NR4A orphan nuclear receptors in glucose homeostasis: A minireview

A.F. Close a, C. Rouillard b, J. Buteau a,*

a Department of AFNS, University of Alberta and Alberta Diabetes Institute, Li Ka Shing Centre, Edmonton, AB, T6G 2E1, Canada
b Department of psychiatry and neurosciences, university Laval, centre de recherche du CHU de Québec, 2705, boulevard Laurier, Québec, QC, G1V 4G2, Canada

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Abstract

Type 2 diabetes mellitus is a disorder characterized by insulin resistance and a relative deficit in insulin secretion, both of which result in elevated blood glucose. Understanding the molecular mechanisms underlying the pathophysiology of diabetes could lead to the development of new therapeutic approaches. An ever-growing body of evidence suggests that members of the NR4A family of nuclear receptors could play a pivotal role in glucose homeostasis. This review aims to present and discuss advances so far in the evaluation of the potential role of NR4A in the regulation of glucose homeostasis and the development of type 2 diabetes.

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1. NR4A structure and function

1.1. Structure

NR4A nuclear receptors comprise a family of immediate early response genes, the expression of which is regulated by a variety of extracellular stimuli. The NR4A family includes three members present in mammalian cells: NR4A1 (also known as NGFI-B, NUR77 and TR3); NR4A2 (NURR1, NOT); and NR4A3 (NOR1, MINOR) [1]. All these three share a high degree of homology and a common structure consisting of a ligand–independent activation–function (AF)-1 transactivation domain in the N-terminal region, a DNA-binding domain composed of two zinc fingers and a ligand-dependent AF-2 transactivation domain in its C-terminal region [2].

1.2. Regulation

To date, the natural ligands of the NR4A nuclear receptors remain unidentified and, for this reason, the NR4As have been dubbed `orphan receptors’. Determination of the structure of the ligand-binding domain of NR4A2 by crystallography has revealed two distinct structural characteristics that distinguish NR4A from the rest of the nuclear receptor superfamily:

- the absence of a ligand-binding cavity;
- the absence of a classical binding site for coactivators [16].

These observations point to a ligand-independent function for NR4A nuclear receptors, suggesting that their activity might

* Corresponding author. Tel.: +780 492 8386.
E-mail address: jbuteau@ualberta.ca (J. Buteau).

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instead are regulated by expression and post-translational modifications. NR4A2 has consistently been shown to be constitutively active [3,4] and its transcriptional activity dovetails with its proteasome-dependent turnover [5]. In addition, all three NR4As can be phosphorylated by a selection of kinases, including c-Jun N-terminal kinase (JNK), ribosomal S6 kinase (RSK) and mitogen-activated protein kinase (MAPK) [6–8]. However, the consequences of these regulatory post-translational modifications have not been extensively investigated. Also, NR4As are subject to SUMOylation, which has been proposed to suppress their transcriptional activity [9,10].

1.3. DNA-binding activity

Upon activation, NR4As bind the consensus sequence AAAGGTCA, known as the NGFIB response element (NBRE), as either monomers or homodimers [11]. All three NR4As can also bind the Nur response element (NurRE), composed of an everted repeat of the octanucleotide AAAT(G/A)(C/T)CA separated by six base pairs, as homodimers [12,13]. However, NR4A3 displays much lower affinity for NurRE, which translates into weaker transcriptional activation [12,13]. In addition, both NR4A1 and NR4A2, but not NR4A3, can form heterodimers and interact with retinoid X receptors (RXRs) to target DR5 elements [19]. Fig. 1 illustrates the specific binding characteristics of the NR4A proteins. The fact that these proteins exert different binding affinities for specific response elements and cofactors suggests non-redundant biological functions for each of these three NR4A nuclear receptors [14,15].

1.4. Biological functions

NR4As have been shown to regulate numerous biological processes, including cell proliferation and differentiation, organ development, immune homeostasis and memory formation [16–23]. Thus far, the vast literature on NR4As consistently suggests that these orphan receptors might be involved in a wide array of pathologies. One of the most studied functions of the NR4As is undoubtedly their role in T-cell development. Indeed, NR4A1 has proved to be essential for activation-induced apoptosis in T-cell hybridomas and to participate in the negative selection of autoreactive thymocytes during differentiation into mature T lymphocytes [24,25]. NR4A have also been found necessary for dopamine neuron differentiation and survival [26–28], and NR4A1 was found to mediate behavioural changes in animals with dopamine denervation and L-DOPA treatment [29], thereby marking NR4As as potential targets in the treatment of Parkinson’s disease. In addition, NR4A expression in macrophages, endothelial cells and smooth muscle cells suggests a role in vascular lesion formation. However, whether they have protective or deleterious effects on the formation of atherosclerosis lesions is still a matter of debate [30–32]. Nevertheless, the fact that NR4As are implicated in a variety of malignancies and modulate the response to conventional chemotherapeutic agents highlights their pivotal role in cancer [33,34]. Moreover, NR4As are potential transcriptional mediators of inflammatory signals, and Nr4a2 mRNA levels are significantly increased in psoriasis and osteoarthritis [35–38]. Thus, as NR4As indisputably play a panoply of critical roles in various biological processes, the present review is focused on their putative role in the regulation of glucose homeostasis and the development of diabetes.

2. Potential role of NR4As in diabetes

Several pieces of evidence hint at an important role for NR4As in the control of glucose homeostasis and diabetes. These include:

- changes in NR4A expression in animal models of insulin resistance, diabetes and/or obesity compared with their respective controls;
- the fact that both NR4A expression and activity are under nutritional control;
- recent reports demonstrating important roles for NR4A members in the regulation of glucose transport, glucose utilization, insulin sensitivity, gluconeogenesis and insulin secretion [39–41].

These putative roles in metabolic tissues are summarized in Table 1.

2.1. Expression in obesity and in diabetes

Nr4a expression is known to vary in a tissue-dependent fashion in animal models of insulin resistance, diabetes and obesity compared with controls. Indeed, while all three Nr4as were increased in the liver of streptozotocin-treated and db/db diabetic mice [39,40], Nr4a1 and Nr4a3 mRNA levels were decreased in the skeletal muscle and adipose tissue of Zucker diabetic fatty rats and odbdb and db/db mice compared with controls [39,40]. Moreover, Nr4a1 expression was reduced by 50% in the skeletal muscle of rats fed a high-fat diet compared with standard chow-fed specimens [41].

In humans, Nr4a expression appears to be influenced differently by body weight and fat mass. Veum et al. [42] recorded significantly elevated Nr4a1–3 mRNA levels in the subcutaneous adipose tissue of severely obese patients compared with lean subjects, whereas this expression was normalized a year after bariatric surgery and fat loss. Also, the same team observed upregulated Nr4a expression in the omental fat of obese patients compared with subcutaneous fat. Such a change was not seen in lean subjects. An attractive possibility is that elevated circulating fatty acids, a hallmark of obesity and insulin resistance,
could be responsible for this increased expression of \textit{Nr4a}s. In fact, saturated long-chain fatty acids are able to upregulate \textit{Nr4a} expression \textit{both in vitro and in vivo} [43–45]. Another possible explanation is that the low-grade systemic inflammation observed in obesity could be involved, as both cytokines and adenosine induce \textit{Nr4a} expression [43,46].

Exercise is an important part of type 2 diabetes treatment as it helps improve insulin sensitivity. It is therefore noteworthy that \textit{Nr4a1–3} mRNA levels were rapidly increased in muscles after a bout of endurance exercise in people [47,48]. Low-intensity exercise induced increases in \textit{Nr4a1} and \textit{Nr4a3} expression in rat skeletal muscle, a result that was reproduced by muscle electrical stimulation [49]. In contrast, immobilization decreased \textit{Nr4a1} and \textit{Nr4a3} mRNA and protein levels, while high-intensity exercise selectively enhanced \textit{Nr4a3} expression in a separate study [49].

2.2. Nutritional and hormonal control of \textit{Nr4a}s

Saturated long-chain fatty acids are robustly incriminated in the development of both insulin resistance and pancreatic beta-cell deterioration, two defects leading to the onset of diabetes [50]. For this reason it is of particular interest that infusion of clonal rat beta cells and isolated rat islets with palmitate (C16:0) and oleate (C18:1, \(\omega 9\)) caused a rapid increase in \textit{Nr4a1} gene transcription in a dose-dependent manner [45], an effect that has also been implicated in the deleterious effects of free fatty acids on glucose-stimulated insulin secretion [43]. In addition, \textit{Nr4a1} subcellular localization was found to be cytoplasmic in clonal MIN6 beta cells, and stimulation with either palmitate or oleate provoked its translocation to the nucleus [43]. Whether or not long-chain fatty acids are able to increase \textit{Nr4a} mRNA levels in hepatocytes [44] and in beta cells, this action appears to be tissue-specific. Indeed, palmitate, oleate and linoleate free fatty acids all failed to alter \textit{Nr4a1} expression in L6 human myotubes [41]. Glucose acted synergistically with glucagon-like peptide-1 (GLP-1) and PACAP-38, two cAMP-raising hormones of the secretin/glucagon family, to increase \textit{Nr4a1} mRNA expression in beta-cell lines [51]. Taken altogether, these studies suggest that both \textit{Nr4a} expression and activity are under nutritional control, and raise the strong possibility that this family of nuclear receptors acts as transcriptional effectors of calorigenic nutrients.

The effect of insulin on \textit{Nr4a} expression is also tissue-dependent. Insulin as well as pioglitazone and troglitazone, two thiazolidinedione insulin-sensitizing drugs, induced a rapid increase in \textit{Nr4a1} and \textit{Nr4a3} transcripts in adipocytes in culture [40]. However, the effect of insulin on \textit{Nr4a} expression in skeletal muscle is more controversial. One study conducted in humans showed that \textit{Nr4a1} mRNA levels were upregulated in skeletal muscle after a hyperinsulinaemic–euglycaemic clamp test [52]. The authors also confirmed the effect of insulin in L6 myotubes \textit{in vitro}, which was in contradiction to a recent study where a hyperinsulinaemic–euglycaemic clamp in rats failed to modify \textit{Nr4a1} expression in muscle [41]. Further confusion of the issue was added by the fact that insulin infusion in clinical volunteers was reported to influence the expression of \textit{762} genes in skeletal muscle without affecting any \textit{Nr4a} family members [53]. In mouse liver, \textit{Nr4a1–3} mRNA levels were increased by glucagon, but were unaffected by insulin [39]. The regulation of \textit{Nr4a} expression and activity by the nutritional and hormonal status of the organism underpins their putative role in glucose homeostasis and type 2 diabetes.

2.3. Glucose transport and utilization in peripheral tissues

In rodents, \textit{Nr4a1} overexpression in skeletal muscle promotes the expression of genes implicated in glucose metabolism, including the glucose transporter \textit{Glut4} and other genes involved in glycolysis and glyogenolysis [54]. Also, silencing \textit{Nr4a1} in rodents and C2C12 in mouse myoblasts leads to a decrease in \textit{Glut4} mRNA and protein levels as well as other genes with critical roles in glucose utilization [17,54]. In addition, several glycolytic genes, such as \textit{enolase 3}, were downregulated in the skeletal muscle of \textit{Nr4a1-null} mice [55,56]. In fact, some of
these effects of NR4A1 were reiterated by NR4A3. Notably, transgenic mice with muscle-specific NR4A3 gain of function displayed increased muscle GLUT4 protein levels [57]. Collectively, these results indicate an important role for both NR4A1 and NR4A3 in enhancing glucose transport and utilization in skeletal muscle. Moreover, Nr4a1 and Nr4a2 expression in C2C12 cells have consistently resulted in an increase in glucose transport [54,58], while glycolytic activity was elevated in Nr4a1 overexpression in mouse myoblasts in vitro [55]. Skeletal muscle isolated from Nr4a1 transgenic mice showed higher mitochondrial content and function than their wild-type littermates. This was due to an increase in complexes I and IV of the electron transport chain, which translated into enhanced oxidative metabolism [56]. Transgenic mice with muscle-specific Nr4a3 overexpression also showed higher mitochondrial DNA content compared with controls, and this was associated with increased O2 consumption and energy expenditure [57].

Such beneficial effects of NR4As on glucose metabolism would be expected to result in improved glucose tolerance and insulin sensitivity in physiological as well as pathological situations. Indeed, Nr4a1-null mice developed muscle insulin resistance, lower glucose tolerance and higher fasting insulin blood levels compared with controls after being fed a high-fat diet [55]. However, transgenic mice with muscle-specific NR4A1 gain of function were not protected against diet-induced glucose intolerance [56]. Mice with muscle-specific Nr4a3 overexpression showed better glucose tolerance, but no insulin sensitivity, compared with wild-type animals [57].

In mouse adipocytes in culture, silencing Nr4a1 blunted α-melanocyte-stimulating hormone (α-MSH)-induced increases in glucose uptake [59]. In addition, transduction of mouse adipocytes with Nr4a3 potentiated insulin-stimulated glucose uptake, whereas silencing Nr4a3 had the opposite effect [40]. This "insulin-sensitizing" effect of NR4A3 was attributed to higher phosphorylation levels of insulin receptor substrate 1 (IRS-1) and Akt as well as the subsequent recruitment of GLUT4 to the cell membrane.

Whether or not NR4A1 and NR4A3 have beneficial effects on glucose metabolism and insulin sensitivity in skeletal muscle and adipose tissue, there is evidence that they both may have rather deleterious effects in the liver. Whole-body Nr4a1 knockout mice displayed greater liver insulin sensitivity with a normal chow diet, an effect that was absent in animals fed a high-fat diet [55]. In contrast to observations in skeletal muscle and adipocytes, loss of Nr4a1 in the liver led to upregulation of glycolytic genes compared with controls.

2.4. Hepatic glucose production

Elevated hepatic glucose output is an important contributor to both fasting and exaggerated postprandial hyperglycaemia in type 2 diabetes. Indeed, these patients display increased hepatic glucose production that is positively correlated with their fasting plasma glucose levels [60]. Also, raising hepatic glucose production in mice by overexpressing phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme of gluconeogenesis, results in increased fasting blood glucose levels and impaired glucose tolerance, two hallmarks of type 2 diabetes [61]. In the fasting state, circulating glucagon induces hepatic gluconeogenesis and glucose output through a cAMP-mediated pathway, an action antagonized by insulin in the postprandial state.

Pei et al. [39] showed that Nr4a1 overexpression in mouse hepatocytes in vitro and in mouse liver in vivo induced the expression of genes involved in gluconeogenesis, most notably glucose-6-phosphatase (G6pc), fructose-1,6-bisphosphatase 1 (Fbp1) and enolase 3. NR4A1 also induced the expression of Slc2a2, a glucose transporter thought to mediate hepatic glucose output. In addition, the same team also showed that transgenic mice with liver-specific NR4A1 gain of function displayed elevated hepatic glucose output and fasting glucose levels. These effects were independent of the established cAMP–CREB pathway, suggesting that NR4A1 might constitute an alternative pathway governing gluconeogenesis [62]. In contrast, silencing Nr4a1 in db/db mice reduced expression of the gluconeogenic genes Fbp1 and Slc2a2, which translate into lower blood glucose levels compared with control db/db mice. Interestingly, Nr4a1 expression was elevated in db/db mice, thereby supporting the hypothesis that the orphan nuclear receptor plays a critical role in hepatic glucose production in this animal model of diabetes.

2.5. Fatty acid metabolism

An increased intramuscular lipid content has been associated with the development of insulin resistance in human and rodent models [63,64]. In the liver, elevated triglyceride (TG) and diacylglycerol (DAG) levels are strongly associated with insulin resistance in people [65,66]. This suggests that the effect of NR4As on lipogenesis and lipid accumulation in peripheral tissue is likely to have major clinical significance.

Nr4a1 knockdown in C2C12 myoblasts induced the expression of sterol regulatory element-binding protein 1c (Srebp-1c), a major regulator of lipogenesis [17], thereby predicting a role for NR4A1 in TG accumulation. Indeed, Nr4a1-null mice fed a high-fat diet showed greater intramuscular TG and DAG content, possibly due to increased fatty acid uptake and impaired β-oxidation [55]. The rise of intracellular lipids in Nr4a1 knockout animals was concomitant with an increase in acylcarnitine and lipoprotein lipase expression [55]. In the same model, lipid and cholesterol accumulation was also observed in the liver, an effect that could be attributed, at least in part, to increased Srebp-1c mRNA levels [55,67]. These findings may have systemic significance, as female Nr4a1-deficient mice presented with higher fasting blood glucose levels and insulin resistance compared with controls [67]. However, their glucose tolerance, as assessed by intraperitoneal glucose tolerance test (ipGTT), was not impaired. Consistent with a role of NR4A1 in the regulation of fat accumulation, overexpression of Nr4a1 in mice reduced hepatic TG levels, possibly through downregulation of Srebp-1c and its canonical target genes (Ldlr, Scd1, Gpam and Fas) [68]. Thus, NR4As may have a protective effect against hepatic fat accumulation [68].

To further explore the mechanism by which NR4As might regulate lipid metabolism, their effect on carnitine...
palmitoyltransferase 1 (Cpt1) has been studied. This enzyme regulates the entry of long-chain fatty acylCoA into mitochondria and is a rate-limiting enzyme in fatty acid β-oxidation [69,70]. Muscle-specific overexpression of Cpt1 in rats improved high-fat-diet-induced insulin resistance [71]. Moreover, when fed a high-fat diet, Cpt1 knockout mice developed more severe insulin resistance and impaired glucose tolerance than their wild-type littermates [72]. Knocking down Nr4a1 in skeletal muscle cells also stunted Cpt1 expression [17]. In addition, both NR4A1 and NR4A2 were found to mediate 9-cis retinoic acid and HX600-induced expression of Cpt1-a in HEK293 kidney-derived cells [73], thereby supporting the possibility that NR4As could promote β-oxidation to limit lipid accumulation.

2.6. Insulin secretion

In the pancreas, Nr4a expression has proved to be restricted to pancreatic islet beta cells [43], and Nr4a1 overexpression in the clonal MIN6 beta-cell line impaired glucose-stimulated insulin secretion [43]. This effect was associated with a decrease in the expression of several transcription factors regulating insulin gene transcription, including MafA, Pdx-1 and NeuroD1. As a consequence, insulin expression and protein content were also reduced in MIN6 cells overexpressing Nr4a1. In the same report, both NR4A1 and NR4A2 modulated the expression of genes involved in cation homeostasis, including the zinc transporters Scl30a1 and Scl39a8. This resulted in perturbation of zinc homeostasis, thereby providing another potential mechanism by which NR4As might influence insulin secretion [74]. Interestingly, common polymorphisms located within the Nr4a3 locus were associated with significantly higher insulin secretion in a clinical trial of non-diabetic subjects, a finding confirmed in a larger study involving diabetic as well as non-diabetic men [75]. However, in that study, no association with either diabetes or glucose tolerance status could be established [75].

2.7. Central regulation of food intake and energy expenditure

Central regulation of food intake is orchestrated by hypothalamic neural circuits [76]. In the rat hypothalamus, Nr4a2 is moderately expressed in the interpeduncular nucleus, whereas Nr4a1 is strongly expressed in the supraoptic nucleus and, to a lesser extent, in the paraventricular nucleus [77]. Both NR4A orthologues have been found to play an active part in the hypothalamic–pituitary–adrenal axis [78]. Nr4a3 expression is dramatically downregulated in the hypothalamus in hyperphagic and obese db/db and β-endorphin null mice compared with controls [79]. Intracerebral silencing of Nr4a3 significantly decreases daily food intake and body weight in mice [79]. This growing body of evidence strongly suggests that NR4As could play an important role in the regulation of food intake and energy expenditure. In line with this view, silencing of Nr4a1 in C2C12 mouse myoblasts suppressed the expression of several genes involved in energy expenditure, including uncoupling protein 3 (Ucp3), AMP-activated protein kinase γ3 (Arkγ3) and adiponectin receptor 2 [17]. NR4A1-dependent regulation of Ucp3 has been confirmed in vivo following the delivery of small inhibitory RNA into mouse tibialis cranialis muscle [17]. Furthermore, Nr4a1-deficient female mice fed a high-fat diet gained significantly more weight and fat mass than their wild-type littermates, possibly as a result of decreased energy expenditure in Nr4a1-deficient mice [67].

2.8. DHR38

Similar to its mammalian counterpart, the Drosophila NR4A orthologue DHR38 [80] has been shown to be under nutritional control and to play a critical role in carbohydrate metabolism, including glycogen synthesis [81]. However, regulation of DHR38 may well differ from that of its mammalian orthologues, thereby preventing the gathering of valuable mechanistic clues from the lesser organism. Indeed, Dhr38 expression was upregulated in fed Drosophila larvae and reduced by starvation, and was significantly lower in animals maintained on a pure protein diet [81]. This contradicts the results obtained in rodents wherein Nr4a1-3 mRNA levels were increased in mouse liver after 24 h of fasting compared with fed controls [39]. Furthermore, in Drosophila, neither insulin/insulin-like growth factor (IGF) signalling nor the glucagon analogue adipokine hormone (AKH) regulated Dhr38 expression [81]. These discrepancies raise doubts as to the applicability of findings obtained in Drosophila to mammalian cells.

3. Conclusion

Compelling evidence described in this review suggests a potential role for NR4A nuclear receptors in the regulation of insulin sensitivity, glucose homeostasis and the development of type 2 diabetes. Taken altogether, these observations identify NR4A nuclear receptors as potential molecular targets for diabetes treatment. However, their precise biological roles in each metabolic tissue have yet to be fully elucidated and are deserving of scrutiny before NR4As can be considered bona fide therapeutic targets. In addition, the mechanisms responsible for their regulation remain relatively unexplored. It is our hope that, in the near future, further investigation with genetically engineered animals will provide additional information concerning the involvement of these nuclear receptors in the pathophysiology of obesity/diabetes and whether or not they truly represent meaningful therapeutic targets in the treatment of metabolic disorders.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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