Charles University in Prague, Faculty of Science
Department of Biochemistry

The role of protein kinase C in the pathogenesis of insulin resistance and its complications

Mgr. Irena Marková

Ph.D. thesis

Supervisor: Doc. RNDr. František Novák, CSc.
Faculty of Science, Charles University in Prague
Department of Biochemistry

Consultant Supervisor: Ing. Ludmila Kazdová, CSc.
Institute for Clinical and Experimental Medicine in Prague
Department of Metabolism and Diabetes

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INTRODUCTION

Metabolic syndrome (MS) represents a cluster of metabolic disorders that increase the risk of type 2 diabetes (DM2) and cardiovascular diseases [1]. The relevancy of MS is shown by its high prevalence and also by the fact that it plays a key role in the pathogenesis of many disorders, especially DM2, obesity, hypertension, cardiovascular diseases and others [2]. MS is characterized by tissue resistance to insulin action, hyperinsulinemia, impaired glucose tolerance and dyslipidemia, essential hypertension, abdominal (visceral) obesity, hyperuricaemia, coagulation disturbances and fibrinolysis, and endothelial dysfunction [3].

Insulin resistance (IR) is a basic metabolic abnormality of MS. It is a common pathological state in which target cells fail to respond to ordinary levels of circulating insulin [4]. It results in an inability of insulin to provide normal glucose and lipid homeostasis. IR increases the demand for insulin secretion by pancreatic β-cells and leads to compensatory hyperinsulinemia. IR involves metabolic disturbances in skeletal muscle, liver and adipose tissue, resulting in reduced insulin-mediated glucose utilization.

The causes of the rise and development of IR, according to current ideas are multifactorial. The aetiology of IR includes genetic factors, increasing age as well as environmental factors related to lifestyle such as food intake associated with obesity and reduced physical activity. The causes of IR are particularly disorders in the insulin signaling cascade, the defect may occur at any stage in the cascade of events beginning with the binding of insulin to insulin receptors, tyrosine kinase activation, the cascade of phosphorylation and dephosphorylation reactions and terminating with the activation of effector systems that are responsible

for mediating the biological effects of insulin. Protein kinase C (PKC) is an important component of the insulin signaling cascade and numerous recent studies have demonstrated that PKC plays a pivotal role in the development of IR.

Great attention is also paid to the role of adipose tissue, which does not function merely as a reservoir of energy in the form of triglycerides, but produces a range of mediators (adipokines) that are involved in the inflammatory process and that may also interact with components of the insulin signaling cascade, thereby contributing to the development of IR. Among adipokines, TNFα and resistin are particularly involved in the development of IR, while in contrast adiponectin increases insulin sensitivity [5].

The increased availability and utilization of free fatty acids (FFA) play a critical role in the development of IR [2, 6]. In IR, visceral adipose tissue is resistant to the antilipolytic effect of insulin and consequently releases excessive amounts of FFA. Elevated FFA impair insulin sensitivity in insulin responsive tissues and negatively affect insulin transduction through a well known cascade of events (activation of cellular kinases). FFA also induce IR in skeletal muscles [7].

PKC is a serine/threonine kinase, which is involved in the pathogenesis of IR and its related complications [8, 9]. The PKC family comprises 12 isoforms that have been subdivided into three groups based on structural similarities and mechanisms of activation: the conventional, novel and atypical PKCs [10]. Individual PKC isoforms differ in their functions, co-factor requirements, substrates, and different tissue and intracellular localization. PKCs play a dual role in insulin signaling. They are involved in the transduction of specific insulin signals but also

contribute to the generation of IR. PKC ζ and λ are the most important isoforms in mediating the effect of insulin on glucose transporter translocation and glucose uptake [11]. PKC ε and PKC θ play a key role in skeletal muscle IR, their selective activation was demonstrated in a number of dietary-induced IR models [8, 9, 12]. These isoforms act as inhibitors of insulin signal transduction and interfere with insulin signaling, leading to reduced insulin signal transmission and ultimately to reduced skeletal muscle insulin sensitivity. Insulin sensitivity of the muscle is closely associated with the triglyceride content. Elevated concentrations of diacylglycerol (DAG) (an intermediate of triglyceride and phospholipid synthesis) are considered to be a link between PKC activation, lipid metabolism disorders and increased IR of skeletal muscle.

AIMS OF THE THESIS

Studying the pathogenesis of IR

- studying the pathogenesis of IR in relation to the cellular localization and the relative amounts of PKC ε and PKC θ in the skeletal muscle of HHTg rats
- developing a method for measuring the activity of glycogen synthase (GS) and analysing its role in the pathogenesis of IR
- monitoring the parameters of IR in relation to PKC ε and PKC θ after nutritionally induced obesity in HHTg rats and during the ontogenesis of HHTg rats

Studying the role of resistin in the pathogenesis of IR

- monitoring the effect of increased resistin expression in the adipose tissue of SHR rats on lipid and carbohydrate metabolism in relation to

the cellular localization and relative amounts of PKC ε and PKC θ in skeletal muscle

Possibilities for pharmacological treatment of IR

- studying the metabolic effects of long-term pioglitazone treatment of SHR-4 rats on ectopic lipid accumulation, skeletal muscle and adipose tissue resistance and on the relative amounts of PKC ε and PKC θ in skeletal muscle

MATERIAL AND METHODS

Experimental models

Strain of hereditary hypertriglyceridemic rats (HHTg) - a suitable non-obese model for studying the pathogenesis of IR, exhibits almost all the symptoms of MS (dyslipidemia, hyperinsulinemia, impaired glucose tolerance, hypertension). Hypertriglyceridemia is genetically fixed and potentiated by a sucrose-rich diet (SRD).

Strain of Wistar rats - Wistar rats were used as controls to HHTg rats

Strain of spontaneously hypertensive rats (SHR-4) – the congenic SHR-4 strain is genetically identical to the SHR strain except for the differential segment of chromosome 4, including FAT/Cd36. In addition to genetically fixed hypertension, this strain exhibits other metabolic abnormalities (dyslipidemia, hyperinsulinemia, glucose intolerance), which can be potentiated by SRD or a fructose-rich diet (FRD).

Transgenic strain of SHR rats expressing mouse resistin – a new transgenic strain of SHR rats expressing a nonsecreted form of mouse resistin under the control of the aP2 promoter predominantly in adipose tissue [13]. This strain allows the study the potential role of resistin in the pathogenesis of IR in SHR rats.

Methods

Biochemical analysis – determination of serum triglycerides, FFA, glucose, cholesterol, insulin, adiponectin and tissue concentrations of proteins and triglycerides using commercially available analytical kits

Analysis to assess insulin resistance in peripheral tissues and glucose tolerance - *in vitro* incorporation of ^{14}C-U-glucose into skeletal muscle/diaphragm glycogen or epididymal fat pad lipids in the basal state or after insulin stimulation and the oral glucose tolerance test (OGTT)

PKC analysis - homogenization and fractionation of skeletal muscle to obtain cytosolic and membrane fractions, protein quantification of the fractions, electrophoresis and Western blotting, immunodetection, enhanced chemiluminescence, computer processing

Activity of GS - activity assayed by measuring the incorporation of ^{14}C-glucose from uridine 5'-diphosphate glucose into glycogen

RESULTS AND DISCUSSION

Effect of nutritionally induced obesity on PKC and insulin resistance in HHTg rats

Experiments were performed in 18-months-old control Wistar rats, HHTg and obese HHTg rats. The obesity of HHTg rats was induced by diet: HHTg rats were fed a SRD for seven months and became obese, while control and HHTg rats were fed a standard laboratory diet. Obesity is one of the factors that contribute to the development of IR, and it is associated with a higher prevalence of DM2 and cardiovascular complications. In numerous studies performed in both humans and

animals, it has been shown that a decrease or increase of body weight is closely correlated with increasing or decreasing insulin sensitivity, respectively [14; 15; 16].

The serum concentrations of triglycerides and FFA were significantly higher in HHTg rats when compared with control rats. In addition, HHTg rats exhibited an impaired sensitivity of the peripheral tissues to insulin action. Another characteristic of these rats is ectopic storage of triglycerides in the liver and skeletal muscle, which negatively affects the insulin sensitivity of these tissues.

Feeding HHTg rats a SRD compared with HHTg rats fed a standard diet resulted in increased body weight, increased weight of the epididymal fat pad, and increased serum concentrations of glucose, triglycerides and FFA. This model of nutritionally induced obesity corresponds fairly well to the situation in humans, because the increased intake of sucrose over the past few decades is considered to be one of the reasons for the significant increase in obesity and its related complications. Increased levels of plasma triglycerides, glucose and also insulin were found in obese insulin-resistant Zucker rats [17]. A common finding in obese subjects is elevated serum FFA concentrations caused by their increased release from excessive fatty tissue [18]. FFA affect metabolic functions in many tissues and contribute to the reduced insulin sensitivity of peripheral tissues. Obesity is also associated with ectopic lipid storage [19]. Nutritionally induced obesity in HHTg rats was accompanied by a significant accumulation of triglycerides in the liver.

In the membrane fraction of skeletal muscle of HHTg rats, we found a lower relative amount of PKC ε when compared to controls. The amount of PKC ε in HHTg rats was not affected by obesity. In HHTg rats, we

found a lower amount of PKC θ in the cytosol and a higher relative amount of PKC θ in the membrane fraction when compared to controls. A decrease of PKC θ in the cytosol and an increase of membrane-associated PKC θ are considered to be an indicator of PKC translocation and activation [8; 20]. Nutritionally induced obesity in HHTg rats tended to further increase PKC θ activation. Decreased skeletal muscle insulin sensitivity in HHTg rats was accompanied by PKC θ activation.

In most studies on IR and the possible role of PKC ε in its pathogenesis, increased PKC ε activity has been found. However, in our study we found lower activation of PKC ε in HHTg rats compared to controls. A similar finding was described in further research in obese Zucker rats. Cooper et al. found reduced PKC activity and smaller quantities of PKC ε in skeletal muscle compared to Zucker lean control animals [21]. There could be various explanations for these different findings concerning PKC ε. The increased activation of PKC ε may increase the translocation, protein turnover but also the degradation of PKC. A genetic mutation causing the altered function or inactivation of PKC may also play a role [21].

The finding of increased PKC θ activation in insulin-resistant HHTg rats is consistent with the literature. In our study, we measured the accumulation of triglycerides in skeletal muscle, which contributes to PKC activation. Triglyceride metabolism is associated with the production of biologically active metabolites such as DAG, which is the PKC activator [19]. Activated PKC θ phosphorylates serin hydroxyl residues of IRS1 (insulin receptor substrate) and IRS2. It reduces IRS1 and IRS2 tyrosine phosphorylation, resulting in the reduced activity of

PI3K (fosfatidylinositol-3-kinase) and leads to reduced glucose transport [22]. These effects of PKC θ decreases insulin signal transduction and contribute to IR.

Our findings show that HHTg rats are a suitable model for the study of IR. Nutritionally induced obesity in HHTg rats further enhanced a number of abnormalities of lipid and carbohydrate metabolism associated with IR. The results suggest that PKC θ could play an important role in the pathogenesis of IR in skeletal muscle.

Glycogen synthase activity and insulin resistance

GS is one of the effector molecules responsible for mediating the biological effects of insulin. GS activity is reduced in the skeletal muscles of patients with DM2 [23]. In our study, we measured GS activity in the skeletal muscle of HHTg rats and control Wistar rats. At the age of four months, all animals were fed a SRD for two weeks. Feeding a SRD to HHTg rats resulted in an exaggeration of metabolic abnormalities. After overnight fasting (16 hours), half of the animals in each group received an oral single dose of glucose (3 g/kg b. wt.) and were killed 30 minutes later. This experimental design allowed us to test the response of the organism to the rise of endogenous insulinemia, which reflects its actual metabolic state. The aim of our study was to identify the mechanism of impaired glucose utilization in muscle tissue during the initial phase of OGTT.

In the fasting state, GS activity did not differ between control and HHTg rats. After a single dose of glucose, the enzyme activity significantly increased in controls, while in HHTg rats it remained unchanged compared with the fasting state. This finding was supported

by the finding of reduced levels of glycogen in the skeletal muscle of HHTg rats [24]. Several studies have shown decreased insulin-stimulated glucose uptake in DM2, which was associated with impaired GS activity and glycogen synthesis [25; 26].

We have demonstrated that impaired glucose utilization in the peripheral tissues of HHTg rats is accompanied by the reduced activity of GS in skeletal muscle. Metabolic maladaptation to the transition from fasting to the fed state may be one of the mechanisms underlying the impaired glucose utilization in the non-obese model of IR. Decreased GS activity and glucose utilization in peripheral tissues suggest a possible defect in insulin signal transduction in the cells. A defect in one or more steps in this signaling cascade, where one of the important effector molecules is also GS, plays a role in the development of IR.

Influence of aging on PKC and insulin resistance

It has been long known that IR progressively increases with age in both humans and rats [27; 28], but the mechanisms implicated in the worsening of IR and other accompanying symptoms with increasing age, are not fully understood. It is assumed that these disorders are associated with changes in body composition, i.e. an increased proportion of body fat and obesity, rather than with aging itself [29; 30].

Experiments were conducted in HHTg rats and control Wistar rats aged 4- and 18- months. The animals were fed a standard laboratory diet, then received a SRD for two weeks before the end of the study.

The relative weight of the epididymal fat pads, which is considered as an indicator of visceral obesity, remained unaltered in HHTg rats with age. As this parameter highly correlates with body fat percent [31], it can

be claimed that the cause of IR deterioration with age was not obesity. This claim is opposed to the theory proposing that the cause of the age-related deterioration of IR is a higher amount of body fat due to obesity. Here a link may exist that reconciles both contradictory claims, i.e. that age-related deteriorating IR is caused by obesity and our finding that it is independent of obesity. It has been speculated that increased serum FFA levels may be the primary defect resulting in decreased glucose utilization in tissues, and, subsequently, in hyperinsulinemia [32]. Hyperinsulinemia may be the compensatory mechanism of IR in the peripheral tissue preventing an increase in gluconeogenesis in the liver and, hence, hyperglycemia [33]. In our study, HHTg rats showed increased serum FFA levels that tended to rise with age and increase following SRD.

Serum triglyceride levels were higher in HHTg rats fed a standart diet compared with controls and tended to rise with age. A SRD for two weeks raised triglyceridemia irrespective of age, by 80 % in the HHTg strain, and by 35 % in the control group. In the HHTg rat strain, hypertriglyceridemia is genetically fixed and, as indicated by the study results, it continues to increase with age.

Another finding in HHTg rats was reduction in insulin-stimulated glucose utilization in adipose tissue and the diaphragm. Defects of glucose utilization were more apparent in 18-months-old animals compared with younger ones. At the age of 18 months, the impaired glucose tolerance of the HHTg strain became even more pronounced and also became partly manifest in the control group, although, at that age, the sensitivity of the peripheral tissue to insulin action in the control

group was only slightly greater than in the completely resistant tissues of the HHTg group.

Decreased cellular insulin signaling is a potential mechanism responsible for the defects in insulin action characteristic of old age. PKC may negatively affect insulin signal transmission. We found no differences in the relative amount of PKC ε protein between young and old HHTg and control rats in the cytosol and membrane fraction. These findings are consistent with the results of Qu et al, who, also found no changes in PKC activity or PKC ε and PKC θ subcellular distribution in skeletal muscle between young and old nondiabetic rats [34]. Unlike the results of the above-mentioned study, we observed differences in PKC θ levels, but at an advanced age. In HHTg rats, we found significantly larger amounts of PKC θ in the cytosol and membrane fraction. IR in 18-months-old HHTg rats was accompanied by an overall increase in the relative amount of protein PKC θ in skeletal muscle. Individual PKC isoforms may differ in their expression in muscle and may have different functions. PKC θ is the major PKC isoform expressed in skeletal muscle, and most studies point to a relationship between PKC θ and IR [35; 36]. The increased amount of PKC θ in old HHTg rats may be related to elevated concentrations of triglycerides in skeletal muscle. Triglycerides are indicators of the presence of other lipid intermediates, such as DAG, and LCACoA (long-chain acyl-CoA), which are PKC activators [37]. An accumulation of triglycerides was found in the skeletal muscle of HHTg rats in the previous study [38].

The results indicate that the age-related increase in IR and the deterioration of some parameters of carbohydrate and lipid metabolism

are associated with an overall increase in the relative amount of PKC θ in the skeletal muscle of HHTg rats. These findings suggest the possible involvement of PKC θ in IR of skeletal muscle disorders.

Effect of resistin overexpression in adipose tissue on PKC and insulin resistance

Resistin is a protein produced by adipose tissue and which has been suggested to represent a molecular link between obesity, IR and DM2 [39]. This hypothesis is supported by studies in which the serum concentrations of resistin were increased in rodent models of obesity and DM2 [39] and by the observation that acute *in vivo* administration of large amounts of recombinant resistin is associated with hepatic IR and impaired glucose tolerance [40].

Experiments were performed in one-year-old SHR rats with the resistin transgene. This strain expresses mouse resistin in adipose tissue that is not detectable in the circulation, thus providing a unique opportunity to study the autocrine effects of resistin in adipose tissue [13]. The control group comprised age-matched genetically identical rats without the transgene. All animals were fed a FRD two weeks before the end of the study.

The transgenic rats exhibited increased serum concentrations of insulin and triglycerides and markedly impaired glucose tolerance. These findings are consistent with the results of previous studies in other transgenic models, such as Wistar rats with chronic resistin over-expression**.** These rats showed glucose intolerance during a glucose tolerance test, hyperinsulinemia and elevated serum levels of

triglycerides [41]. Resistin expression in SHR rats did not affect skeletal muscle insulin sensitivity and was not accompanied by an accumulation of triglycerides in skeletal muscle or the liver. In addition, it did not lead to elevated serum concentrations of FFA.

The used animal model expresses mouse resistin in adipose tissue and showed a near total resistance of the adipose tissue to insulin action. This finding suggests an autocrine effect of resistin in adipose tissue, and it appears that resistin may negatively affect insulin signaling. A number of *in vivo* studies have shown that resistin negatively influences insulin signal transduction not only in adipose tissue, but also in the liver and skeletal muscle [42; 43].

Chronic transgenic expression of resistin did not affect the relative amount of PKC ε in the studied cellular fractions, but a significant increase in PKC θ levels was found in both the cytosolic and membrane fractions. Increased skeletal muscle PKC θ activity was found in transgenic mice expressing human resistin which were fed a high-fat diet [44]. Increased amounts of skeletal muscle triglycerides and DAGs were found in these animals. The reason for the increased relative amount of PKC θ found in our study is not clear because in SHR transgenic rats we did not detect ectopic storage of triglycerides in the skeletal muscle or increased FFA levels in the circulation. FFA in the circulation can directly stimulate production of DAG in muscle and activate PKC θ [18].

The results of our study support the hypothesis of a role of resistin in the pathogenesis of IR. They suggest its autocrine effects in adipose tissue that may be, together with increasing age, considered to be a predisposition to diabetes. The role of PKC in the mechanisms

underlying the deterioration of metabolic abnormalities that accompany increased resistin expression in adipose tissue has not yet been clarified.

Effect of long-term pioglitazone treatment on PKC and insulin resistance

Thiazolidinediones (TZDs) such as pioglitazone are insulin sensitizing drugs used for the treatment of DM2. TZDs improve glycemic control by reducing insulin resistance in the target tissues. They are PPARγ agonists (peroxisome proliferator-activated receptor γ), which regulate the transcription of genes sensitive to insulin and are involved in regulating glucose and lipid metabolism in adipose and muscle tissue. Their mechanism of action is not yet fully understood [45].

In these study, we tested a hypothesis that TZDs ameliorate IR in muscle tissue by suppressing muscle lipid storage and the activation of novel PKC isozymes. Specifically, we analyzed the long-term metabolic effects of pioglitazone in an animal model of MS, SHR-4 rats. SHR-4 rats in an experimental group were fed a SRD supplemented with pioglitazone (300 mg/ kg diet) from the age of 4 to 8 months. SHR-4 controls were fed a SRD without pioglitazone for the same time. The SHR-4 congenic strain with the wild type FAT/Cd36 gene was selected instead of the SHR that harbors a deletion variant because FAT/Cd36 is a target gene involved in the insulin sensitizing actions of pioglitazone [46].

Pioglitazone-treated SHR-4 rats showed decreased serum triglycerides and FFA, which are likely metabolic consequences of PPARγ activation in adipose tissue. The activation of these receptors by

TZDs reduces the rate of FFA release and amplifies insulin-stimulated glucose transport.

Long-term pioglitazone treatment of SHR-4 rats increased adipose tissue insulin sensitivity, which was accompanied by increased protein content in adipose tissue. This finding suggests a remodeling of adipose tissue, involving a multiplication of small, metabolically active, insulin-sensitive fat cells [47]. Our findings are consistent with the hypothesis that TZDs influence adipose tissue metabolism. They can influence the differentiation, size and total number of adipocytes within a particular fat depot as well as regulating insulin signaling, glucose and lipid metabolism in mature adipocytes [47]. In addition, pioglitazone-treated rats exhibited significantly decreased levels of serum insulin while hyperinsulinemia was observed in SHR-4 control rats fed a SRD. Decreased levels of serum insulin, triglycerides and FFA suggest the amelioration of systemic IR and dyslipidemia.

TZD treatment is usually associated with significantly decreased intramyocellular lipid levels [48; 49]. According to a widely accepted hypothesis, the lipid reducing effects of TZDs in skeletal muscles are considered as possible insulin sensitizing mechanisms acting through reducing the activity of novel PKC isoforms in particular, thereby ameliorating insulin signaling. Surprisingly, in the current study, we observed that long-term pioglitazone treatment was associated with increased triglyceride levels in the gastrocnemius muscle, with activation of the PKC ε and PKC θ isoforms, and with amelioration of insulin-stimulated glycogenesis. These findings are similar to the results of Lessard et al. [50], who found ameliorated glucose tolerance in obese Zucker rats treated with rosiglitazone despite the fact that these rats

exhibited significantly increased intramyocellular triglycerides in the soleus muscle when compared to glucose intolerant controls. The reason for these discrepant results regarding the effects of TZD on skeletal muscle lipid accumulation is unclear. It has been suggested that the effect of TZD on lipid storage might depend on the predominant type of muscle fibers. For instance, most of studies showing reduced skeletal muscle lipid content after TZD treatment analyzed muscles with a large percentage of type II fibers (glycolytic fibers), while Lessard et al. [50] analyzed the soleus muscle containing predominantly type I fibers (oxidative fibers). The gastrocnemius muscle used in the current study, contains both type I and type II fibers, suggesting that the differential effects of TZD on lipid storage in skeletal muscle might not depend on the muscle type. It is also possible that increased triglyceride levels in the gastrocnemius muscle might be explained by the reduced activity of hormone sensitive lipase (HSL), which is a lipase for DAGs [51]. Downregulation of HSL could be associated with increased DAG, which in turn might affect the activation of PKC ε and PKC θ.

Serum concentrations of adiponectin in pioglitazone-treated rats were significantly higher compared to controls. The positive effect of TZDs on IR is related to the stimulation of adiponectin production. Adiponectin can play an important role in the mechanism responsible for enhancing the insulin sensitivity of peripheral tissues [52].

In conclusion, the results of the current study demonstrate that long-term pioglitazone treatment can ameliorate IR in muscle tissue as well as many other parameters of lipid and carbohydrate metabolism. Increased skeletal muscle triglyceride accumulation after pioglitazone treatment was associated with the activation of PKC ε and PKC θ. The favourable

effects of TZDs are probably due to the remodeling of adipose tissue and increased adiponectin secretion.

SUMMARY

Studying the pathogenesis of insulin resistance and the role of PKC in insulin resistance

• In HHTg rats, elevated serum triglycerides and FFA were associated with the ectopic accumulation of triglycerides in tissues and reduced insulin sensitivity of peripheral tissues. Impaired glucose utilization in the peripheral tissues was associated with the reduced activity of GS in skeletal muscle. Decreased GS activity and glucose utilization in peripheral tissues indicate a possible defect in insulin signal transduction. In line with this, our results show that skeletal muscle IR was associated with the increased activation and translocation of PKC θ.

• Nutritionally induced obesity of HHTg rats resulted, in many cases, in the further deterioration of metabolic abnormalities associated with IR. We found that PKC θ, in particular, could contribute to the metabolic abnormalities associated with IR and obesity.

• The age-related increase in IR and deterioration of some parametres of carbohydrate and lipid metabolism, were not associated, in HHTg rats, with obesity but with increased serum levels of triglycerides and FFA.

• The age-related worsening of IR in HHTg rats was accompanied by increased relative amounts of PKC θ in skeletal muscle. These findings

suggesting the possible involvement of PKC θ in skeletal muscle IR disorders.

Studying the potential role of resistin in the pathogenesis of insulin resistance

• The chronic transgenic expression of resistin in adipose tissue in SHR rats was associated with disorders of lipid and carbohydrate metabolism, the resistance of adipose tissue to insulin action and a significant increase in the relative amount of PKC θ in skeletal muscle.

• The results support the hypothesis of a role of resistin in the mechanisms underlying IR, suggesting its autocrine effects on adipose tissue. Whether PKC may play a role in the deterioration of metabolic abnormalities, which is accompanied by the increased expression of resistin in adipose tissue, was not completely resolved.

Effects of pharmacological intervention in insulin resistance

• Long-term pioglitazone treatment positively influenced the metabolic abnormalities associated with IR. It decreased the serum concentrations of triglycerides and FFA, increased the insulin sensitivity of adipose and muscle tissue, increased the serum levels of adiponectin and avoided the development of hyperinsulinemia. By contrast, pioglitazone increased skeletal muscle triglyceride accumulation and activated PKC ε and PKC θ.

• The beneficial effects of TZDs can be explained by the remodeling of adipose tissue and increased adiponectin secretion.

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LIST OF ABBREVIATIONS

DAG diacylglycerol

DM2 type 2 diabetes mellitus

FFA free fatty acids

FRD fructose rich diet

GS glycogen synthase

HHTg hereditary hypertriglyceridemic rat

HSL hormone sensitive lipase

IR insulin resistance

IRS insulin receptor substrate

MS metabolic syndrome

OGTT oral glucose tolerance test

PKC protein kinase C

PPARγ peroxisome proliferator-activated receptor γ

SHR spontaneously hypertensive rat

SRD sucrose rich diet

TZDs thiazolidinediones