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Binding and Antilipolytic Action of Insulin in Isolated Adipocytes from Cortisol-Treated Rats

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The effects of an *in vivo* cortisol-treatment to rats (2 × 2 mg/rat/day, for one week) on insulin plasma levels, insulin binding and antilipolytic activity in rat adipose tissue were investigated. Hyperinsulinemia together with an increase in insulin degradation in the serum of cortisol-treated rats were observed. The adipocytes from cortisol-treated animals showed a statistically significant decrease in insulin binding but no change in receptor numbers [cortisol-treated 103,000 ± 8,000 (n = 8) receptors/cell and controls 138,000 ± 15,000 (n = 16) receptors/cell], together with unchanged receptor affinity [ED50: cortisol-treated 3×10^{-9} M and controls 3.2×10^{-9} M], and a decreased sensitivity to the antilipolytic effect of insulin. The evidence presented for pre-receptor, receptor and post-receptor insulin defects on the action of cortisol in isolated rat adipocytes could represent a coordinated mechanism by which cortisol exerts «insulin resistance» in this tissue.

Key words: Cortisol, Adipocytes, Insulin-degradation, Insulin-receptor, Antilipolysis.

Contradictory results have been reported concerning the effects of glucocorticoids on insulin receptor binding and/or their actions on different tissues. The differences in the results may be explained on the basis of different experimental set-ups. These include dose and type of glucocorticoid used, administration route and the duration of *in vivo* treatment or the time-course of *in vitro* exposure, and the type of tissue in which insulin binding and action were studied.

The effect of *in vivo* glucocorticoid-treatment to rats on insulin binding in adipose tissue has been reported to be decreased (5, 16, 18), increased (5), or unchanged (23). In addition, under the same conditions, some of the effects of insulin proved to be impaired (5, 16, 23), while others were found to be increased (5).

The purpose of the present study was therefore to evaluate the binding —a receptor-event— and the antilipolytic action of insulin —a post-receptor event hitherto

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unstudied in glucocorticoid-treated animals—, using isolated adipocytes from cortisol-treated rats. Additionally, insulin degradation in the serum of these cortisol-treated rats as a pre-receptor event probably involved in the process was studied.

Materials and Methods

Animals.— Male Wistar rats (190-200 g, b.w.) from our own inbred colony were used. They received standard rat chow and tap water *ad libitum*. The animals were injected s.c. with cortisol (Sigma) (2 × 2 mg/rat/day) for one week. The glucocorticoid solution was freshly prepared in ethanol:H_2O (1:1), the last injection being given 3-4 hours before sacrifice. Control rats received similar amounts of the ethanol solution. No weight differences were found in cortisol-treated rats as compared to the controls. At the end of the treatment period, the animals were decapitated (11.00 - 12.00 a.m.) and blood samples and epididymal adipose tissue were extracted.

Isolation of adipocytes.— Isolated adipocytes were prepared by collagenase (Worthington) digestion according to the method reported by RODBELL (19). Adipocyte numbers were determined with a NEUBAUER-type haemocytometer and cell viability —assessed by the ability to exclude 0.2 % Tripan blue— was greater than 90 %. Measurements of cell diameter were also performed as reported by DI GIROLAMO *et al* (6). A minimum of 100 cells was measured from each rat.

Insulin binding studies.— Mono-[^{125}I] insulin was prepared (9) with specific activities of 200-300 μCi/μg using Na^{125}I (Amersham) and porcine monocomponent insulin (Novo). Isolated adipocytes (0.3-0.5 × 10^6 cells/ml) were incubated at a final volume of 350 μl with mono-[^{125}I]-insulin (0.1-0.2 × 10^{-9}M) at 30 °C for 30 min. in Krebs-Hepes buffer pH 7.4 containing glucose (3.3 mM), BSA (1 %) and bacitracin (0.9 mM) (Sigma) either in the absence or the presence of unlabeled insulin at increasing concentrations from 0.25 × 10^{-9}M to 0.5 × 10^{-7}M. Adipocytes were separated from the medium by centrifugation through dinonyl-phthalate according to the method of GLIEMANN *et al.* (10). The amount of ^{125}I-insulin bound in the presence of 0.25 × 10^{-6} M unlabeled insulin was considered as non-specific binding. Binding data were expressed as ^{125}I-insulin specifically bound/10^6 cells.

Insulin degradation in the incubation medium of the adipocytes at the time of binding was determined by TCA-precipitaton.

Antilipolysis studies.— A hundred μl of isolated adipocytes (0.3-0.5 × 10^6 cells/ml) were incubated with Krebs-Hepes buffer pH 7.4 without glucose and with 1 % FFA-free BSA (Sigma) and bacitracin (0.9 mM). The cells were incubated at 37 °C either in the absence or presence of 10^{-6}M isoproterenol or 10^{-6}M isoproterenol plus different concentrations of insulin (5 × 10^{-12} M − 1.25 × 10^{-10} M) for 60 min. The glycerol content was determined by the method of LAMBERT and NEISH (13) with some of our own modifications (22). The data were expressed as nmol/10^5 cells/h.

Blood determinations.— Plasma samples were analyzed for insulin content by RIA (12) and for glucose using a commercial glucose-oxidase method. Serum samples were incubated with mono-[^{125}I] insulin (0.1 × 10^{-9} M) for one and two hours at 37 °C and the disappearance of intact insulin was measured by the decrease in radioactivity precipitable in 10 % TCA.

Statistical analysis.— The results were expressed as means ± SEM. For statistical comparisons Student's «t»-test was used.

Results

Plasma insulin levels were elevated in cortisol-treated rats [1.5 ± 0.06 (n = 20) ng/ml] as compared to controls [1.0 ± 0.01 (n = 33) ng/ml, $p < 0.05$]. Plasma glucose was also increased in treated rats [129 ± 0.9 (n = 40) mg/dl vs. 112 ± 1.2 (n = 33) mg/dl, $p < 0.05$].

The rate of insulin degradation in serum was increased in cortisol-treated rats both at 1 h [5 ± 0.6 % (n = 24) vs. 3 ± 0.4 % (n = 28), $p < 0.05$] and 2 h of incubation [8 ± 0.8 % (n = 22) vs. 6 ± 0.6 % (n = 30), $p < 0.05$].

No changes were found in adipocyte cell diameter between the two groups of animals: 49.5 ± 4.1 μm in cortisol-treated rats (n = 6) and 48.8 ± 4.0 μm in control rats (n = 6).

Cortisol-treatment induced a statistically significant decrease in insulin binding to adipocytes. Figure 1 shows the competitive displacement of ^{125}I-insulin by different concentrations of unlabeled insulin in adipocytes from cortisol-treated and control rats. The concentration of unlabeled insulin required to displace half of the maximal ^{125}I-insulin bound was similar in both the treated and control groups (3×10^{-9} M and 3.2×10^{-9} M respectively) indicating no change in receptor affinity.

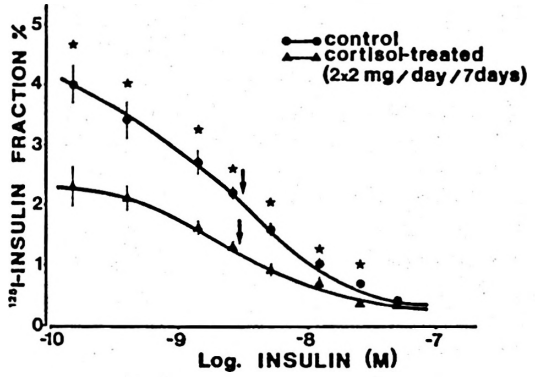

Fig. 1. *Displacement curves of mono-[^{125}I] insulin binding in adipocytes from cortisol-treated rats (n = 8) as compared to controls (n = 16).*
Each point is the mean ± S.E.M. obtained from separate determinations carried out in triplicate with isolated adipocytes from individual rats. Arrows represent the insulin concentration causing 50 % inhibition of the receptor-bound fraction of Mono-[^{125}I] insulin (apparent receptor affinity) in each group of animals.

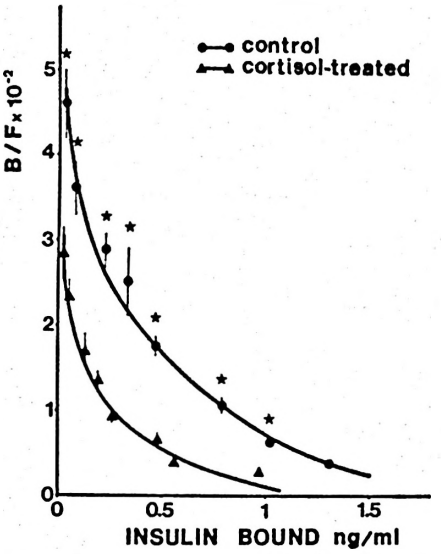

Fig. 2. *Scatchard analysis of insulin binding data in adipocytes from cortisol-treated rats (n = 8) as compared to controls (n = 16).*
Each point is the mean ± S.E.M. obtained from separate determinations carried out in triplicate with isolated adipocytes from individual rats. The asterisks represent statistically significant differences between the two groups ($p < 0.05$).

Figure 2 shows a Scatchard analysis (20) of the binding data. A significant decrease in binding was observed up to concentrations of unlabeled insulin of 0.25×10^{-7} M. However the differences in the total number of insulin receptors were not statistically significant between the groups: 103,000 ± 8,000 (n = 8) receptors/cell in adipocytes from cortisol-treated rats and 138,000 ± 15,000 (n = 16) receptors/cell in controls.

Adipocytes from rats treated with cortisol (n = 4) were found to inactivate in-

Table I. *Antilipolytic action of insulin in the presence in all groups of 10^{-6} M isoproterenol on isolated adipocytes from five cortisol-treated and five control rats.*
Values (Means ± S.E.M.; n = 5) are percent of maximal glycerol release.

Insulin [M]				
0	5×10^{-12}	1.25×10^{-11}	5×10^{-11}	1.25×10^{-10}
Control				
100	89.7±2.6	85.1±4.8	69.0±3.0	62.1±8.6
Cortisol				
100	95.2±3.9	92.9±2.4	79.9±3.9[a]	72.0±9.3

a $p < 0.05$.

sulin to the same extent as those from control rats (n = 4): 2.8-2.9 % at the time of binding.

Basal values of glycerol release were 54 ± 12 nmol/10^5 cells/h in adipocytes from treated rats (n = 5) and 34 ± 7 nmol/10^5 cells/h in controls (n = 5), with no statistically significant differences. Glycerol values in the presence of 10^{-6} M isoproterenol were elevated in adipocytes from treated rats [106 ± 12 (n = 5) nmol/10^5 cells/h] as compared to controls [75 ± 10 (n = 5) nmol/10^5 cells/h, $p < 0.05$]. However the differences in the rates of glycerol release (96 % and 120 % respectively) were not statistically significant.

The antilipolytic activity of insulin in the presence of isoproterenol on isolated rat adipocytes from cortisol-treated and control rats is shown in Table I. Results are indicated as means ±S.E.M. of % maximal glycerol release in the presence of 10^{-6} M isorproterenol. Fat cells prepared from the cortisol-treated rats were significantly less sensitive to the antilipolytic effect of insulin.

Discussion

Glucocorticoids have been described to increase insulin secretion (14, 15), and in the present study has also detected high levels of plasma insulin in cortisol-treated rats. The significance of the hyperinsulinemia accompanying the increased insulin degradation in the serum of our cortisol-treated rats remains to be elucidated. In certain situations of «insulin resistance», the presence of a serum factor with increased insulin degradating activity has been reported (1, 17). Glucocorticoid excess results in «insulin resistance», and it is possible that in this situation a serum factor might also be induced which could cause an increase in insulin degradation.

Our data pointing to a decrease in insulin receptor binding with no changes in receptor affinity and capacity in adipocytes from cortisol-treated rats are partially in accordance with previous *in vivo* studies in rats after dexamethasone administration (5,18) or cortisol implants (16). In this sense, De Pirro *et al.* (5) reportet that dexamethasone administration to rats resulted in a decrease in insulin binding to adipocytes without changes in receptor affinity. Olefsky *et al* (18) reported that adipocytes from dexamethasone-treated rats had decreased insulin binding and decreased receptor affinity and Mendes *et al.* (16) detected that cortisol implants produced a decreased insulin binding to isolated adipocytes per cell although not per unit surface area.

Our binding results do not agree with the findings of other authors after prednisolone (5) or prednisone (23) treatment to rats. De Pirro *et al.* (5) detected an increased insulin binding without changes in receptor affinity and Vann Bennett and Cuatrecasas (23) did not detect any change either in receptor concentrations or in receptor affinity.

The discrepancies between our results and those of the aforementioned authors could be explained in terms of different factors. These could involve differences in the dose and type of glucocorticoid used and the duration of the *in vivo* treatment.

The increased insulin levels in our cor-

tisol-treated rats could be one of the causes of the decrease in insulin receptors in these animals since hyperinsulinemia has consistently been associated with decreased insulin binding although exceptions have also been observed. However, a similar decrease in insulin receptor binding in adipocytes from streptozotocin-diabetic rats with cortisol-implants has been described (16), suggesting that hyperinsulinemia is not involved in the effect of cortisol on insulin receptors. Additionally, we have recently put forward a similar hypothesis following study of patients with Cushing's syndrome (3).

There has been found (2) a direct effect of cortisol on insulin binding using a preparation of isolated rat adipocytes. Therefore, it seems likely that the increased cortisol levels (11) in our cortisol-treated rats might induce the decrease in insulin receptor binding in the adipose tissue of these animals.

On the other hand, fat cells from cortisol-treated rats were found to inactivate insulin to the same extent as in control rats, showing that the changes in insulin binding were not due to differences in insulin degradation at the time of binding.

The decrease in insulin binding in adipocytes from cortisol-treated rats was accompanied by a decrease in the antilipolytic action of insulin in terms of decreased sensitivity. Concerning this, «insulin resistance», as shown in other two insulin activities, has also been detected in isolated adipocytes from glucocorticoid-treated rats i.e.: glucose-transport (23) in prednisone-treated rats, and glucose-transport and oxidation (5) after dexamethasone-treatment. Moreover, cortisol implants in normal and diabetic rats reduced both basal and insulin-stimulated lipogenesis in isolated adipocytes, although insulin sensitivity was unchanged (16).

Recently, a possibly specific role for the insulin-sensitive particulate phosphodiesterase in the antilipolytic action of insulin has been reported (7, 21). Moreover, glucocorticoid exposure of 3T3L1 adipocytes with dexamethasone results in an inhibition of the acute stimulation of cyclic AMP phosphodiesterase by insulin (8). Decreased phosphodiesterase activity would presumably potentiate cAMP accumulation in adipocytes from cortisol-treated rats as previously suggested by us (4). Therefore, decreased cAMP-phosphodiesterase activity could be closely related to a decreased antilipolytic action of insulin in adipocytes from cortisol-treated rats.

In conclusion, the aforementioned alterations could represent a coordinated mechanism by which cortisol exerts «insulin resistance» in rat adipose tissue.

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Resumen

Se estudia en ratas el efecto del tratamiento con cortisol (2 × 2 mg/rata/día, durante una semana), sobre los niveles circulantes de insulina, y a nivel de la unión de la insulina a sus receptores y su acción antilipolítica en adipocitos de tejido adiposo epididimal. Los resultados indican hiperinsulinemia e incremento en la degradación de la insulina en suero; disminución en la unión de la insulina a sus receptores sin cambios en la capacidad [ratas tratadas 103,000 ± 8, 000 (n = 8) receptores/cel y controles 138,000 ± 15.000 (n = 16) receptores/cel], ni en la afinidad del receptor [ED50: ratas tratadas 3×10^{-9} M y controles $3{,}2 \times 10^{-9}$ M] y, una disminución de la sensibilidad de los adipocitos a la acción antilipolítica de la insulina. Estos efectos de la insulina a nivel prereceptor, receptor y post-receptor pueden representar un mecanismo coordinado por el cual el cortisol induce un estado de «resistencia a la insulina», en el tejido adiposo de rata.

Palabras clave: Cortisol, Adipocitos, Degradación de la insulina, Receptor de insulina, Antilipolisis.

References

1. Blazar, B. R., Whitley, C. B., Kitabchi, A. E., Tsai, M. E., Santiago, J., White, N., Stentz, F. B. and Brown, D. M.: *Diabetes*, 33, 1133-1137, 1984.
2. Calle, C., Carranza, M. C., Sánchez-Casas, P., Simón, M. A. and Mayor, P.: VII Congr. Nac. Diabetes (Santiago de Compostela), 1984. Abst. p. 11.
3. Calle, C., Carranza, M. C., Simón, M. A., Torres, A. and Mayor, P.: *Biosci. Rep.*, 7, 713-719, 1987.
4. Calle, C., Sánchez-Casas, P., Simón, M. A. and Mayor, P.: *Biochem. Biophys. Res. Commun.*, 145, 90-95, 1987.
5. De Pirro, R., Green, A., Yung-Chin Kao, M. and Olefsky, J. M.: *Diabetología*, 21, 149-153, 1981.
6. Di Girolamo, M., Mendlinger, S. and Fertig, J. W.: *Am. J. Physiol.*, 221, 850-858, 1971.
7. Elks, M. L. and Manganiello, V. C.: *Endocrinology*, 116, 2119-2121, 1985.
8. Elks, M. L., Manganiello, V. C. and Vaughan, M.: *J. Biol. Chem.*, 258, 8582-8587, 1983.
9. Freychet, P., Roth, J. and Neville Jr, D. M.: *Biochem. Biophys. Res. Commun.*, 43, 400-408, 1971.
10. Gliemann, W. J., Østerlind, K., Vinter, J. and Gammeltoft, S.: *Biochem. Biophys. Acta*, 286, 1-9, 1972.
11. Häring, H., Calle, C., Bug, A., Renner, R., Hepp, K. D. and Kemmler, W.: *Diabetologia*, 19, 379-385, 1980.
12. Herbert, V., Lau, K. S., Gottlieb, C. W. and Bleicher, S. J.: *J. Clin. Endocr. Metab.*, 25, 1375-1384, 1965.
13. Lambert, M. and Neish, A.: *Can. J. Res.*, 83, 29-30, 1950.
14. Marco, J., Calle, C., Hedo, J. A. and Villanueva, M. L.: *Diabetología*, 12, 307-311, 1976.
15. Marco, J., Calle, C., Roman, D., Díaz-Fierros, M., Villanueva, M. L. and Valverde, I.: *N. Engl. J. Med.*, 288, 128-131, 1973.
16. Mendes, A. M., Madon, R. J. and Flint, D. J.: *J. Endocr.*, 106, 225-231, 1985.
17. Mishim, R. I., Almira, E. C. and Cleman M. W.: *J. Clin. Endocrinol. Metab.*, 52, 177-180, 1981.
18. Olefsky, J. M., Johnson, J., Liu, F., Jen, P. and Reaven, G. M.: *Metabolism*, 24, 517-572, 1972.
19. Rodbell, M.: *J. Biol. Chem.*, 235, 375-380, 1964.
20. Scatchard, G.: *Ann. N. Y. Acad. Sci.*, 51, 660-672, 1949.
21. Saltiel, A. R. and Steigerwalt, R. W.: *Diabetes*, 35, 698-704, 1986.
22. Simón, M. A. and Calle, C.: In «Gut Regulatory Peptides: Their role in health and disease. Series of Frontiers of Hormone Research», Vol. 16 (E. Blázquez ed.). S. Karger, A. G., Basel. 1987, pp. 111-120.
23. Vann Bennett, G. and Cuatrecasas, P.: *Science*, 176, 805-806, 1972.