Research Signpost
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

Recent Advances in Pharmaceutical Sciences VI, 2016: 77-90 ISBN: 978-81-308-0566-5
Editors: Diego Muñoz-Torrero, Àngela Domínguez and Àngels Manresa

5. The role of the c-Jun N-terminal kinase (JNK) pathway in insulin resistance

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Abstract. Obesity is usually associated with a decreased response to insulin, a major metabolic defect known as insulin resistance and an early trait in the development of type 2 diabetes. The c-Jun N-terminal kinase (JNK) pathway has emerged as a central regulator of insulin sensitivity, locally and systemically, thereby, of body's metabolic homeostasis. As the incidence of obesity and type 2 diabetes has alarmingly increased in the last few decades, there is a tremendous necessity to identify novel pharmacological targets to efficiently improve the therapeutic outcome. In this regard, the JNK pathway seems to meet most of the requirements for being an adequate candidate to direct pharmacological intervention.

Introduction

The incidence of type 2 diabetes is alarmingly increasing worldwide specially as a result of the tremendous rise in obesity, a major risk factor for

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the development of this disease. Decreased sensitivity of target tissues to insulin, known as insulin resistance, is an early trait in the development of type 2 diabetes and results in glucose intolerance; afterwards, failure of pancreatic insulin-secreting cells (β-cells) is the major determinant of progression to hyperglycemia, the hallmark of diabetes. Insulin resistance is strongly associated to obesity; its incidence in the population parallels that of obesity; and represents the link of obesity to type 2 diabetes [1].

Insulin resistance is a major metabolic disorder and develops because defective insulin receptor (IR) signaling in insulin-target tissues such as liver, adipose tissue and skeletal muscle, among others. The etiology of insulin resistance has been the focus of intensive research, and it is currently accepted that chronic inflammation is an important cause [2]. In this regard, obesity, which is the most common origin for the development of insulin resistance, is usually associated with a low-grade, chronic inflammatory state that initially affects the adipose tissue but at latter stages spreads out to other tissues because the fat accumulation at these anomalous locations [3]. Other conditions associated to the expansion of the adipose tissue such as the increased production of reactive oxygen species (ROS) or the endoplasmic reticulum (ER) stress also promote insulin resistance either directly or through the enhancement of the inflammatory response [4]. At molecular level, pro-inflammatory cytokines are main players in promoting insulin resistance, as they are activators of signaling pathways, such as the c-Jun N-terminal kinase (JNK) and the inhibitor of nuclear factor κ-B kinase (IKK) pathway, that negatively interfere with the early steps in the IR signaling cascade [5]. In this chapter we are going to focus the attention on the physiopathological role of the JNK pathway on the regulation of insulin sensitivity.

1. The JNK signaling pathway

JNK comprises a group of serine/threonine protein kinases that belongs to the stress-activated protein kinase (SAPK) subfamily of mitogen-activated protein kinases (MAPKs). MAPKs participate in ubiquitous and evolutionary conserved signal transduction pathways in eukaryotes. MAPK pathways enable coordinated responses to a vast array of stimuli regulating different cellular processes such as gene transcription, mRNA stability, protein synthesis, cell proliferation, cell differentiation, or apoptosis. The

MAPKs are the final step of a protein kinase cascade (the MAPK module) composed, in addition to the MAPK, by a MAPK kinase (MAP2K), and a MAP2K kinase (MAP3K), which are sequentially activated through phosphorylation by the upstream kinase. In addition, non-enzymatic scaffold proteins may also participate in these MAPK modules to stabilize physical interactions and/or promote recruitment of the components. MAPK modules enhance efficiency and selectivity in signal transmission. MAPKs are activated by dual phosphorylation on tyrosine and threonine residues within the evolutionary conserved Thr-Xxx-Tyr motif located in their activation loop. MAPKs are proline directed kinases thus; they phosphorylate serine and threonine residues followed by a proline, though engagement of substrates requires additional interactions out of the catalytic site mediated by specific docking sites located apart of the phosphorylation sites. MAPKs are inactivated by dephosphorylation performed by protein phosphatases such as the MAP kinase phosphatase (MKP)/dual specificity phosphatase (DUSP)-1 [6].

JNK was initially identified as the major protein kinase responsible for the phosphorylation of residues at the N-terminal/transactivation domain of c-Jun, a component of the AP-1 complex, triggering its transactivation function (Fig. 1) [7-9]. The JNK signalling pathway is preferentially activated by pro-inflammatory signals, such as tumor necrosis factor (TNF)-α or interleukin (IL)-1β, and stress stimuli, such as ultraviolet (UV), ER stress and ROS. Consistently, JNK pathway is a target of anti-inflammatory drugs such as glucocorticoids, which efficiently repress the activation of the JNK pathway by different mechanisms [10-12].

JNKs are encoded by three genes, *jnk*-1, *jnk*-2, and *jnk*-3, which through alternative splicing mechanisms give rise up to twelve different isoforms. Expression of *jnk*-1 and *jnk*-2 is ubiquitous whereas *jnk*-3 is expressed in a tissue specific manner [13,14]. The MAP2K MKK4 and MKK7 catalyze the phosphorylation, and concomitant activation, of JNK. Whereas MKK4 also phosphorylates the MAPK p38, MKK7 is highly specific for JNK and other substrates have not been identified so far for this protein kinase. Accordingly, MKK4 and MKK7 show nonredundant functions *in vivo*. For instance, MKK4 activates JNK in response to stress stimuli, such as anisomycin or heat-shock, whereas MKK7 is required for JNK activation by pro-inflammatory signals [15,16]. Consistent with the variety of stimuli that activate the JNK pathway, a diverse group of MAP3Ks phosphorylate, and concomitantly activate, MKK4 and/or MKK7 [6].

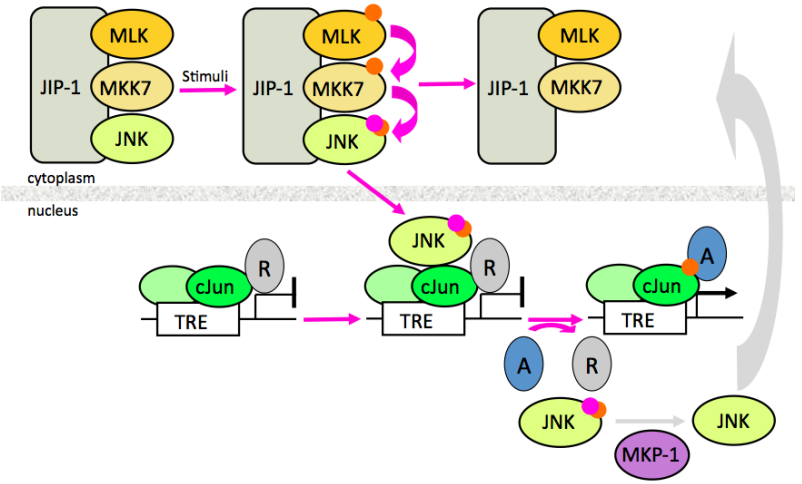

Figure 1. Regulation of the AP-1-dependent gene transcription by the JNK pathway. In non-stimulated conditions, protein kinases (MAP3K: MLK, MAP2K: MKK7, and MAPK: JNK) and scaffolds (JIP-1) of the JNK pathway module are pre-assembled in the cytoplasm but inactive. In the nucleus, AP-1 complexes are associated to their cognate DNA binding elements, known as TPA response elements (TREs), but the interaction of co-repressors (R) with c-Jun, a component of the AP-1 complex, inhibits transcription of the AP-1 target genes. Upon stimulation, the protein kinases in the module are sequentially activated by phosphorylation (phosphotyrosine and phosphoserine/phosphothreonine are indicated in magenta and orange dots, respectively). Activated JNK dissociates from the module and translocates into the nucleus where interacts and phosphorylates c-Jun at specific sites in its transactivation domain. c-Jun phosphorylation induces the dissociation of co-repressors allowing the interaction with co-activators (A), consistently, the AP-1-dependent gene transcription is induced. MKP-1, a nuclear protein phosphatase, inactivates JNK by dephosphorylation, and inactive JNK returns to the cytoplasm.

2. The IR signaling pathway

Insulin is a hormone secreted by the pancreatic β-cells in response to an increase in glycemia. It has very important endocrine functions mostly, but not exclusively, related to carbohydrate and lipid homeostasis. Insulin reduces glycemia by promoting glucose uptake in adipose tissue and skeletal muscle and suppressing hepatic gluconeogenesis. In addition, insulin promotes glycogen, lipid and protein synthesis in target tissues. Insulin signaling is triggered by its binding of the IR, a receptor that belongs to the

tyrosine kinase receptor family and that is composed by two extracellular subunits (α chains) and two transmembrane subunits (β chains). Hormone engagement activates the intrinsic tyrosine kinase activity of the IR resulting in the autophosphorylation on the β subunit cytoplasmic tails. These phosphotyrosine residues are docking sites for the recruitment of proteins from the IR substrate (IRS) family, such as IRS-1 or IRS-2. IRS-1/2 are also phosphorylated on tyrosine residues by the IR resulting in the generation of a docking platform for proteins of the IR signaling, such as the subunit of the phosphatidylinositol-3-kinase (PI3K), p85, or the Grb2-SOS adaptor Shc.

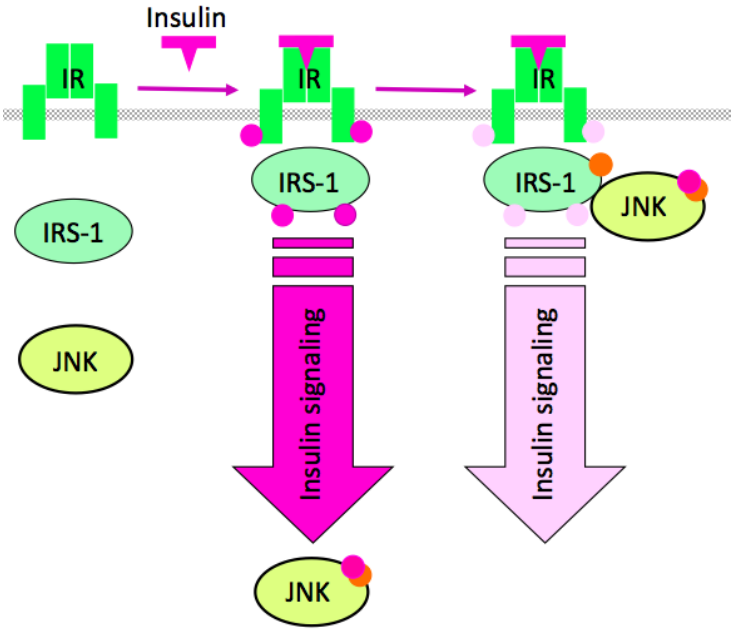

Figure 2. Insulin-induced JNK activation inhibits IR signaling by phosphorylation of IRS-1 on serine-307. Insulin binding to the IR triggers its tyrosine kinase activity eliciting the autophosphorylation on tyrosine residues (magenta dots) of the cytoplasmic tails of the IR β subunits. These phosphotyrosine residues are docking sites for the recruitment of proteins from the IRS family such as IRS-1. Upon interaction, IRS-1 is phosphorylated on tyrosine residues by the IR resulting in the generation of a docking platform for the interaction with a variety of proteins involved in IR signaling. Insulin induces the activation of JNK, which in turn binds to IRS-1 and phosphorylates it on serine-307 (orange dot). IRS-1 phosphorylation on serine-307 disrupts its interaction with the IR ensuing the physiological down-regulation of IR signalling.

IR-activated PI3K promotes AKT activation, which eventually elicits the regulation of glucose metabolism and protein synthesis, and, through the sterol-regulatory element binding protein (SREBP)-1c, the metabolism of lipids. In contrast, insulin mitogenic effects are mostly mediated by the induction of the SOS-Ras-extracellular signal-regulated protein kinase (ERK) signaling pathway (Fig. 2) [17].

IRS-1 and IRS-2 are tightly regulated by phosphorylation on multiple serine/threonine residues mediated by several insulin-stimulated protein kinases, an autologous mechanism that regulates, positively or negatively, insulin sensitivity [18]. Concretely, IRS-1 phosphorylation on serine-307 is induced by insulin and inhibits IRS-1 interaction with the insulin-activated IR, thereby inhibits IR signaling. Serine-307 is targeted by JNK, which is activated in response to insulin and in this manner operates as a negative feedback mechanism to turn off IR signalling in physiological conditions (Fig. 2) [19-21]. Likewise, IRS-2 is also phosphorylated by JNK on serine-488 with similar functional consequences [22].

3. Obesity-induced insulin resistance and the JNK pathway

The relationship between obesity-induced insulin resistance and inflammation originates with the observation of an increased TNF-α gene expression in adipose tissue of obese rodents, which turns into increased levels of this cytokine locally and, eventually, systemically. Moreover, TNF-α neutralization in these animals improved insulin sensitivity [23]. In the following years, many studies have contributed to decipher the cellular and molecular mechanisms underlying the role of inflammation in the promotion and progression of obesity-induced insulin resistance, however most of them are out of the scope of this revision hence they will not be mentioned but are profoundly reviewed in references 2 to 5.

In obesity there is an overexpansion of the adipose tissue that is accomplished by processes of adipocyte hyperplasia and hypertrophy. Hypertrophic adipocytes undergo a series of perturbations such as an increased demand on the synthetic machinery, an accumulation of unfolded proteins in the ER or an excessive production of ROS that generate stress in the ER. To restore organelle functionality a response known as the unfolded protein response (UPR) emanates from the ER. The UPR is a complex response system with different branches but notably one of them, mediated by the inositol-requiring enzyme (IRE)-1, activates JNK through a pathway involving TNF-receptor associated factor (TRAF)-2 [24]. This activation of JNK leads to IRS-1/2 phosphorylation with the concomitant inhibition of IR

signaling. In addition, the JNK pathway-induction of pro-inflammatory gene expression works as a feed-forward mechanism to further increase JNK activity. In this scenario, this chronically activated JNK leads to the promotion of insulin resistance (Fig. 3). In addition, in obesity there is an increase in the systemic level of free fatty acids (FAA), which by binding to the Toll-like receptors activate the JNK pathway and, thereby, foster insulin resistance [25].

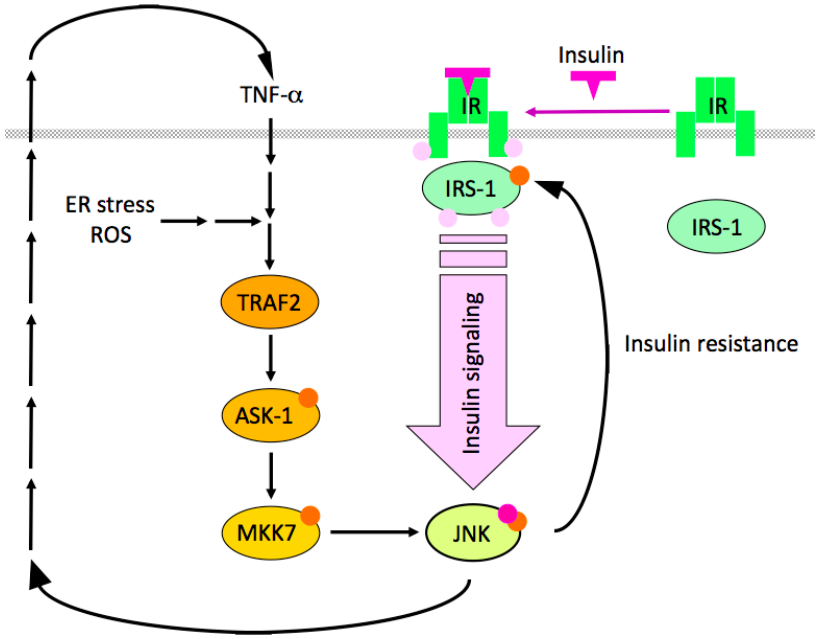

Figure 3. Obesity-induced JNK activation induces insulin resistance through IRS-1 phosphorylation on serine-307. In obesity there are conditions, such as the increased level of pro-inflammatory cytokines (TNF-α) and ROS, or the induction of ER stress, that exacerbate the activity of the JNK pathway by a TRAF-2-dependent induction of phosphorylation, and concomitant activation, of the protein kinases of the JNK module (magenta and orange dots on ASK-1, MKK7 and JNK, respectively). This obesity-induced JNK activity inhibits IR signaling through the phosphorylation of IRS-1 on serine-307 (orange dot). In addition, enhanced expression of pro-inflammatory mediators, such as the TNF-α, mediated by the JNK pathway further contributes to the inhibition of IR signaling mediated by the JNK-dependent IRS-1 phosphorylation. Thereby, in this scenario of chronically exacerbated JNK activity insulin resistance develops.

In accordance with this, JNK activity is augmented in tissues such as liver, adipose tissue, and skeletal muscle of obese humans [26] and rodents [27]. Moreover, a central role of the JNK pathway on obesity-induced insulin resistance is also substantiated by genetic data.

Epidemiologic studies identified the gene encoding the JNK interacting protein (JIP)-1, a scaffold protein of the JNK module, as a candidate for type 2 diabetes [28]. In addition, JIP-1-genetic deficiency in mice prevents obesity-induced activation of JNK in adipose tissue and protects from diet-induced obesity and insulin resistance [29].

Moreover, *jnk*-1-deficient mice are protected from the development of adiposity and show significantly improved insulin sensitivity and enhanced IR signalling in diet- and genetically-induced obesity mouse models [27]. This study demonstrated that JNK-1 isoform has a prominent role in obesity-induced insulin resistance given that *jnk*-2-deficient mice showed a wild type phenotype [27]. However, the fact that *jnk*-2-deficient mice heterozygous for *jnk*-1 are also protected from diet-induced obesity and insulin resistance points to a compensatory role of JNK-1 in the absence of JNK-2 [30].

The pivotal role of JNK in obesity-induced insulin resistance has strongly instigated the pharmacological targeting of the JNK pathway as a therapeutic approach for the treatment of type 2 diabetes. In this regard, treatment of obese mice with a small molecule inhibitor of JNK improved insulin sensitivity [31].

Notably, it has been described that thiazolidinediones (TZDs), a group of synthetic peroxisome proliferator-activated receptor (PPAR)-γ ligands used in clinics because their insulin-sensitizing activity [32], are active in the inhibition of obesity-induced JNK activity in insulin-target tissues such as liver and adipose tissue. Indeed, the lack of TZD antidiabetes action in obese *jnk*-1-deficient mice indicates that JNK inhibition mediates this pharmacological property. Moreover, PPAR-γ is expressed in immune cells, such as macrophages, and in adipocytes; therefore, TZDs may inhibit JNK in both cellular compartments, immune and metabolic, of the adipose tissue, and regardless the nature of the stimuli, a pro-inflammatory cytokine or a stress mediator (Fig. 4) [33].

The contribution of tissue-specific JNK activity on insulin sensitivity has been studied in transgenic mice by tissue specific genetic inactivation of *jnk*-1, or to a lesser extend *jnk*-2, or over-expression of dominant-negative or constitutively-activated forms of JNK or its immediately activating kinase, MKK7.

Adipose-tissue specific *jnk*-1 genetic ablation does not prevent from adiposity but protects from the development of insulin resistance in adipose tissue and liver induced by a high-fat diet. This effect on hepatic insulin sensitivity seems to be mediated by the reduction in the interleukin (IL)-6 release from the adipose tissue [34].

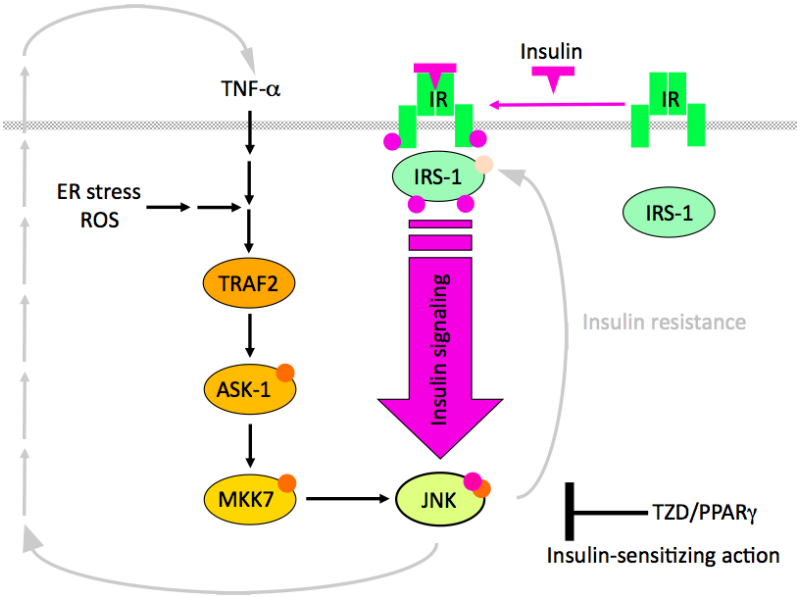

Figure 4. TZD insulin-sensitizing action is mediated by inhibition of the JNK pathway in adipocytes and macrophages. In obesity, the JNK pathway is activated by different stimuli and inhibits IR signaling through IRS-1 phosphorylation on serine-307 (see text and legend of Fig. 3). TZDs inhibit obesity-induced JNK activity, an action that requires their interaction with PPAR-γ, and, in this way, restore IR signaling and, thereby, insulin sensitivity.

In the liver, overall JNK activity inhibition by adenovirus-enforced overexpression of a dominant-negative version of JNK or by simultaneous inactivation of *jnk*-1 and *jnk*-2 protects against insulin resistance in diet-induced obese mice [35,36]. In addition to the negative action of JNK on IR signaling through IRS-1/2 phosphorylation, the inhibitory role of JNK on the PPAR-α/fibroblast growth factor (FGF)21 pathway also contributes to this protective phenotype [36-38]. Nonetheless, there is some

controversy regarding the specific role of each liver JNK isoform on systemic glucose metabolism. On one hand, knock down of *jnk*-1 or *jnk*-2 gene expression in the liver of obese animals resulted in reduced hyperglycemia and hyperinsulinemia but with distinct effects on hepatic steatosis [39,40]. However, single *jnk*-1 genetic ablation specifically in hepatocytes induced glucose intolerance, insulin resistance and hepatic steatosis in lean mice and had no effect on obese animals, indicating that this isoform has a protective function in this particular cell type [41]. This discrepancies may be due to a dual role of JNK-1 in hepatocytes that is, in addition to down-regulate IR signalling, JNK-1 is required for gluconeogenesis, lipogenesis and clearance of circulating insulin [41], in addition to not yet characterized interactions among the distinct cell types in the liver. Finally, activation of the JNK pathway by adenovirus-mediated over-expression of wild type JNK decreased insulin sensitivity and augmented insulinemia due to an increased hepatic glucose production [35].

Regarding skeletal muscle, whereas *jnk*-1-deficiency specifically in this tissue does not protect from diet-induced obesity, it ameliorates systemic insulin resistance by preserving IR signalling locally. This protective effect is not widespread to adipose tissue or liver, in fact, theses mice showed enhanced hypertriglyceridemia, due to a reduction on muscle lipoprotein lipase, macrophage infiltration in adipose tissue and hepatic steatosis [42]. In contrast, JNK over-expression studies in skeletal muscle have rendered contradictory and therefore, require further analysis [43,44].

JNK has also been implicated in promoting insulin resistance in pancreatic β-cells. In particular, FFA administration results in JNK activation with the concomitant inhibition of IR signalling in these cells [45]. Moreover, *in vivo* activation of JNK by over-expression of a constitutively active version of MKK7 in pancreatic β-cells leads to glucose intolerance due to the failure to secrete insulin in response to glycemia. Static insulin secretion assays with isolated pancreatic islets showed that JNK activation blocks the second phase of glucose-induced secretion of insulin, which depends on IR signalling [46]. In addition, JNK might be involved in pancreatic β-cell failure, as it is required, though not sufficient, for pro-inflammatory cytokine-induced apoptosis in this cell type [46,47].

Finally, JNK also regulates glucose metabolism from the central nervous system (CNS). As in peripheral tissues, obesity increases JNK activity in the hypothalamus [48], and CNS-specific *jnk*-1-genetic ablation

protects mice from diet-induced obesity, glucose intolerance and insulin resistance [49,50]. This phenotype is achieved through augmented energy expenditure and locomotor activity [49,50]. Moreover, a similar phenotype was also observed in genetically modified mice lacking JNK-1 and JNK-2 in the adenohypophysis [51]. In contrast, JNK activation in the agouti-related peptide (AgRP)-expressing neurons of the hypothalamus induces obesity as a consequence of hyperphagia [52]. Altogether, these results indicate that at the CNS JNK regulates the activity of the hypothalamic-pituitary-adrenal axis [49-52].

4. Conclusion

The JNK pathway is currently regarded as a promising candidate for the pharmacological treatment of insulin resistance and type 2 diabetes because its pivotal role in the control of insulin sensitivity. For instance, the ability of the insulin-sensitizing drugs TZDs to inhibit the JNK pathway is fundamental for their pharmacological action. Even though in most of the experimental models studied exacerbated activity of the JNK pathway is detrimental for metabolic homeostasis, some examples showed that inhibition of JNK pathway activity in a particular tissue has negative consequences in others. In addition, incipient studies have demonstrated compensatory as well as non-redundant functions between the different JNK isoforms. Therefore, tissue-specific and isoform-specific JNK actions on metabolic control require further investigations for the design of the most advantageous therapeutic strategy.

Acknowledgements

This work was supported by the Plan Nacional I+D grants SAF2013-40832-P and SAF2014-57856-P from the Spanish Ministry of Economy and Competitivity. JL-M and JD-D, and MIA were recipients of fellowships from FPI, and FPU programs from the Spanish Government, respectively. CB was supported by an ADR fellowship from University of Barcelona, Spain.

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