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Dexamethasone Inhibits Insulin Binding to Insulin-Degrading Enzyme and Cytosolic Insulin-Binding Protein p82

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We recently demonstrated that insulin specifically binds to several cytosolic insulin-binding proteins (CIBPs) including insulin-degrading enzyme (IDE) and CIBP p82 in cytosol isolated from H35 rat hepatoma cells. Insulin binding to these CIBPs was regulated by culture conditions, such as serum or insulin. In the present study, we examined the effect of dexamethasone on insulin binding to CIBPs in H35 cells. When the cells were treated with 100 nM dexamethasone for 24 hrs, insulin binding to IDE and CIBP p82 was decreased by about 50% without decreasing the expression level of IDE. Insulin added with the dexamethasone prevented the steroid's effect. Furthermore, dexamethasone directly blocked insulin binding to CIBPs in isolated cytosol. These results suggest that dexamethasone, directly or as a complex with other proteins, binds to IDE and CIBP p82 and changes their ability to bind insulin, possibly by inducing a conformational change or by blocking insulin binding sites. IDE was recently identified as a receptor accessory factor for androgen and glucocorticoid receptors and plays an important role in the regulation of gene transcriptional responses. Combined with previous reports, our findings suggest IDE and other CIBPs such as CIBP p82 may play a role in the cross-talk between insulin and the signal transduction pathways of steroid hormones.

Insulin accumulates in the nucleus in various cell types (1–4) and follows the translocation of internalized insulin to the cell cytoplasm (5, 6). We have recently demonstrated that insulin specifically binds to several cytosolic proteins called CIBPs including IDE in cytosol isolated from rat liver, muscle or H35 rat hepatoma cells (7). Some CIBPs are common and others are tissue specific or increased during cell differentiation (8). H35 cell cytosol has two major CIBPs; IDE and an 82 kDa protein (CIBP p82). Insulin binding to CIBPs was regulated by cultured conditions in H35 cells; serum and insulin treatment increased insulin binding to CIBPs (7). These results suggest that insulin binding to CIBPs, including IDE, may play a physiological role in insulin action. IDE, a zinc-sensitive metalloendoprotease, is the primary enzyme responsible for cellular insulin degradation (9). The majority of its activity is found in the cytoplasm (10). Insulin binds to IDE in intact cells (11) and in isolated cytosol (12) and is one of the important factors regulating translocation of insulin to the nucleus (5). IDE has been hypothesized to play a role in the intracellular degradation of not only insulin but other hormones or growth factors. The degradation of TGF-α and EGF was also a metalloprotease sensitive process (13). EGF inhibited the degradation of insulin by purified IDE (14), suggesting competition for the enzyme. Adding glucocorticoid to conditioned medium decreased insulin degradation by intact cells (15). Interestingly, IDE was recently identified as a receptor accessory factor for androgen and glucocorticoid receptors, and shown to regulate the DNA binding of androgen and glucocorticoid receptors (16). The interaction of IDE with androgen and glucocorticoid receptors may couple insulin and steroid hormone signaling pathways.

In the present study, we examined the effects of the glucocorticoid dexamethasone on insulin

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Abbreviations: IDE, insulin degrading enzyme; CIBP, cytosolic insulin binding protein; TGF-α, transforming growth factor-α; EGF, epidermal growth factor; CTHBP, cellular thyroid hormone binding protein.

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binding to CIBPs in cytosol isolated from H35 rat hepatoma cells treated with dexamethasone for 24 hrs as well as its effects when added directly to the cytosol. Our results showed the dexamethasone inhibited insulin binding to IDE and CIBP p82 without decreasing the concentrations of CIBPs, at least for IDE. These results suggest that dexamethasone, directly or as a complex with some proteins, binds to IDE and changes the ability of IDE to bind insulin, possibly by causing a conformational change or by blocking the insulin binding site. Combined with previous reports, these findings suggest IDE and other CIBPs may play a role in the cross-talk between insulin and the signal transduction pathways of steroid and other polypeptide hormones.

MATERIALS AND METHODS

Cell Culture and Preparation of Cytosol Fraction

H35 rat hepatoma cells were cultured for three days after passage as described before (17). In most experiments, when the cells reached 60–70% of confluency, the cells were serum-deprived with DMEM with 0.1% BSA for 24 hrs. Then the cells were cultured for 24 hrs with 0.1% BSA with no addition, 17 nM insulin, 100 nM dexamethasone, or both insulin and dexamethasone. Cytosol fractions were prepared as previously described (7, 8).

^{125}I-B26-Insulin Binding to CIBPs

^{125}I-B26-insulin was prepared and purified on a reverse-phase high-performance liquid chromatography as previously described (7). The cytosol, at the concentration of 1 mg/ml, was incubated at 4°C for 15 min with 1.7 nM ^{125}I-B26-insulin in the presence or absence of 4.2 μM unlabeled insulin or various concentrations of dexamethasone and crosslinked with 0.5 mM disuccinimidyl suberate for 15 min as described before (7). We chose a 15 min incubation time because the maximum labeling was obtained at 10–15 min in H35 cell cytosol (7). 25 μg of cytosol samples were dissolved in Laemmli buffer with 50 mM DTT, were subjected to 7.5% SDS-PAGE and ^{125}I-insulin-protein complexes were detected by Phosphorlmager (Molecular Dynamics) and quantitatively analyzed with ImageQuant software (Molecular Dynamics).

Western Blot Analysis

Anti-IDE antibody (2BS, rabbit polyclonal) was kindly provided by Dr. M.R. Rosner (Univ. of Chicago). 10 μg of cytosol protein was electrophoresed on 6% SDS-PAGE and electro-transferred onto PDVF membrane (Immobilon-P, Millipore) using a Bio-Rad miniature slab gel apparatus (Mini-Protean II). Western blot analysis was performed as described before (18) and the labeled proteins were visualized by PhosphorImager.

RESULTS

We first examined the effect of dexamethasone treatment of intact H35 rat hepatoma cells on ^{125}I-B26-insulin binding to CIBPs. As shown in Figure 1, insulin treatment of intact cells did not have any effect on ^{125}I-B26-insulin binding to IDE in isolated cytosol, but increased its binding to CIBP p82 (open bar). Dexamethasone treatment decreased the labeling of both CIBP p82 and IDE by about 50% (solid bar). When dexamethasone and insulin were added together, insulin overcame dexamethasone's effects (diagonal stripes). These results suggest that insulin binding to IDE and CIBP p82 was regulated by dexamethasone in intact cells either by changing CIBP protein concentrations or by changing their insulin binding affinity.

We next determined whether or not dexamethasone treatment had an effect on IDE concentrations in the cytosol fractions from H35 cells treated for 24 hrs with 17 nM insulin and/or 100 nM dexamethasone. As shown in Figure 2, IDE concentrations were virtually identical in cytosols isolated from insulin, dexamethasone or insulin plus dexamethasone treated cells (lane 2, 3 and 4 respectively). The slightly lower concentration of IDE in the control cells in this experiment (lane 1) compared to the treated cells was not a consistent observation. These results suggest that the changes in Figure 1 were not due to changes in the concentration of IDE. It was not possible to quantitate CIBP p82, which has not been identified, but is a unique protein based on amino-acid sequence analysis.

Lastly, to determine whether or not dexamethasone directly inhibited insulin binding to CIBP, we examined the effects of dexamethasone added to isolated cytosol on insulin binding to CIBPs.

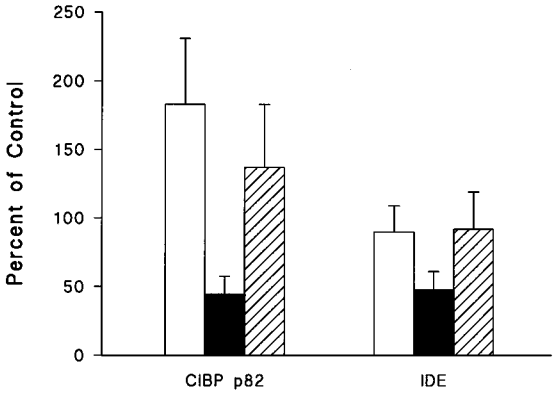

FIG. 1. Effect of dexamethasone and/or insulin on ^{125}I-B26-insulin binding to CIBP p82 and IDE in H35 cell cytosol. Serum-deprived H35 cells were incubated with no addition (control, 100% level), 17 nM insulin (open bar), 100 nM dexamethasone (solid bar) or both insulin and dexamethasone (diagonal stripes) for 24 hrs. The cytosol was isolated and ^{125}I-B26-insulin binding to CIBP p82 and IDE was determined as described in Materials and Methods. The quantitative data were expressed as a percentage of the control. Results are mean (± SEM) of three experiments.

The cytosol fraction was prepared from serum-fed cells since the serum deprivation decreased insulin binding to CIBP compared to serum-fed cells (7). As shown Figure 3, 0.1% ethanol (lane 2), the solvent for dexamethasone solution, and 100 nM dexamethasone (lane 3) did not have a significant effect on insulin binding to CIBP compared to control (lane 1). In contrast, 1 μM dexamethasone (lane 4) almost completely blocked insulin binding to IDE and CIBP p82 and was similar to the decrease caused by 4.2 μM unlabeled insulin (lane 5). These results suggest that dexamethasone interferes directly with insulin binding to IDE and CIBP p82.

DISCUSSION

In a series of studies from our laboratory, we have demonstrated that insulin accumulates in the cell nucleus in various different cell types (1–4). Insulin is internalized by both receptor-mediated and fluid-phase endocytosis (17) and is translocated to the cytoplasm from the endosomes (5,6) although the mechanisms which are involved are still not clear. Nuclear translocation of hormones and growth factors has been widely reported. EGF (19), acidic FGF (FGF-1) (20), basic FGF (FGF-2) (21), interleukin-1 (22), prolactin (23), angiogenin (24), NGF (25), growth hormone (26), and IGF-1 (27) all accumulate in the nucleus. Several laboratories have reported that the nuclear accumulation of ligands, irrespective of membrane signaling events through their receptor, is required for full biological response. For example, the effect of FGF-1 on DNA synthesis requires nuclear translocation since it is lost if the nuclear localization sequence is removed from the ligand (20). Prolactin signaling in T-lymphocytes appears to utilize a classical receptor-mediated kinase cascade and a novel peptide hormone activation pathway involving nuclear translocation (23). These observations have led to the suggestion that the soluble, or non-plasma membrane associated receptors, may be a significant part of the signal transduction system for these hormones.

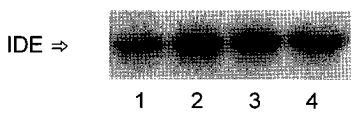

FIG. 2. Effect of dexamethasone and/or insulin on IDE expression level. Serum-deprived H35 cells were incubated with no addition (lane 1), 17 nM insulin (lane 2), 100 nM dexamethasone (lane 3) or both insulin and dexamethasone (lane 4) for 24 hrs. The cytosol was isolated and equal amounts of cytosol protein were subjected to SDS-PAGE and Western blot analysis with anti-IDE antibody (2BS) as described in Materials and Methods.

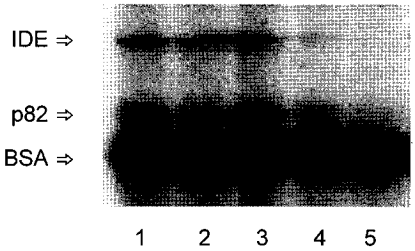

FIG. 3. Direct effect of dexamethasone on ^{125}I-B26-insulin binding to CIBP in isolated H35 cytosol. Isolated H35 cell cytosol was incubated with ^{125}I-B26-insulin in the presence of no addition (lane 1), 0.1% ethanol (lane 2), 100 nM dexamethasone (lane 3), 1 μM dexamethasone (lane 4) or 4.2 μM unlabeled insulin (lane 5), and ^{125}I-B26-insulin binding was analyzed as described in Materials and Methods.

The existence of insulin receptors on intracellular organelles (28), particularly the nucleus (29,30), suggests that intracellular insulin could play an important role in insulin regulated nuclear events if these receptors were exposed to insulin. This hypothesis is supported by studies from several laboratories. For example, microinjection of insulin into the cytoplasm of Xenopus oocytes increased RNA and protein synthesis (31). When added to isolated nuclei, insulin affected various nuclear processes, such as nucleo-cytoplasmic transport of macromolecules (32), protein phosphorylation (33), enzymatic activities (34) and mRNA release from nuclei (35).

Once insulin is internalized and translocated to the cytoplasm, the enzyme IDE is responsible for cellular insulin degradation. IDE is one of the important factors which regulate translocation of insulin into the nucleus. Inhibition of IDE activity increased nuclear accumulation of insulin (5). Insulin has been shown to bind IDE in intact cells (11) and in isolated cytosol (12). We have recently demonstrated that insulin specifically binds to other proteins in cytosol isolated from rat liver, muscle or H35 rat hepatoma cells (7). H35 cell cytosol has two major CIBPs; one is IDE and the other is CIBP p82, which has not been identified, but is a unique protein based on amino-acid sequence analysis. Insulin binding to CIBPs is regulated in various conditions. Serum or insulin treatment increases insulin binding to CIBPs in H35 cells (7). In the present study, we demonstrated that 100 nM dexamethasone treatment of intact cells for 24 hrs decreased insulin binding to CIBP p82 and IDE by about 50% (Figure 1) without decreasing the expression levels of IDE (Figure 2). Furthermore, dexamethasone directly blocked insulin binding to both IDE and CIBP p82 in isolated cytosol (Figure 3). These results suggest that dexamethasone, directly or as a complex with some proteins, binds to IDE and changes IDE's abilities to bind to insulin, either by causing a conformational change in IDE or by blocking the insulin binding site.

IDE, a zinc-sensitive metalloendoprotease, has been hypothesized to play a role in the intracellular degradation of not only insulin but other hormones or growth factors. The degradation of TGF-α and EGF is also a metalloprotease sensitive process (13). EGF inhibits the degradation of insulin by the purified IDE (14). Adding glucocorticoid to the conditioned medium decreases insulin degradation (15). Interestingly, IDE has been recently identified as a receptor accessory factor for androgen and glucocorticoid receptors, and shown to regulate the DNA binding of androgen and glucocorticoid receptors (16). The interaction of IDE with androgen and glucocorticoid receptor may couple insulin and steroid hormone signaling pathways.

Recently we tested whether the expression of or insulin binding to CIBPs are regulated by the adipocyte differentiation process using 3T3-L1 cells (8). Only two CIBPs, IDE and CIBP p55, which is identified as CTHBP, were found in this cell system. We showed that both the expression of and insulin binding to CIBP p55\CTHBP increased during 3T3-L1 adipocyte differentiation. Ashizawa and Cheng (36) showed that CTHBP was important in regulating 3,3′,5-triiodo-L-thyronine transcriptional responses. We do not think CIBPs, IDE and CTHBP, are coincidentally known as binding proteins for other hormones or their receptors, but may be physiologically

relevant and coordinate the antagonistic or synergistic regulation of gene expression by steroid hormones, peptide hormones and cytokines. Further studies are necessary to document the roles of CIBPs in insulin action.

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