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Is the metabolic syndrome an intracellular Cushing state? Effects of multiple humoral factors on the transcriptional activity of the hepatic glucocorticoid-activating enzyme (11β-hydroxysteroid dehydrogenase type 1) gene

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Abstract

Although glucocorticoid, as "gluco-" literally implies, plays an important role in maintaining the blood glucose level, excess of glucocorticoid production/action is known to cause impaired glucose tolerance and diabetes. Since 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which converts inactive cortisone to active cortisol, is primarily expressed in the liver, an enhanced expression of the enzyme may increase the intracellular glucocorticoid level and thus increase the hepatic glucose production. In this study, we examined the effects of multiple humoral factors related to the metabolic syndrome on the transcriptional activity of 11β-HSD1 gene in hepatocytes in vitro. We found that, among the factors examined, adipocyte-derived cytokines (adipokines), like TNFα and IL-1β, potently stimulated the transcriptional activity of 11β-HSD1 gene in human HuH7 cells. In contrast, only minimal effects of other humoral factors were observed when they were used alone. Interestingly, however, when applied in combination, they synergistically enhanced the transcriptional activity of 11β-HSD1 gene. They also potentiated the effects of cytokines. Glucocorticoid receptor (GR)-dependent transcription was indeed increased even with an inactive glucocorticoid cortisone following TNFα pretreatment, indicating the enhanced intracellular conversion. Finally, PPARγ/PPARα agonists, clinically used as anti-diabetic drugs, significantly inhibited the transcriptional activity of 11β-HSD1. Altogether, our data strongly suggest that combination of the humoral factors related to the metabolic syndrome, including the adipokines, synergistically enhances the hepatic expression of 11β-HSD1 gene and causes the intracellular Cushing state in the liver by increasing the intracellular glucocorticoid level. We assume that the observed synergistic effects of these factors on 11β-HSD1 may, at least partly, explain the reason whereby accumulation of the multiple risk factors facilitates the derangement of glucose and lipid metabolism in the metabolic syndrome.

Keywords: Glucocorticoid; 11β-Hydroxysteroid dehydrogenase; Cushing syndrome; Metabolic syndrome; Cytokine

1. Introduction

Recent clinical and epidemiological studies strongly suggest that accumulation of metabolic risk factors is responsible for the occurrence of cardiovascular events such as coronary heart disease or stroke (Reaven, 1988; Kaplan, 1989; DeFronzo and Ferrannini, 1991; Matsuzawa et al., 1993). Reaven (1988) proposed syndrome X, in which a cluster of metabolic disorders such as impaired glucose tolerance, dyslipidemia, obesity, and hypertension, causes a variety of vascular disorders. The concept of the "multiple risk factor syndrome" including the deadly quartet, insulin resistance syndrome, and visceral obesity syndrome, has recently been standardized as "the metabolic syndrome", the clinical guidelines of which is now being proposed (WHO report, 1999; Final Report of the NCEP–ATP III, 2002).

The molecular mechanism of the syndrome, whereby the accumulation of the factors synergistically increases the risk of

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cardiovascular diseases, is yet to be determined. It is assumed that a variety of metabolic stress is merged within the cell to cause an altered profile of gene expression, which subsequently causes the clinical picture of the metabolic syndrome. Interestingly, the phenotype of the metabolic syndrome is fairly similar to that of Cushing's syndrome such as central (abdominal) obesity, glucose intolerance, dyslipidemia/fatty liver, and hypertension, suggesting that enhanced glucocorticoid action may, at least partly, be responsible for the pathogenesis of the syndrome. Indeed, Bujalska et al. (1997) have shown that the expression of 11β-hydroxysteroid dehydrogenase (11β-HSD1) is increased in visceral adipose tissue and suggest that visceral obesity is "a Cushing syndrome of the omentum". 11β-HSD1 is an enzyme which converts inactive cortisone to active cortisol in vivo (Tomlinson et al., 2004; Seckl, 2004), and thus the enhanced expression of the enzyme is expected to raise the concentration of cortisol within the adipose tissue and/or in the liver. More recently, Masuzaki and Flier have developed a strain of transgenic mice with an adipocyte-specific over expression of 11β-HSD1, which showed a phenotype similar to the metabolic syndrome (Masuzaki et al., 2001). Thus, 11β-HSD1 ectopically expressed in the abdominal adipose tissue may be one of the key molecules for the organization of the metabolic syndrome.

More important, however, is that 11β-HSD1 is expressed in target organs of glucocorticoid hormone such as the liver, brain and anterior pituitary. Among them, the liver is a key organ of glucose metabolism (Seckl and Walker, 2001), and glucocorticoid plays an important role in maintaining the blood glucose level (Kraus-Friedmann, 1984). The indispensable role of the hormone is recognized when hypoglycemia occurs during adrenal insufficiency. Thus, 11β-HSD1 is assumed to be acting as an amplifier of glucocorticoid action to maintain blood level in healthy subjects. It is also assumed that, if the expression of the enzyme is unusually increased, the intracellular glucocorticoid level will also be over activated and may cause the metabolic derangements similar to Cushing syndrome within the liver. Indeed, recent basic studies suggest that the hepatic 11β-HSD1 expression is increased in db/db mice or polygenic obese mice, both of which are animal models of the metabolic syndrome (Liu et al., 2005; Morton et al., 2005).

In this study, we tried to elucidate the effects of the humoral factors related to the metabolic syndrome on the transcriptional activity of 11β-HSD1 gene using the HuH7 human hepatocyte cell line in vitro. We found that the known humoral factors such as insulin, proinflammatory cytokines, glucocorticoid and endotoxin, exerted positive effects on the transcriptional regulation of 11β-HSD1 gene in a synergistic manner. In contrast, ligands for PPARα/PPARγ, widely used as anti-diabetic and antihyperlipidemic drugs, are found to have inhibitory effects on the promoter activity of 11β-HSD1 gene.

2. Materials and methods

2.1. Reagents

Dexamethasone, cortisone, cortisol, human insulin, oleic acid, lipopolysaccharide (LPS), clofibrate, and metformin were obtained from Sigma (St. Louis, MO). Human interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) were from PeproTech (Rocky Hill, NJ). Troglitazone was a generous gift from Sankyo Pharmaceutical Co. (Tokyo, Japan).

2.2. Plasmids

Expression vectors for cFos and cJun were described previously (Yoshida et al., 2006). pGRE-Luc reporter plasmid was purchased from Clontech (Palo Alto, CA, USA), and pAP1-Luc and pNFκB-Luc were from Stratagene (La Jolla, CA). NF-κB p65 (RelA) expression vector was a generous gift from Dr. Alcami (Instituto de Salud Carlos III, Madrid, Spain).

2.3. Cell culture and transfection

HuH7, a human hepatoma cell line, was obtained from RIKEN cell bank (Saitama, Japan), and maintained in DMEM (high glucose) supplemented with 10% bovine serum (FBS) under a 5% CO_2/95% air atmosphere at 37 °C. For stable transfection experiments, a mixed polyclonal HuH7 cell line incorporated with the 5′-promoter region of the human 11β-HSD1 gene (−1348/+47 bp, +1 designates the transcription start site)-luciferase reporter plasmid, designated as HuH7HSD1, was established with FuGene 6 transfection reagents (Roche Applied Science, Indianapolis, IN, USA), and applied for the subsequent studies. For transient transfection experiments, HuH7 cells were co-transfected with the test plasmids and the RSV-β-galactosidase (β-Gal) expression vector using FuGene 6 for 24 h, and β-Gal activity in each sample was used as an internal control. For a glucocorticoid bioassay experiment, the HuH7GRE cell line, in which HuH7 cells were incorporated stably with the GRE-luciferase reporter plasmid (Stratagene, CA, USA), was used. Furthermore, the HuH7RSV cell line, in which HuH7 cells were incorporated stably with the constitutive RSV-luciferase reporter plasmid was also established and used as a control.

2.4. Experiments

The HuH7HSD1 cells (or transiently transfected HuH7 cells) were cultured with DMEM containing 1% FBS in 24-well plates. In each experiment, test reagents, in 1000× concentration, were added directly into the culture medium, and the cells were incubated for the defined time interval. At the end of incubation, the culture medium was removed, and the cells were harvested for the determination of luciferase activity.

2.5. Assays

Luciferase assay was performed as previously described (Aoki et al., 1997), and light output was measured for 20 s at room temperature using a luminometer (Berthold Lumat LB9507, Bad Wildbad, Germany). β-Gal activity was determined by Galacto-Light Plus β-gal assay kit (Tropix, Bedford, MA, USA).

2.6. RT-PCR

Endogenous expression of insulin receptor, glucocorticoid receptor (GR) α and β, and 11β-HSD1 mRNAs were examined by RT-PCR using Superscript II (Invitrogen) and Taq DNA polymerase (Takara, Kyoto, Japan). The primer sets used were as follows: sense, 5′-CCTTCAAGAGATGATTCAGATG-3′ and antisense, 5′-GTTCATTAGACAGGCCTTGGT-3′ for insulin receptor; sense, 5′-ACACAGGCTTCAGGTATCTT-3′ and antisense, 5′-ACTGCTTCTGTTGCCAAG-3′ for GRα; sense, 5′-ACACAGGCTTCAGGTATCTT-3′ and antisense, 5′-CGCCAAGATTGTTGGGATGA-3′ for GRβ; sense, 5′-CTGCAAACGAGGAATTCAGAC-3′ and antisense, 5′-GGAGACGACAACAATGCTTCC-3′ for 11β-HSD; sense, 5′-GTCACAGTTTTAGCTTCCATG-3′ and antisense, 5′-CTTGCCTAAGTAATGGTCCAC-3′ for hexose-6-phosphate dehydrogenase (H6PD).

2.7. Western blotting

The HuH7HSD1 cells were cultured in 3.5-cm diameter dishes and the cellular proteins were obtained using NE-PER nuclear and cytoplasmic extrac-