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Insulin attenuates the stimulatory effects of tumor necrosis factor α on 11β-hydroxysteroid dehydrogenase 1 in human adipose stromal cells

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Abstract

Obesity is frequently associated with insulin-resistance and abnormal glucose homeostasis. Recent evidence indicates that TNFα may play a role in mediating the insulin-resistance of obesity through its overexpression in adipose tissue. Previously, we have shown that human adipose stromal cells contain 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) mRNA and activity. The present study was designed to examine the effects of insulin on 11β-HSD1 expression in human adipose stromal cells under basal and TNFα-stimulated conditions. The cells were obtained from breast adipose tissue by collagenase digestion, and grown to confluence under replicating conditions in 10% fetal bovine serum. The cells were transferred to serum-free medium for 24 h prior to treatment with either TNFα, insulin or both for a further 24 h. The level of 11β-HSD1 reductase activity was determined by measuring the conversion of [^{3}H]-cortisone to [^{3}H]-cortisol at a substrate concentration of 10 nM. Treatment with TNFα at concentrations of 0.1–10 ng/ml resulted in a dose dependent increase in 11β-HSD1 reductase activity from 1.5 to 10-fold. Insulin (0.1–100 nM) had no effect under basal conditions, but inhibited the stimulatory effects of TNFα (5 ng/ml) on 11β-HSD1 reductase activity in a dose dependent fashion (8–66%) inhibition). Northern blot analysis revealed corresponding changes in the level of 11β-HSD1 mRNA, suggesting that the effects of TNFα and insulin on 11β-HSD1 activity are mediated at the level of gene transcription. The interaction between insulin and TNFα suggests that local and systemic factors may act in a concerted fashion to modulate glucocorticoid activity in adipose and other peripheral tissues.

Keywords: Glucocorticoids; Type 2 diabetes; Insulin-resistance

1. Introduction

11β-Hydroxysteroid dehydrogenase 1 (11β-HSD1) is an enzyme which catalyzes the interconversion of cortisol and cortisone in the liver and most other peripheral tissues [1–6]. In the intact cell, this enzyme favors the

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conversion of cortisone to cortisol while in broken cell preparations, the interconversion of cortisol and cortisone is equal [7]. The conversion of cortisone to cortisol in the in vivo setting provides a mechanism through which the pool of circulating cortisone which does not bind to corticosteroid-binding globulin (CBG), can be converted to cortisol, the active glucocorticoid within peripheral cells where 11β-HSD1 is expressed. The degree of this cortisol biogenesis will depend on the intracellular level of 11β-HSD1 which is known to be controlled by local and systemic factors. Hamani and Siiteri, demonstrated that 11β-HSD activity was present in cultured skin fibroblasts and this activity could be stimulated by glucocorticoids and inhibited by insulin [8]. Using primary cultures of rat hepatocytes, Liu et al. demonstrated that hepatic 11β-HSD was stimulated by glucocorticoids (dexamethasone) and inhibited by growth hormone and insulin [9].

Previous studies from our laboratory have shown that 11β-HSD1 activity and mRNA are present in human adipose stromal cells. We have also shown that the conversion of cortisone to cortisol by 11β-HSD1 stimulates aromatase activity in these cells and that carbenoxolone, an inhibitor of 11β-HSD, blocks this stimulation [10]. Bujalska et al. compared 11β-HSD1 activity in stromal cells from omental and subcutaneous fat. They found activity in omental fat but not in subcutaneous fat and demonstrated stimulation of omental 11β-HSD1 by glucocorticoids [11].

Adipose tissue is an important site for insulin action. Approximately 15% of insulin-mediated glucose uptake occurs in adipose tissue [12] and fat distribution can independently influence insulin sensitivity[13,14]. A preponderance of upper body fat is associated with insulin resistance in polycystic ovarian disease and type 2 diabetes mellitus. One of the possible factors associated with resistance to insulin action is TNFα [15–17]. This factor can stimulate serine phosphorylation of IRS-1 which inhibits tyrosine phosphorylation in the insulin signaling pathway. The present studies were carried out to determine the effects of TNFα and insulin on 11β-HSD1 expression and activity in stromal cells from human subcutaneous adipose tissue to gain further insight into factors regulating 11β-HSD1 activity in peripheral tissues.

2. Materials and methods

2.1. Reagents and supplies

[1,2,6,8-^{3}H(N)]-Cortisol (80 Ci/mmol) was purchased from Du Pont Canada (Markham, Ontario). [1,2,6,7-^{3}H(N)]-Cortisone was prepared from [1,2,6,8-^{3}H(N)]cortisol in our laboratory, as described previously [19]. Non-radioactive steroids were obtained from Steraloids (Wilton, NH). Polyester-backed thin-layer chromatography (TLC) plates were obtained from Fisher Scientific (Unionville, Ontario). All solvents used were OmniSolv grade from BDH (Toronto, Ontario). General molecular biology reagents were from Gibco BRL (Burlington, Ontario) or Pharmacia Canada (Baie D'Urte, Quebec). Cell culture supplies were obtained from Gibco BRL or Fisher Scientific. The cDNAs used in this study, including a mouse 18S rRNA cDNA (Dr. D.T. Denhardt, Rutgers University, Newmark, NJ),were labelled with [^{32}P]dCTP (Du Pont Canada; 3000 Ci/mmol) by random priming. TNFα was obtained from collaborative research and human insulin from Lily Pharmaceuticals.

2.2. Cell culture and treatments

Adipose tissue was obtained from patients undergoing reduction mammoplasty. The tissue from each patient was processed, and the cells were cultured at 37°C in DMEM supplemented with 10% FBS, as described previously [18]. At confluence (after 10–16 days), cells were incubated in serum free medium for 24 h prior to treatment, and the treatments were carried out under serum-free conditions. Cells in triplicate wells were exposed to TNFα and insulin singly or in combination for 24 h, unless otherwise stated. Controls (also in triplicates) were treated similarly but without the addition of TNFα or insulin. Studies were carried out on cells from three subjects with the exception of the TNFα dose response experiments which were carried out on cells from two subjects.

2.3. Assay of 11β-HSD activity

At the end of treatment, the cells were washed three times in serum-free medium. The level of 11β-HSD1 reductase activity in intact cells was determined by measuring the rate of cortisone to cortisol conversion, as described previously [19,20]. Briefly, the cells were incubated for 2 h at 37°C in serum-free medium containing approximately. 100,000 cpm [^{3}H]-cortisone and 10 nM unlabelled cortisone. At the end of incubation, the medium was collected, and steroids extracted. The extracts were dried, and the residues resuspended in methanol. A fraction of the resuspension was spotted on a TLC plate which was developed in chloroform/methanol (9.1 v/v). The bands containing the cortisone and cortisol were identified by UV light, cut out into scintillation vials and counted in Scintisafe™ Econol 1 (Fisher Scientific, Toronto, Canada). The rate of cortisone to cortisol conversion was calculated, and the blank values (defined as the amount of conversion in the absence of cells) were subtracted, and expressed as

a percentage of control. Blank values were less than 2% in all experiments.

2.4. Northern blot analysis

Total RNA was extracted from cultured cells using RNeasy kit (QIAGEN, Mississauga, Ontario, Canada) according to the manufacturer's instructions. Prior to use, samples (10 μg) were checked by agarose gel electrophoresis in the presence of formaldehyde, and the integrity of the RNA was assessed by the presence of two sharp bands representing 28S and 18S rRNA after staining with ethidium bromide. To determine the relative abundance of 11β-HSD1 mRNA, total RNA samples were subjected to Northern blot analysis as described previously [5]. Briefly, denatured RNA samples (30 μg) were subjected to agarose gel (1%) electrophoresis in the presence of formaldehyde, and transferred overnight by capillary blotting to a Zeto-Probe membrane (Bio-Rad Canada, Mississauga, Ontario). The RNA was fixed by UV cross-linking (Gene Cross-Linker, Bio-Rad) to the membrane which was then baked under vacuum at 80°C for 60 min. The blot was hybridized with a [^{32}P]-human 11β-HSD1 cDNA at 42°C for 16 h in the presence of formamide (50%). The same blot was then stripped and reprobed with [^{32}P]-mouse 18S rRNA to serve as an internal control for gel loading and efficiency of RNA transfer, as described previously [5].

To determine the relative abundance of 11β-HSD1 mRNA and 18S rRNA, the relative optical density of the corresponding signals on autoradiographic films were measured by scanning a laser densitometer (LKB 2222-020 UtraScan XL; LKB Produkter AB, Bromma, Sweden). In all cases, the signals were detected within the linear scan range of the densitometer. For each RNA sample, the ratio of 11β-HSD1 mRNA signal to 18S rRNA signal was calculated.

3. Results

In previous studies of steroid metabolism in human adipose stromal cells, it was found that enzyme activity was influenced by the state of cell confluence and by the presence of serum. In the present investigation, cells were grown to confluence in the presence of 10% fetal bovine serum and the effect of 1 and 5 ng/ml of TNFα on 11β-HSD1 reductase activity was studied both in the presence and absence of FBS. In the presence of 10% FBS, 5 ng/ml TNFα stimulated 11β-HSD1 activity by approximately 100% (Fig. 1). When serum was removed from the culture medium for 24 h prior to the addition of TNFα, there was an eight-fold increase inx 11β-HSD1 activity with 1 ng/ml TNFα and greater than a 20-fold increase with 5 ng/ml TNFα. Subsequent studies of 11β-HSD1 activity were carried out in the absence of serum. Fig. 2 shows the effect of incubation time with TNFα prior to the assay of 11β-HSD1 activity. There was minimal stimulation of activity during the first 4 h. of incubation. At 8 h, activity had increased by 100% and at 24 h it had increased by greater than 10-fold. The effect of increasing concentrations of TNFα (0.1–10 ng/ml) on 11β-HSD1 activity is shown in Fig. 3. In these studies, TNFα was added for 48 h prior to measurement of enzyme activity to obtain maximum stimulation. Studies were carried out at a substrate concentration of 10 and 100 nM to be certain that substrate concentration would not be a limiting factor with such large stimulation of enzyme activity. The studies with both substrate concentrations were similar showing a progressive increase in 11β-HSD1 activity with 0.5–10 ng/

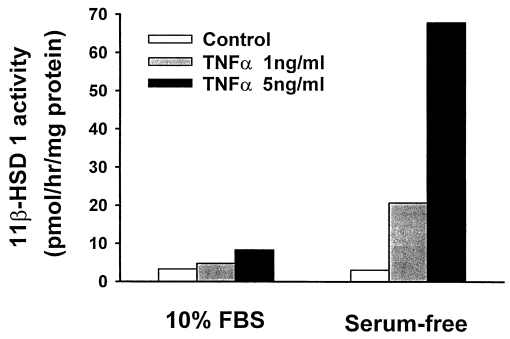

Fig. 1. The effect of serum on 11β-HSD1 stimulation by TNFα. Human adipose stromal cells were grown to confluence in the presence of 10% fetal bovine serum (FBS). In the serum free study, serum was removed for 24 h prior to the addition of TNFα 1 ng/ml (black bars) or 5 ng/ml (grey bars). 11β-HSD1 reductase activity was assayed 24 h after the addition of TNFα. The data are the mean of triplicate incubations of cells from a single individual.

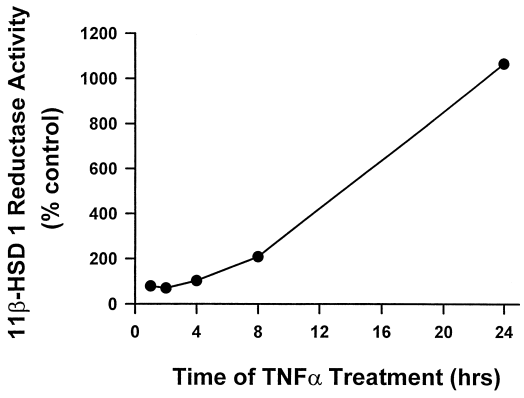

Fig. 2. Induction of 11β-HSD1 by TNFα (5 ng/ml)–time course. Cells were grown to confluence in the presence of 10% FBS. Serum was removed for 24 h prior to addition of TNFα. 11β-HSD1 reductase activity was assayed 1–24 h after addition of TNFα. The study was carried out in triplicate from cells from a single individual.

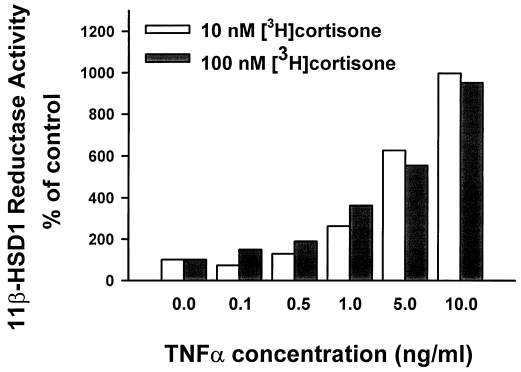

Fig. 3. Induction of 11β-HSD1 by TNFα–dose response curve. The study was carried out on cells grown to confluence in the presence of 10% FBS then deprived of serum for 24 h. 11β-HSD1 activity was assayed 48 h after addition of TNFα. Substrate concentration was 10 nM cortisone (white bars) or 100 nM cortisone (black bars). The results shown are from duplicate incubations on cells from two subjects.

ml TNFα. A concentration of 5 ng/ml TNFα was used for the remaining studies since it provided an intermediate stimulation which would be sensitive to both stimulatory and inhibitory factors.

Fig. 4 shows the effect of increasing concentrations of insulin (0.1–100 nM) on basal and TNFα stimulated 11β-HSD1 activity. There was no effect of insulin on basal 11β-HSD1 activity. There was a progressive inhibition of TNFα stimulated 11β-HSD1 activity with increasing concentrations of insulin. There was a 10% inhibition at 0.5 nM, a 40% inhibition at 10.0 nM and a 50% inhibition at 100 nM.

In order to gain further insight into the mechanism of insulin induced attenuation of the TNFα stimulation of 11β-HSD1, a study was carried out in which insulin (100 nM) was added simultaneously with TNFα (5 ng/ml) or at 16, 20, 22 or 23 h after the addition of TNFα. These data are shown in Fig. 5. Insulin had no effect on basal 11β-HSD1 activity. When insulin and TNFα were both present for 24 h, the TNFα stimulation of 11β-HSD1 activity was inhibited. When insulin was added 16 h after the TNFα, there was partial inhibition. When added more than 20 h after the TNFα, there was no inhibition. Total RNA samples from adipose stromal cells treated with TNFα and insulin were subjected to Northern blot analysis using human 11β-HSD1. cDNA probe revealed a single 1.8 kb mRNA in all samples. The level of 11β-HSD1 mRNA was increased eight-fold by TNFα. Insulin alone did not alter the level of 11β-HSD1 mRNA but attenuated the TNFα stimulated increase. Changes in 11β-HSD1 reductase activity mirror mRNA levels (Fig. 6.

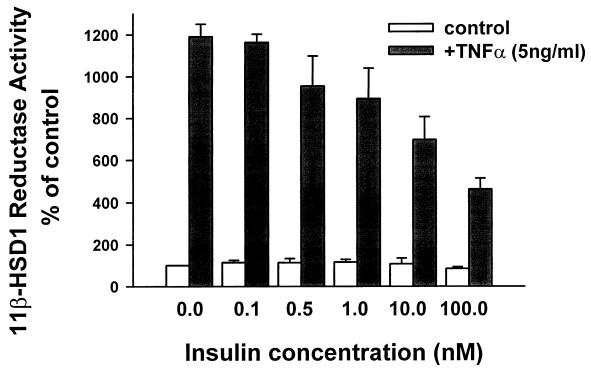

Fig. 4. The effect of increasing concentrations of insulin on 11β-HSD1 reductase activity in human adipose stromal cells in the presence (dark bars) and absence (white bars) of TNFα (5 ng/ml). Results are the mean of triplicate incubations carried out on cells from three individuals.

4. Discussion

The present study demonstrates that TNFα is a potent stimulus for 11β-HSD1 reductase activity in human adipose stromal cells. The magnitude of this stimulation by concentrations of TNFα, which are within the range found under physiological conditions suggests that TNFα may be a major factor in regulating 11β-HSD1 activity. Initial studies of the effect of TNFα on both reductase and dehydrogenase activity showed that the stimulatory effect of TNFα was primarily on the reductase component. The net effect of TNFα in this system is to increase the intracellular content of cortisol. Since TNFα is produced by fat cells [21,22] and decreases with weight loss [23], this system may play an important role in regulating adipocyte metabolism and differentiation.

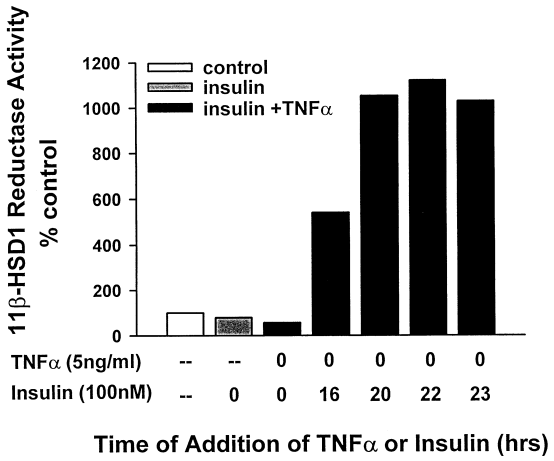

Fig. 5. The effect of the time of the addition of insulin (10 nM) after the addition of TNFα on 11β-HSD1 reductase activity in human adipose stromal cells. Confluent cells were deprived of insulin for 24 h prior to the addition of TNFα (5 ng/ml). Insulin was added simultaneously with TNFα or 16, 20, 22 or 23 h after the addition of TNFα. The results shown are from a single experiment with duplicate incubations.

The stimulatory effect of TNFα on 11β-HSD1 had been previously reported by Escher et al. [24] using rat glomerular mesangial cells. They noted that both IL1β and TNFα stimulated 11β-HSD1 but IL-3, IL-6, platelet derived growth factor, PMA and forskolin were without effect. The present study demonstrates that insulin inhibits TNFα stimulation of 11β-HSD1 activity. This interaction between TNFα and insulin is of interest because of the evidence that TNFα may be a factor in causing insulin resistance [15–17]. In the present situation, TNFα stimulates the formation of 11β-HSD1 mRNA and 11β-HSD1 activity and this process is inhibited by insulin. This suggests that insulin may play a role in regulating intracellular cortisol levels. The concentrations of insulin required to inhibit 11β-HSD1 activity are within the physiological range. Fasting insulin in normal subjects is in the 0.1 nM range and levels increase to the 1.0 nM range during glucose stimulation. In insulin resistant states, insulin levels can reach up to 10 nM or greater. The results in this study are in keeping with those of Hammami and Siiteri [8]. They demonstrated that insulin inhibited 11β-HSD activity when cells were incubated over a period of 5 days with a half maximal inhibition of 1–10 nM. They noted that 10 μM insulin inhibited the induction of 11β-HSD by glucocorticoids (Dexamethasone 100 nM). The present studies demonstrate that the inhibitory effect of insulin is through the inhibition of mRNA synthesis. This effect can be demonstrated within 6–8 h and is dependent on other factors such as glucocorticoids or TNFα which are acting to stimulate 11β-HSD1 activity. It is uncertain whether the resistance to the action of insulin on glucose transport would be associated with resistance to insulin inhibition of TNFα stimulation of 11β-HSD1 activity.

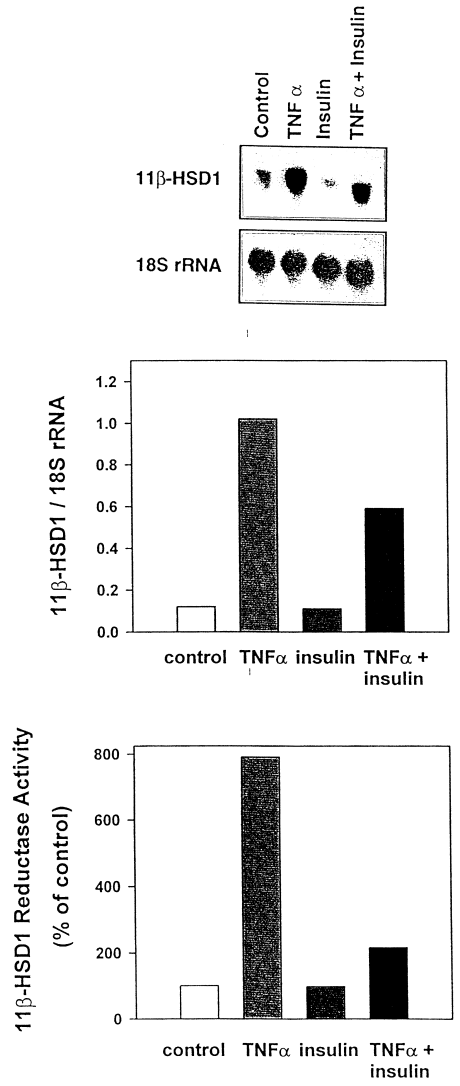

Fig. 6. The effect of TNFα (5 ng/ml) and insulin (100 ng/ml) alone or in combination on 11β-HSD1 activity, and mRNA levels in human adipose stromal cells. Cells were grown to confluence and deprived of serum for 24 h prior to addition of insulin, TNFα or both for 24 h prior to assay of 11β-HSD1 reductase activity (Panel C) and harvesting of cells for Northern blot analysis of total RNA hybridized sequentially with [32P]-human 11β-HSD1 cDNA and [32P]-mouse 18s rRNA cDNA (Panel A). Panel B shows the intensity of the radiographic signals measured by the densitometer.

The interaction between TNFα and insulin may play a role in the metabolic syndrome associated with insulin resistance. An increase in the intracellular levels of glucocorticoids in response to cytokine stimulation of 11β-HSD1 may be incompletely regulated by insulin. This increase in glucocorticoids may further contribute to local insulin resistance and lead to adipocyte differentiation. 11β-HSD1 is present in vascular smooth muscle cells [24] and can regulate the vasoconstrictor effect of cortisol. The interaction between TNFα and insulin on 11β-HSD may be a factor in the hypertension seen in patients with insulin resistance and type 2 diabetes. Walker et al studied the possible role of 11β-HSD1 in influencing insulin sensitivity [25]. They found that inhibiting 11β-HSD1 by administration of carbenoxolone to male subjects for 7 days resulted in an overall increase in insulin sensitivity which was mediated by decreased hepatic glucose production with no effect on insulin sensitivity in the forearm. It is possible that some of the beneficial effects of insulin sensitizing drugs such as metformin and troglitazone may be acting either by inhibiting 11β-HSD1 directly or by augmenting the inhibitory effects of insulin. The regulation of 11β-HSD1 may provide an additional approach in the management of type 2 diabetes mellitus.

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