$ (Amersham International), according to FREYCHET *et al.* (14). The specific activity of mono^{125}I-insulin was 250-400 µCi/µg and degradation of this fraction was less than 5 % by precipitation with 10 % TCA.

Plasma measurements. — Plasma PRL levels were measured by a specific double-antibody radioimmunoassay (RIA) system using materials kindly supplied by NIAMDD (NIH, Bethesda, MD) and previously validated (39). Plasma PRL levels were expressed in terms of rat PRL-RP-3. Plasma insulin levels were also measured by RIA, as previously described

(26), using porcine monocomponent insulin (Novo) as standard. Basal plasma glucose levels were determined using a commercial glucose-oxidase kit.

Binding experiments. — Insulin binding experiments were performed as previously described (9, 28). Isolated adipocytes (0.2-0.5 × 10^6 cells/ml) were incubated with mono-^{125}I-insulin (0.1-0.2 × 10^{-9} M) at 30 °C for 30 min in Krebs-HEPES buffer containing glucose 3.3 mM, 1 % bovine serum albumin (BSA) and bacitracin (0.9 mM) (Sigma), pH 7.4, either in the absence or presence of unlabeled insulin at increasing concentrations (0.2 × 10^{-10}-0.5 × 10^{-7} M). Cell-bound radioactivity was separated from the medium by centrifugation through dinonyl phthalate. All results are expressed in terms of specific binding. The non-specific binding (in the presence of 0.25 × 10^{-6} M unlabeled insulin) did not differ between either group of rats: 12-15 %. Binding data were analysed by the method of SCATCHARD (33) using the ISIS-12 curve-fitting program of THAKUR *et al.* (37). Insulin degradation in the incubation medium of the adipocytes at the time of binding was determined by TCA-precipitation using appropriate controls in which fat cells were omitted.

Antilipolytic activity of insulin. — It was determined by its ability to inhibit glycerol release from adipocytes in the presence of 10^{-6} M isoproterenol. Glycerol production was determined by our own modification (35) of the method of KORN (23) after incubation of adipocytes for 1 h at 37 °C essentially as described previously (9, 28), in the absence or presence of 10^{-6} M isoproterenol or 10^{-6} M isoproterenol plus insulin (0.25 × 10^{-11}-0.25 × 10^{-9} M).

Statistical analysis. — The results are expressed as means ± S.E.M. For statistical comparisons Student's t-test for unpaired data was used.

Results

Plasma PRL levels were found to be significantly greater in male grafted rats than in the controls (18.9 ± 1.9 (n = 6) ng/ml vs. 5.5 ± 0.3 (n = 6) ng/ml; $p < 0.01$). This expected increase in circulating PRL levels was not associated with changes in the circulating levels of insulin, which

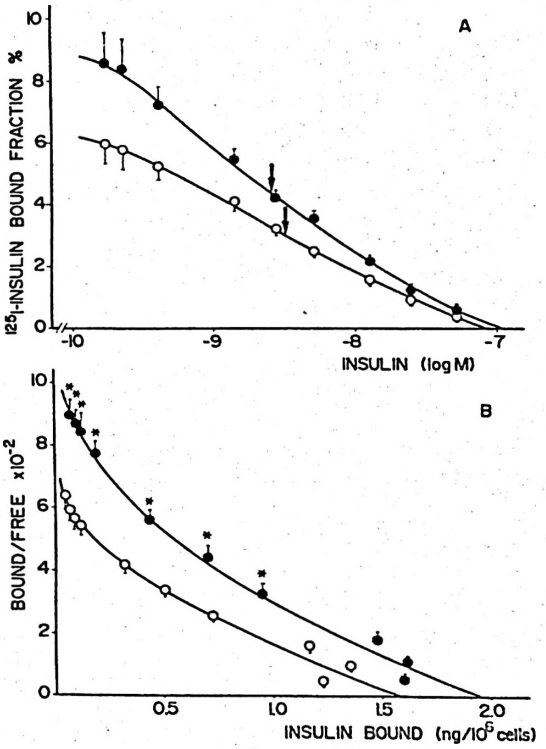

Fig. 1. *Displacement curves and Scatchard analysis of mono-^{125}I-insulin binding in adipocytes from five grafted (•) and seven control (○) male rats.* Each point is the mean ± S.E.M. obtained from separate determinations carried out in duplicate with isolated adipocytes from individual rats. Panel A: Arrows indicate the respective amounts of insulin required for a 50 % displacement of maximal specific binding (ED_{50}) in each group. Panel B: Asterisks represent statistically significant differences between values of the grafted and control rats ($p < 0.05$).

Table I. *Parameters of insulin binding in adipocytes from grafted male and control male rats (means ± S.E.M.)*

	High-affinity binding		Low-affinity binding	
	K_{D1} (nM)	Sites per cell	K_{D2} (nM)	Sites per cell
Control male rats (n = 7)	0.56 ± 0.15	5,600 ± 300	5.13 ± 0.69	156,000 ± 10,000
Grafted male rats (n = 5)	0.61 ± 0.17	12,700 ± 500*	4.60 ± 0.63	180,000 ± 12,000

* $p < 0.001$ between values of the control and grafted rats.

were similar in both grafted and control rats (1.4 ± 0,1 (n = 28) ng/ml vs. 1.3 ± 0,1 (n = 21) ng/ml; p = NS). No changes were found in basal glucose values between the two groups (grafted rats: 121 ± 4 (n = 13) mg/dl and controls: 117 ± 6 (n = 12) mg/dl, p = NS).

Adipocyte cell diameters were similar in the two groups of animals. The values were: 49.8 ± 4.2 μm in grafted rats (n = 5) and 48.0 ± 3.8 μm in control rats (n = 7, p = NS).

Adipocytes from male grafted rats bound more insulin than did adipocytes from controls (fig. 1A). The insulin concentrations required for 50 % displacement of maximal specific binding (ED_{50}) were 2.5×10^{-9} M in adipocytes from grafted rats and 3.0×10^{-9} M in adipocytes from the controls. The binding data were further analyzed according to SCATCHARD (33), and the curvilinear plots obtained were interpreted in terms of two components of differing affinity for in-

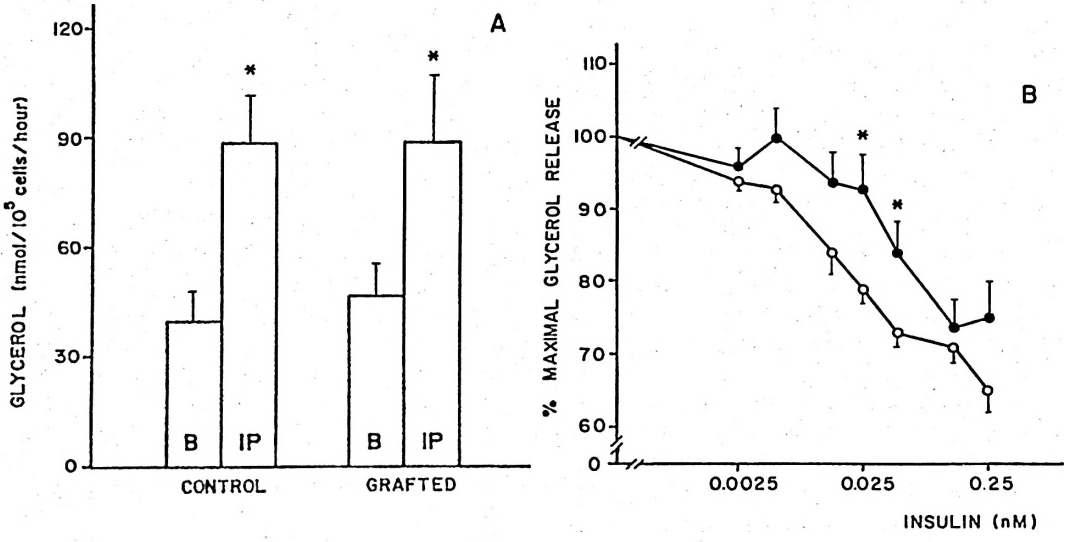

Fig. 2. *Lipolytic effect of isoproterenol and antilipolytic effect of insulin in adipocytes from rats with chronic hyperprolactinemia.*

(A) Glycerol production by adipocytes from seven grafted and seven control male rats. B: basal production. IP: in the presence of 10^{-6} M isoproterenol. Means ± S.E.M. Asterisks represent statistically significant differences ($p < 0.05$). (B) Antilipolytic action of insulin (0.0025-0.25 nM) in adipocytes from seven grafted (•) and seven controls (○) male rats. Results are indicated as means ± S.E.M. of % maximal glycerol release in the presente of 10^{-6} M isoproterenol. Asterisks represent statistically significant differences between the two groups ($p < 0.05$).

sulin (37). A significant increase in insulin binding to adipocytes from hyperprolactinemic rats was observed up to unlabeled insulin concentrations of 5×10^{-9} M (fig. 1B). This difference was accounted for mainly by a two-fold greater number of the high affinity binding sites, while the number of the low affinity sites was only 15 % greater (table I). The dissociation constants (KD) were not different between the groups.

The percentage of labeled insulin degraded by cells during the binding experiments was similar in both groups of adipocytes and ranged between 6 % and 8 %.

Glycerol production by the adipocytes of male grafted rats as compared to the controls is shown in fig. 2A. The basal values of glycerol release were similar in both groups. The addition of isoproterenol (10^{-6} M) significantly enhanced glycerol production and no differences were found in the isoproterenol stimulus between either group: 94 ± 16 % (n = 7) stimulus in adipocytes from grafted rats and 130 ± 18 % (n = 7) in those from controls (p = NS). Fig. 2B shows the antilipolytic activity of insulin in the presence of isoproterenol on isolated adipocytes from male grafted rats and controls. A clear antilipolytic effect of insulin was observed in both groups of adipocytes. The percentages of inhibition of glycerol production at insulin concentrations of 0.0125 nM; 0.025 nM; 0.05 nM; 0.125 nM and 0.25 nM were, respectively: 6.2 % (p = NS), 6.8 % (p = NS), 16.1 % (p = NS), 26.4 % ($p < 0.05$), 25.1 % ($p < 0.05$) in adipocytes from hyperprolactinemic rats, and 16.1 %, 20.9 %, 26.9 %, 29.0 %, 34.9 % ($p < 0.05$) in all cases) in adipocytes from controls. The maximal percentage of inhibition of glycerol production in adipocytes from hyperprolactinemic rats (26 %) was achieved in the presence of 0.125-0.25 nM insulin, while a similar percentage of inhibition (26 %), that was not the maximum (34.9 %), was obtained with a concentration of 0.05 nM insulin in adipocytes from control rats.

Discussion

As expected, a significant increase in plasma PRL levels after grafting an anterior pituitary gland under the kidney capsule of prepubertal male rats has been detected (2, 7). Moreover, in agreement with other authors in male rats (1) no changes in basal glucose or insulin plasma levels have been observed.

An increase in insulin binding at low unlabeled-insulin concentrations on adipocytes from grafted male rats was detected. This increase in binding cannot be explained by alterations in receptor affinity, as may be seen on comparing the ED_{50} values obtained from the displacement curves, and by the dissociation constants obtained from Scatchard analysis assuming two populations of sites. Thus, the observed increase in insulin binding at low unlabeled insulin concentrations can be mainly attributed to a 127 % increase in the number of high affinity binding sites, while the low affinity sites remain unaltered. These results cannot be related to alterations in adipocyte size or plasma insulin levels, since the two latter parameters remained unchanged in the hyperprolactinemic rats. Moreover, adipocytes from rats with hyperprolactinemia were found to inactivate insulin to the same extent as in the control rats, showing that the changes in insulin binding were not due to differences in insulin degradation at the time of binding.

A comparison with our binding results in male grafted rats can only be made with our previous binding results from female rats, because the literature does not contain any data on insulin receptors in this experimental model. Female grafted rats showed an increase in the total number of insulin receptors on parametrial adipocytes, with no changes in the apparent af-

finity of the receptors (7). These findings could indicate that the sex status does not alter the primary effects of hyperprolactinemia on insulin receptors in rat adipocytes.

There was no correlation between the increase in insulin binding and the decrease in insulin antilipolytic activity in adipocytes from hyperprolactinemic rats. The amount of insulin required to achieve a significant antilipolytic response was ten times higher in adipocytes from hyperprolactinemic rats than in those from controls. A decrease in insulin biological activity on adipose tissue appears to be a general feature of physiological situations of hyperprolactinemia (5, 7, 12, 20, 26, 38), although these insulin-resistant states are not always accompanied by an increase in insulin binding (13, 31, 38). The differences in the hormonal status present in these hyperprolactinemic situations should also be taken into account. In this sense, apart from their high PRL levels, grafted rats have been described to show increased circulating levels of corticosterone (29) and catecholamines (11). Other authors along with us have reported that glucocorticoids decrease insulin binding and induce insulin resistance in rat (30) and human adipocytes (9), and catecholamines also induce similar effects in rat (17, 22) and human adipocytes (8). Therefore, the results obtained by us at receptor level cannot be attributed to the aforementioned hormone alterations; however their involvement in the post-receptor effects observed cannot be excluded.

Since the molecular mechanism(s) of insulin action have not been elucidated, it is difficult to clarify the possible mechanism(s) involved in the uncoupling between increased high affinity insulin receptors and decreased antilipolytic activity. A possibility could be that PRL in some way acts by impairing the ability of the insulin receptor complex to transmit its transmembrane signalling. This alteration in signal generation would then lead to insulin resistance. It has been hypothesized that the phosphorylation and protein kinase activity of insulin receptors might mediate the action of insulin (15). Accordingly, the possible diminished protein kinase activity of insulin receptors, as has been described in other insulin-resistant situations (17, 19, 25), could provide a possible mechanism for the post-insulin binding defect of insulin action in adipocytes from grafted rats.

Furthermore, it has also been suggested that insulin promotes the release of an insulin mediator from the plasma membrane, possibly a glycophospholipid, which modulates the activity of some enzymes (4, 27). In this sense, another possible mechanism could be related with a defect in the release of this insulin mediator, as has been recently reported in adipocytes from lactating rats (21).

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Resumen

Se estudia el modelo experimental de hiperprolactinemia crónica, inducido por trasplante de una glándula pituitaria anterior bajo la cápsula renal de ratas Wistar machos. En estos animales no resultan alterados los niveles plasmáticos de glucosa e insulina. En adipocitos procedentes del tejido adiposo epididimal de ratas hiperprolactinémicas, se detecta un aumento estadísticamente significativo en la unión de la insulina a sus receptores específicos a bajas concentraciones de la hormona. Este incremento en la unión puede ser atribuido, principalmente, a un aumento en el número de receptores de alta afinidad y baja capacidad, según se deduce de la interpretación del análisis de Scatchard en términos de dos tipos de receptores de insulina. Las constantes de disociación

(K_{D1} y K_{D2}) no difieren entre los dos grupos de ratas estudiados. La afinidad aparente del receptor de insulina tampoco resulta alterada. Además, los adipocitos procedentes de ratas hiperprolactinémicas muestran una disminución de sensibilidad a la acción antilipolítica de la insulina. Por tanto, en adipocitos procedentes de tejido adiposo epididimal de ratas, con hiperprolactinemia crónica, se induce un incremento en el número de receptores de insulina de alta afinidad acompañado de una pérdida de respuesta en estos adipocitos a la acción antilipolítica de la insulina. Estos efectos sugieren una falta de acoplamiento entre la unión de la insulina y su actividad biológica en el tejido adiposo de estos animales. Se proponen algunos de los mecanismos posibles que pueden estar involucrados.

Palabras clave: Hyperprolactinemia, Adipocitos, Receptor de insulina, Antilipolisis.

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