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BINDING AND ACTION OF GLUCAGON IN ISOLATED ADIPOCYTES FROM CORTISOL-TREATED RATS

C. Calle, P. Sanchez-Casas, M.A. Simón, and P. Mayor

Department of Biochemistry, Faculty of Medicine,
Universidad Complutense. Madrid 28040. SPAIN

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SUMMARY: Evidence for pre-receptor, receptor and post-receptor glucagon defects was investigated in adipocytes from cortisol-treated rats. A decrease in glucagon binding due to a decreased number of receptors was observed. No changes in receptor affinity were detected. Both, the lipolytic response of glucagon and the ability of glucagon to increase basal and theophylline-stimulated cAMP accumulation remained unaltered. Moreover, a hyperglucagonemia accompanied by an increase in glucagon degradation in the serum of cortisol-treated rats was observed. Such alterations could represent a new mechanism by which glucocorticoids exert their biological actions.

We have previously reported that the high glucagon levels detected in adrenalectomized rats are related to a patent decrease in glucagon binding receptors in isolated adipocytes (1) and decreased lipolytic activity (2). Adrenalectomy has also been reported to elevate phosphodiesterase activity in adipose tissue (3). Contrariwise, in circumstances of glucocorticoid excess, data from our laboratory have shown that prednisolone treatment is also associated with hyperglucagonemia in man (4) and with enhanced glucagon secretion by isolated pancreatic islets from prednisolone-treated mice (5). To our knowledge, the binding and action of glucagon in isolated adipocytes from cortisol-treated rats has not yet been investigated and the purpose of the present study was thus to evaluate these events. In addition, glucagon degradation in the serum of these cortisol-treated animals as a pre-binding event probably involved in the process was studied.

MATERIALS AND METHODS

Male Wistar rats, fed ad lib., with body weights of 140-160 g, were used. The treated rats were injected subcutaneously with freshly prepared

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cortisol-solutions in ethanol: H_2O (1:1). The last injection was administered 3-4 hours before sacrifice. Control rats received similar amounts of the ethanol solution. No weight differences were found in the cortisol-treated rats as compared to the controls. Isolated adipocytes were prepared from epididymal fat pads by collagenase digestion (6). No differences in the size of the adipocytes from treated and control rats were found. Mono-[^{125}I]glucagon at a specific activity of 400-500 μCi/μg was prepared in our laboratory (7) using Na^{125}I (Radiochemical Center, Amershan). Glucagon binding studies were carried out essentially as described previously (1). The adipocytes (0.2 - 0.5 x 10^6 cells/ml) were incubated at a final volume of 350 μl with Mono-[^{125}I]glucagon (0.3 - 0.4 x 10^{-9}M) at 30° C for 30 min in Krebs-Hepes buffer containing glucose (3.3 mM), BSA (1%) and bacitracin (0.9 mM), pH 7.4, in the absence/presence of unlabelled glucagon (Novo Ind. Bagsvaerd) at different concentrations (4.07 x 10^{-10} - 8.14 x 10^{-6} M). Incubations were terminated according to Gliemann et al. (8). All binding data are expressed as specific binding. The non-specific binding (in the presence of 4.07 x 10^{-7} M unlabelled glucagon) did not differ between groups. Glucagon degradation in the incubation medium of the adipocytes at the time of binding was determined by TCA-precipitation using appropriate controls where fat cells were omitted. Glycerol production was determined by a modification of the method of Lambert and Neish (9) after incubation of adipocytes for 2 h at 37° C essentially as described previously (10), in basal medium and in the presence of glucagon (2.03 x 10^{-8} - 2.03 x 10^{-7} M). Cyclic AMP accumulation was determined by RIA (11) after 2 min of cell incubation as previously described (10), in basal medium and in the presence of glucagon (8.14 x 10^{-8} M), theophylline (2.5 mM) or both. Plasma samples were analyzed for glucagon content by RIA (12) and for glucose using a commercial method. Serum samples were incubated with Mono-[^{125}I]glucagon (0.1 x 10^{-9} M) for one and two hours at 37° C and the disappearance of intact glucagon was measured by the decrease in radiactivity precipitable in 10% TCA.

The results were expressed as means ± S.E.M. For statistical comparisons Student's "t"-test was used.

RESULTS

Plasma glucagon levels were elevated in cortisol-treated rats (260 ± 22 (20) pg/ml) as compared to controls (113 ± 3 (33), $p < 0.0001$). Plasma glucose was also increased in treated rats (129 ± 6 (40) mg/dl vs. 112 ± 7 (33), $p < 0.0001$). The rate of glucagon degradation was increased in cortisol-treated rats both at 1 h (11 ± 2% (7) vs. 8 ± 2% (10)) and 2 h of incubation (22 ± 7% (9) vs. 13 ± 8% (13) $p < 0.01$) although the difference only had statistical significance at 2 h.

Specific glucagon binding was significantly reduced at all glucagon concentrations in the adipocytes from cortisol-treated rats as compared to the controls: Figure 1 (left panel). The concentration of unlabelled glucagon required to displace 50% of the maximal specific binding was 3.4 x 10^{-8} M in adipocytes from cortisol-treated animals and 2.9 x 10^{-8}M in controls, indicating no changes in receptor affinity. Figure 1 (right

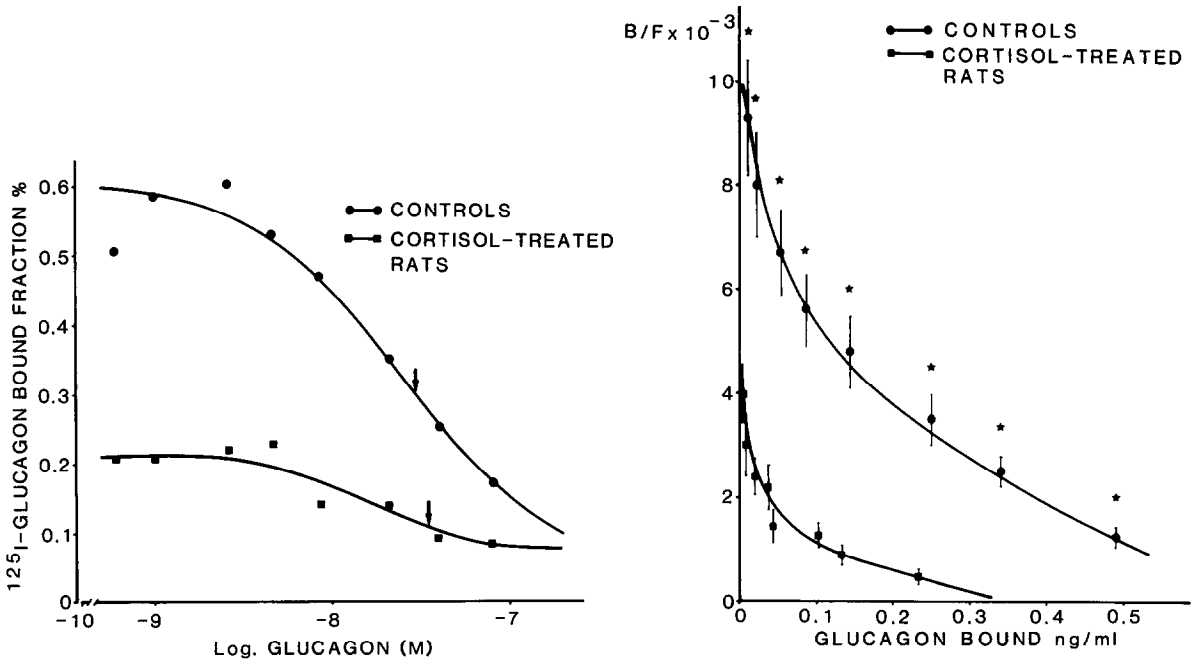

FIGURE 1

Left panel. Displacement curves of Mono-[^{125}I]glucagon binding in adipocytes from cortisol-treated rats as compared to controls. Arrows ( ) represent the glucagon concentration causing 50% inhibition of the receptor-bound fraction of Mono-[^{125}I]glucagon (apparent receptor affinity) in each group of animals.

Right panel. Scatchard plot of binding data. Asterisks ( ) represent statistically significant differences between values of control and cortisol treated rats.

panel) shows a Scatchard analysis of the binding data. The results point to a clear reduction in the number of glucagon receptors in adipocytes from cortisol-treated rats (40,000 ± 10,000 (10) receptors/cell) as compared to controls (84,000 ± 11,000 (16) $p < 0.005$). The percentage of labelled glucagon degraded by cells was similar in both groups of animals: 18-19%.

The lipolytic activity of different glucagon concentrations in adipocytes from cortisol-treated rats is shown in Table I. The basal values of glycerol were similar in both groups. The addition of increasing glucagon concentrations significantly enhanced glycerol production at a similar rate in both treated and control adipocytes. Cyclic AMP accumulation values in the basal medium of adipocytes and in the presence of theophylline, glucagon, or both, were higher in adipocytes from cortisol-

TABLE I

Lipolytic activity of glucagon in isolated adipocytes from cortisol-treated and control rats. Means ± SEM

	GLUCAGON [M]						
	Basal	$2,03x10^{-9}$	$8,14x10^{-9}$	$2,03x10^{-8}$	$4,07x10^{-8}$	$8,14x10^{-8}$	$2,03x10^{-7}$
	Glycerol n mol/10^5 cells/2 h						
Cortisol-treated rats (n = 11)	24±4	21±3	22±3	26±3	38±6 (a)	37±5 (a)	37±5 (a)
Control rats (n = 11)	23±5	25±4	26±4	32±4	32±5	36±6 (b)	39±6 (b)

(a) p vs. basal value < 0.05 in cortisol-treated rats.
(b) p vs. basal value < 0.05 in control rats.

treated rats than in controls. However, both, the rate of cAMP to theophylline and the rate of cAMP response to glucagon or glucagon + theophylline were similar in the two groups of adipocytes (See table II).

TABLE II

Effect of glucagon on basal and theophylline-stimulated cAMP accumulation in isolated adipocytes from cortisol-treated and control rats. Means ± SEM

	cAMP pg/10^6 cells/2 min.	
	Cortisol-treated rats (n = 10)	Control rats (n = 10)
Basal Medium	15.3 ± 0.9	8.4 ± 0.3
Theophylline (2.5 mM)	24.0 ± 3.1 (a,c)	14.1 ± 2.4 (a)
Glucagon ($8.14x10^{-8}$ M)	22.0 ± 2.2 (a,c)	14.5 ± 2.6 (a)
Glucagon ($8.14x10^{-8}$ M) + Theophylline (2.5 mM)	52.6 ± 8.6 (a,b,c)	32.4 ± 4.8 (a,b)

(a) p vs. respective basal value $p < 0.05$
(b) p vs. respective theophylline-stimulated value $p < 0.05$
(c) p vs. between groups $p < 0.05$

DISCUSSION

We have previously reported (4) that prednisolone treatment induces an increase in glucagon plasma levels in man and in the present study we also detected high levels of plasma glucagon in the cortisol-treated rats. The significance of the hyperglucagonemia accompanying the increased glucagon degradation in the serum of our contisol-treated rats remains to be elucidated. In certain types of insulin resistance the presence of a serum factor with increased insulin-degradating activity has been reported (13, 14). Glucocorticoid excess results in insulin resistance and it is posible that in this situation a serum factor might also be induced that could cause an increase in glucagon degradation.

The increased glucagon levels in our cortisol-treated rats may be one of the causes of the decrease in glucagon receptors in these animals since hyperglucagonemia has been consistently associated with decreased glucagon binding although exceptions have also been observed. Furthermore, the decrease in glucagon binding was higher than could be expected according to a down-regulation process alone. Regarding the reports that cAMP potentiates the down-regulation of different hormone receptors (15,16), it seems probable that the increased cAMP accumulation detected by us in adipocytes from cortisol-treated rats could potentiate the down-regulation of glucagon receptors in these cells. On the other hand, fat cells from cortisol-treated rats were found to inactivate glucagon to the same extent as in the control rats, showing that the changes in glucagon binding were not due to differences in glucagon degradation at the time of binding.

Despite the reduced binding of glucagon, the ability of this hormone to stimulate glycerol release in isolated adipocytes from cortisol-treated rats was not reduced. In addition, the rate of cAMP response to glucagon or glucagon + theophylline was also similar in both groups of adipocytes. However, basal cAMP accumulation was elevated in adipocytes from cortisol-treated rats with respect to the control and this increase also persisted in the presence of glucagon, theophylline or both. Decreased cAMP

phosphodiesterase activity (17, 18, 19) would presumably potentiate cAMP accumulation in adipocytes from cortisol-treated rats without altering the cAMP response to glucagon in the lipolytic process.

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