Minireview: Nuclear Hormone Receptor 4A Signaling: Implications for Metabolic Disease

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Numerous members of the nuclear hormone receptor (NR) superfamily have been demonstrated to regulate metabolic function in a cell- and tissue-specific manner. This review brings together recent studies that have associated members of the NR superfamily, the orphan NR4A subgroup, with the regulation of metabolic function and disease. The orphan NR4A subgroup includes Nur77 (NR4A1), Nurr1 (NR4A2), and Nor-1 (NR4A3). Expression of these receptors is induced in multiple tissues by a diverse range of stimuli, including stimuli associated with metabolic function, such as: β-adrenoceptor agonists, cold, fatty acids, glucose, insulin, cholesterol, and thiazolidinediones. *In vitro* and *in vivo* gain- and loss-of-function studies in major metabolic tissues (including skeletal muscle, adipose, and liver cells and tissues) have associated the NR4A subgroup with specific aspects of lipid, carbohydrate, and energy homeostasis. Most excitingly, although these orphan receptors do not have known endogenous ligands, several small molecule agonists have recently been identified. The preliminary studies reviewed in this manuscript suggest that therapeutic exploitation of the NR4A subgroup may show utility against dyslipidemia, obesity, diabetes, and cardiovascular disease. **(*Molecular Endocrinology* 24: 1891–1903, 2010)**

NURSA Molecule Pages: Nuclear Receptors: Nur77 | NURR1 | NOR1.

Metabolic diseases such as obesity and type 2 diabetes are major contributors to morbidity and mortality throughout industrialized nations. At the molecular level, a number of nuclear hormone receptors (NRs), which belong to a superfamily of structurally related ligand-dependent transcription factors, have been shown to regulate metabolic function and the pathophysiology of metabolic disease in an organ-specific manner.

This review focuses on the nuclear hormone receptor 4A (NR4A) subgroup in the context of metabolism. Members of this subgroup have recently emerged as regulators of metabolic function both *in vitro* and *in vivo*. In mammals, the NR4A subgroup consists of three closely related orphan nuclear receptors: neuron-derived *clone 77* [Nur77; NR4A1; also known as NGFI-B, N10, NAK-1, TIS1, 3CH77, and TR3 (1–3)]; nuclear receptor related 1 [Nurr1; NR4A2; also known as RNR-1, NOT, TINUR, and HZF-3 (4)]; and neuron-derived orphan receptor 1 [Nor-1; NR4A3; also known as MINOR, TEC, and CHN (5–7)]. The NR4A subgroup share significant sequence homology. In humans, the three members share 97% homology in their DNA-binding domains, 20–30% homology in their N-terminal transactivation domains, and 60–65% homology in their C-terminal ligand-binding domains based on compatible amino acid residue homology (8).

Members of the NR4A subgroup were categorized as NRs on the basis of structural homology to other members of the NR superfamily without prior identification of an endogenous ligand. Currently, no endogenous/native *bona fide* ligands have been identified, and thus members of this subgroup were designated as orphan NRs. These receptors are also constitutively active and operate in a ligand-independent manner (9). Structural studies on the ligand-binding domains (LBDs) of all three NR4A members have revealed that these receptors do not possess a ligand-binding cavity of conventional size due to the presence of bulky hydrophobic amino acid residue side chains

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Abbreviations: BAT, Brown adipose tissue; LBD, ligand-binding domain; NBRE, NGFI-B response element; NR, nuclear hormone receptor; siRNA, small interfering RNA; SMC, smooth muscle cell; WAT, white adipose tissue.

(10–13). However, several agonists have been recently described that may interact with these receptors in unconventional ways. Interestingly, these compounds can work either by modulating the N-terminal AF-1 [*e.g.* 6-mercaptopurine (11)] or the C-terminal LBD [*e.g.* 1,1-di(3′-indolyl)-1-(*p*-substituted phenyl)methanes (14)].

Concordant with the original cloning of these receptors from the central nervous system (or derived cell lines) (3–5), all three members have strong expression in this tissue (3, 4, 7, 15). The expression profiles of the NR4A subgroup in energy-dependent tissues such as skeletal muscle, brain, adipose tissues, heart, and liver (5, 7, 15–18) suggest a possible functional role in energy metabolism.

Members of the NR4A subgroup are known to bind to DNA as monomers, homodimers, and heterodimers. As monomers, the NR4A subgroup binds to the NGFI-B response element (NBRE; AAAGGTCA). This sequence consists of the canonical NR-binding motif (AGGTCA) proceeded by two adenines (9, 19). As homodimers and heterodimers with other NR4A members, all three members of the NR4A subgroup bind to the Nur-responsive element, which consists of everted repeats of the sequence AAAT(G/A)(C/T)CA similar to the monomeric NBRE motif (20, 21). The protypical and best characterized endogenous Nur-responsive element is found in the promoter of the proopiomelanocortin gene (20). Nur77 and Nurr1 (but not Nor-1) can also heterodimerize with retinoid X receptor (22, 23). NR4A/ retinoid X receptor heterodimers bind to a combination of an NBRE and retinoic acid response element as direct repeats (22).

Functionally, the NR4A subgroup is involved in various hormonal, physiological, and pathophysiological processes including cardiovascular disease (24, 25), apoptosis (26, 27), neurological disease, steroidogenesis (28, 29), inflammation (30), oncogenesis (31, 32), and metabolic disease (33–35).

The Expression of the NR4A Subgroup Is Induced by Diverse Stimuli

The expression of all three members of the NR4A subgroup are known to be rapidly and transiently induced by various diverse stimuli in a wide range of tissues and cultured cells. Briefly, such stimuli includes various receptor agonists [such as G protein-coupled receptors (28, 29, 36, 37) and tyrosine kinase receptors (38, 39)], activators of cAMP and protein kinase signaling (40–43), mechanical stress (44), exercise (45), and UV light (46). A much more comprehensive list of implicated stimuli is detailed in Table 1. Due to the acute induction of expression (typically 15–90 min after the stimuli), the members of NR4A subgroup have been classified as immediate-early response genes (1, 47, 48). In many cases, stimuli that induce one member of the NR4A subgroup also induce the expression of the other two members in a similar spatiotemporal response (36, 37, 49). Because endogenous ligands have not been identified, the activity of the NR4A family may therefore be predominantly regulated by expression.

β-Adrenergic Signaling, cAMP, and Metabolism

Shortly after the discovery of Nur77, the expression of this receptor was found to be induced by β-adrenoceptor agonists (isoprenaline and norepinephrine) in cultured neural cells (50) and by conditions that cause norepinephrine release (stress and yohimbine treatment) in rat cerebral cortex (51).

β-Adrenergic signaling regulates metabolic function, particularly in metabolically active peripheral tissues such as skeletal muscle and adipose (52–54). The importance of β-adrenergic signaling to metabolic homeostasis is highlighted by the unusual susceptibility of β-adrenoceptor knockout mice to diet-induced obesity (55). In skeletal muscle, β-adrenergic stimulation transiently induces the mRNA expression of all three NR4A members both *in vivo* (37, 49, 56, 57) and *in vitro* (16, 37, 57–59). This induction, which is typically 10- to 100-fold, has been observed with pan and subtype-specific β-adrenoceptor agonists (37, 49, 56, 57) and can be blocked by β-adrenoceptor antagonists (37). This induction has also been observed in both slow-twitch oxidative and fast-twitch glycolytic skeletal muscle (56, 57).

Providing further evidence of cross talk between the NR4A subgroup and β-adrenergic signaling in skeletal muscle is the demonstration that the expression of *Nur77* is significantly decreased after skeletal muscle denervation, highlighting that sympathetic innervation is required for *Nur77* expression (57). Exercise has also been noted as inducing all three members of the NR4A subgroup at 3 h (45). Because exercise enhances sympathetic tone (60), this also indirectly supports cross talk between the NR4A subgroup and β-adrenergic signaling.

Like many other systems in which the expression of NR4A subgroup is induced by diverse stimuli (61–64), the induction of the NR4A subgroup in skeletal muscle by β-adrenergic signaling appears mediated by cAMP, protein kinase A, MAPK, and finally cAMP response element-binding protein (56).

In other metabolically active tissues, NR4A mRNA expression has been transiently induced by β-adrenoceptor agonists in brown adipose tissue (BAT)(65, 66), white adipose tissue (WAT) (49, 66), liver, cardiac muscle, ad-

TABLE 1. Stimuli known to induce the NR4A family

Type of stimuli	Comments (with implicated receptor or enzyme where applicable)	References
Agonists of G protein-coupled receptors (GPCRs)	β-Adrenoceptors	16, 37, 50, 56–58, 66
	MSH receptors	36
	ACTH receptor	28, 40
	Angiotensin receptors	29, 103, 104
	Dopamine receptors	105
	Prostaglandin E2 receptor	106
	α-Thrombin receptor	107, 108
	LH/choriogonadotropin receptor	09, 110
	FSH receptor	111
	Glucagon-like peptide 1 receptor	75
Tyrosine kinase receptors (TKRs)	Insulin receptor	39, 79–81
	Vascular endothelial growth factor (VEGF) receptor	38
	Nerve growth factor receptor	3, 41
	Fibroblast growth factor receptors	42, 47, 112
	Platelet-derived growth factor receptors	47, 107
	Epidermal growth factor receptor	107, 112
	Uncharacterized/complex mixtures of serum growth factors	1, 6, 113, 114
Agonists of cytokine receptors	Receptors activated by Interferon-γ	115
	Receptors activated by IL-1	116
	Receptors activated by TNF-α	115
Agonists of toll-like receptors (TLRs)	TLR4 ligand (lipopolysaccharide)	80, 115, 117
	TLR3 ligand (poly(I:C))	115, 118
	TLR2 ligand (lipoteichoic acid)	115, 118
Activators of cAMP and protein kinase signaling	Adenylate cyclase activator (forskolin)	40–43
	Phosphodiesterase inhibitor (isobutylmethylxanthine)	79, 80
	Protein kinase C activator [phorbol-12-myristate-13-acetate (PMA)]	40, 41, 112
Physical stress	Mechanical stress (*in vitro*)	44
	Balloon angioplasty in coronary arteries	107
Exercise stress	Aerobic and resistance exercise	45
Psychological stress	In hypothalamus and cerebral cortex	51, 119
Free fatty acids	Unknown receptor/mechanism	76
Glucose	Unknown receptor/mechanism	75
Cholesterol	Unknown receptor/mechanism	64
Low-density lipoproteins	Low-density lipoprotein receptor	34
Thiazolidinediones	Peroxisome proliferator-activated receptor γ agonist	114, 120
Membrane depolarization	In excitable tissues	40, 105
Neurotransmitters	Dopamine receptors and γ-aminobutyric acid (GABA) receptors	121
UV light	Expression in melanocytes	46
50 Hz electromagnetic radiation	Unknown receptor/mechanism	57
Bisphenol A	Endocrine-disrupting toxin	122

renal glands, kidney, stomach, and pancreas (49). These studies demonstrate that β-adrenergic signaling regulates the expression of the NR4A subgroup in multiple tissues. Given that β-adrenoceptors regulate metabolism, particularly in metabolically active peripheral tissues, the induction of NR4A expression in these tissues by β-adrenoceptors suggested an association between these receptors and metabolism.

NR4A Subgroup and Metabolism in Skeletal Muscle

Given the known regulation of lipid, glucose, and energy metabolism in skeletal muscle by β-adrenergic signaling (52, 53, 67, 68), the acute regulation of NR4A expression by β-adrenoceptor agonists in skeletal muscle implicated the NR4A subgroup as potential mediators of skeletal muscle metabolism after β-adrenergic signaling.

To examine the contribution of the NR4A subgroup to skeletal muscle metabolism *in vitro*, Maxwell *et al.* (58) used small interfering RNA (siRNA) to attenuate *Nur77* expression in C2C12 skeletal muscle cells. Consequently, this repression reduced lipolysis and altered the expression of genes (and proteins) associated with the regulation of lipid, carbohydrate, and energy metabolism (58). Of most interest, the expression of AMP-activated protein kinase subunit γ 3 (*Ampkγ3*), fatty acid translocase (*Fat/Cd36*), and glucose transporter 4 (*Glut4* mRNA and pro-

tein) were repressed after the expression of Nur77 (but not negative control) siRNA, thus directly implicating *Nur77* expression in the regulation of key metabolic genes *in vitro* (see Table 2 for full list of regulated genes). This *in vitro* work was further validated by Chao *et al.* (57), who overexpressed *Nur77* within C2C12 cells via an adenoviral expression system. This resulted in the predominant induction of the expression of key genes associated with glucose transport, insulin signaling (such as *Glut4*), glycolysis, and glycogenolysis (see Table 2 for full list of regulated genes). Consistent with these gene expression changes, glucose transport was also enhanced by adenoviral *Nur77* expression, and Nur77 was shown to interact with the promoters of several identified genes *in vitro* (57). It should be noted that for most expression changes listed in Table 2, it has not been determined whether these are primary or secondary targets of NR4A manipulation.

Using a similar siRNA approach *in vitro*, *Nor-1* expression was repressed in C2C12 skeletal muscle cells (37, 56). Excitingly, biochemical examination of the Nor-1 siRNA cells revealed significantly lower levels of palmitate (fatty acid) oxidation, and significantly greater lactate production, which is consistent with a shift from oxidative to anaerobic (glycolytic) energy production (56). Supporting dependence on anaerobic energy production, ATP levels in the Nor-1 (but not negative control) siRNA-expressing cells were resistant to (azide-mediated) inhibition of oxidative metabolism (56). Also consistent with the shift to anaerobic metabolism was the induction of hypoxia-inducible factor-1α (*Hif1*α) mRNA expression, a key transcriptional regulator of anaerobic gene expression, and this is consistent with the shift to anaerobic metabolism. In terms of gene expression, *Nor-1* attenuation *in vitro* also altered the expression of genes associated with the regulation of lipid, carbohydrate, and energy metabolism (see Table 2 for full list of regulated genes). For example, this included mRNAs that encoded critical regulators of metabolism including *Lipin1, Foxo1,* and *Pgc1*α (56). The mRNA expression of both subunits of pyruvate dehydrogenase phosphatase 1 (*Pdp1c* and *Pdp1r*) was also repressed, which is consistent with the shift to anaerobic metabolism via regulation of pyruvate dehydrogenase (56). Furthermore, attenuation of *Nor-1* expression *in vitro* dramatically induced the expression of myostatin (*Mstn*; >65-fold), a key negative regulator of skeletal muscle hypertrophy (37). Several of the genes identified by Nor-1 knockdown *in vitro* were also up-regulated by β-adrenoceptor agonists *in vivo* in skeletal muscle (*e.g. Ucp2, Ucp3, Lipin1,* and *Pgc1*α) (37, 56), therefore suggesting that Nor-1 regulates acute gene expression associated with β-adrenergic signaling. Furthermore, *in vitro*, Nor-1 was recruited to the promoters of Lipin1 and Pdk4, consistent with the direct transactivation of these genes by this receptor (56).

Interestingly, despite the conserved structural and sequence homology between Nur77 and Nor-1, and common hormone response elements, the expression of only one gene, caveolin 3 (*Cav3*), was repressed by suppression of both *Nur77* and *Nor-1* expression *in vitro* (37, 58). However, complementary genes and pathways were implicated in both the *Nor-1* and *Nur77* siRNA expression studies. For example, *Ucp2* expression was suppressed by *Nor-1* knockdown, whereas *Ucp3* expression was suppressed by *Nur77* attenuation (37, 58).

To date, only Nur77 has been manipulated *in vivo* in the context of skeletal muscle metabolism. *In vivo*, skeletal muscle *Nur77* expression has been manipulated by: 1) electroporation of *Nur77* expression vector in rats (57); 2) electroporation of *Nur77* small hairpin RNA expression vector in rats (57); 3) electroporation of *Nur77* siRNA expression vector in mice (58); 4) the utilization of the previously described (69) Nur77 knockout mouse line (33, 57); and 5) finally the utilization of Nur77 agonists (70). Consistent with the *in vitro* work on Nur77 in skeletal muscle (33, 57), many identical gene expression changes were identified *in vivo* (see Table 2 for full list of regulated genes). *In vivo*, Nur77 appears to regulate the expression of metabolic genes specifically involved in glucose transport, insulin signaling (such as Glut4), glycolysis, and glycogenolysis. Furthermore, Ucp3 also appears regulated in *vivo* (58) (see Table 2 for full list of regulated genes).

Phenotypically, Nur77 null mice exhibited limited metabolic changes on a normal diet; however, major changes are observed after high-fat feeding (33). The following changes related to skeletal muscle have been observed in Nur77 null mice after high-fat feeding compared with wild-type animals: increased weight gain, decreased energy usage (by indirect calorimetry of oxygen), insulin resistance in skeletal muscle, and slower blood glucose clearance. In terms of glucose uptake and insulin signaling, the skeletal muscle of these mice exhibit decreased expression of *Glut4* and other genes related to glycolytic flux in skeletal muscle, impaired insulin receptor substrate 1 phosphorylation, increased im lipid content (triacylglycerols and diacylglycerols), and lower glycolysis byproducts. All these molecular and cellular events are consistent with impaired insulin signaling after loss of Nur77 and with the gene expression changes observed both *in vivo* and *in vitro*. A note of caution with the Nur77 null mice is the dramatic compensatory increase in *Nor-1* expression that has been observed in these animals

TABLE 2. Metabolically associated gene and/or protein expression altered by manipulation of NR4A members (direct or indirect targets)

Gene	Full name	Site of observation and reference(s)
Regulated by alterations to *Nur77* (NR4A1) expression		
AbcG5	ATP-binding cassette subfamily G member 5	Liver (74)
AbcG8	ATP-binding cassette subfamily G member 8	Liver (74)
AdipoR2	Adiponectin receptor 2	C2C12 myotubes (33, 57)
Aldo1	Aldolase 1	Skeletal muscle (57)
Aldo2	Aldolase 2	Skeletal muscle (58)
Ampkγ3	AMP-activated protein kinase subunit γ 3	C2C12 myotubes (57)
ApoB	Apolipoprotein B	Liver (74)
ApoE	Apolipoprotein E	Liver (74)
Atf3	Activating transcription factor 3	Skeletal muscle and C2C12 myotubes (58)
Bpgm	2,3-Bisphosphoglycerate mutase	Skeletal muscle and C2C12 myotubes (58)
Cav3	Caveolin 3	C2C12 myotubes (33)
Ehhadh	enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase	Skeletal muscle (33, 57)
Eno3	Enolase 3	Skeletal muscle, C2C12 myotubes (58) and liver (73, 74)
Fat	Fatty acid translocase	C2C12 myotubes (33)
Fas	Fatty acid synthase	Liver (57, 74)
Fbp1	Fructose-1,6-bisphosphatase 1	Liver (73)
Fbp2	Fructose-1,6-bisphosphatase 2	Skeletal muscle and C2C12 myotubes (33, 57, 58) and liver (73, 74)
Fxr	Farnesoid X receptor	Liver (74)
G6pc	Glucose-6-phosphatase	Liver (73)
Glut4	Glucose transporter 4	Skeletal muscle and C2C12 myotubes (57)
Gpd1	Glycerol-3-phosphate dehydrogenase	Skeletal muscle and C2C12 myotubes (57)
Gys3	Glycogen synthase 3	C2C12 myotubes (33)
Ldlr	LDL receptor	Liver (74)
Lipc	Hepatic lipase	Liver (74)
Lpn1	Lipin1	Skeletal muscle (33, 57)
Lpl	Lipoprotein lipase	Skeletal muscle (33)
Pdk4	Pyruvate dehydrogenase kinase, isozyme 4	Skeletal muscle (57)
Pfkm	Phosphofructokinase, muscle	Skeletal muscle and C2C12 myotubes (57)
Pgam2	Phosphoglycerate mutase 2 (muscle)	Skeletal muscle and C2C12 myotubes (33, 57)
Pgk1	Phosphoglycerate kinase 1	Skeletal muscle and C2C12 myotubes (57)
Phka1	Phosphorylase kinase, α 1	Skeletal muscle and C2C12 myotubes (57)
Phkg1	Phosphorylase kinase, γ 1	Skeletal muscle and C2C12 myotubes (57)
Ppp1r3c	Protein phosphatase 1 regulatory subunit 3C	Skeletal muscle and C2C12 myotubes (57)
Pygm	Phosphorylase, glycogen, muscle	Skeletal muscle and C2C12 myotubes (33)
Rxrα	Retinoid X receptor α	Liver (74)
Scd1	Stearoyl-coenzyme A desaturase 1	Liver (57, 74)
Slc37a2	Solute carrier family 37, member 2 (glycerol-3-phosphate transporter)	C2C12 myotubes (33)
Srebp1c	Sterol regulatory-element-binding protein-1c	C2C12 myotubes (57, 58) and liver (74)
Ucp2	Uncoupling protein 2	C2C12 myotubes (57, 58)
Ucp3	Uncoupling protein 3	Skeletal muscle and C2C12 myotubes (37)
Regulated by alterations to *Nor-1* (NR4A3) expression		
Cav3	Caveolin 3	C2C12 myotubes (37)
Eno3	Enolase 3	Liver (73)
Fabp4	Fatty acid binding protein 4	C2C12 myotubes (56)
Fbp1	Fructose-1,6-bisphosphatase 1	Liver (73)
Fbp2	Fructose-1,6-bisphosphatase 2	Liver (73)
Hif1α	Hypoxia inducible factor-1α	C2C12 myotubes (56)
G6pc	Glucose-6-phosphatase	Liver (73)
Mstn	Myostatin	C2C12 myotubes (56)
Pdp1c	Pyruvate dehydrogenase phosphatase isoform 1 catalytic subunit	C2C12 myotubes (56)
Pdp1r	Pyruvate dehydrogenase phosphatase isoform 1 regulatory subunit	C2C12 myotubes (56)
Pgc1α	Peroxisome proliferator-activated receptor-γ coactivator 1α	C2C12 myotubes (56)

(*Continued*)

TABLE 2. Continued

Gene	Full name	Site of observation and reference(s)
Pgc1β	Peroxisome proliferator-activated receptor-γ coactivator 1β	C2C12 myotubes (66)
Ucp1	Uncoupling protein 1	3T3-L1 cells (37)
Ucp2	Uncoupling protein 2	C2C12 myotubes (37)
Ucp3	Uncoupling protein 3	C2C12 myotubes (10, 11, 13, 91)

(29, 65), potentially masking aspects of the phenotype that could be rescued by enhanced *Nor-1* expression.

Indirect evidence that NR4A expression is associated with skeletal muscle metabolism has also been published. In a recent study of rats selectively bred for low or high endurance capacity, comparisons revealed significantly lower mRNA/protein expression of *Nur77*- and *Nur77*-regulated genes (*e.g. Cd36*, *Ucp3*, and *Ampkγ3*) within the low endurance capacity cohort (71). This is in concordance with the study from Maxwell *et al.* (58). Furthermore, β-adrenoceptor agonist-induced lipolysis and hormone sensitive lipase phosphorylation were both attenuated in the low-endurance capacity cohort (71). In other studies, skeletal muscle *Nur77* expression was found to be repressed in rats after a high-fat diet, and all three NR4A members were repressed in rodent models of obesity and diabetes (34, 59). Conversely, Nur77 was induced by exercise (59), and all three NR4A members are induced by dietary restriction (72). These studies indicate that *Nur77* expression is strongly associated with metabolic activity.

NR4A Subgroup and Metabolism in the Liver and Pancreas

Similar to the emerging role of the NR4A subgroup in skeletal muscle metabolism, the NR4A subgroup has been associated with glucose homeostasis in the liver. Pei *et al.* (73) demonstrated *in vivo* that the NR4A subgroup is induced by glucagon and fasting, and that all three members of the NR4A subgroup induce hepatocyte glucose production *in vitro*. The ectopic adenoviral expression of *Nur77* stimulated glucose production both *in vitro* and *in vivo*, raised blood glucose levels *in vivo*, and induced genes involved in gluconeogenesis (see Table 2 for full list of regulated genes) (73). Conversely, expression of an inhibitory mutant Nur77 receptor reduced hepatic glucose production and lowered blood glucose levels *in vivo* (73). Consistent with these studies, Nur77 null mice on a high-fat diet also exhibit decreased hepatic glucose production and liver insulin resistance (33), whereas treatment with the Nur77 agonist, cytosporone B, was observed to increase blood glucose levels in fasting mice (70). Interestingly, Pei *et al.* (73) also demonstrated that hepatic expression of *Nur77*, *Nurr1*, and *Nor-1* is induced by the cAMP axis in response to glucagon in a very similar fashion to β-adrenoceptor signaling in skeletal muscle.

In terms of liver lipid metabolism, hepatic expression of *Nur77* via adenoviral overexpression vectors in mice modulated the plasma lipid profile, reduced hepatic triglyceride content, and reduced expression of *Srebp1c*, a master regulator of lipid and cholesterol metabolism (74). Consequently, downstream lipogenic target genes of *Srebp1c* were also reduced (see Table 2 for full list of regulated genes) (74). Conversely, Nur77 null mice on a high-fat diet exhibit increased hepatic steatosis and enhanced expression of lipogenic genes in liver (33).

Further evidence for the role of the NR4A subgroup in liver, is that ip injection of β-adrenoceptor agonists significantly induces the NR4A subgroup in liver (49). Moreover, various metabolic genes were regulated by β-adrenoceptor signaling in liver, including genes previously associated with the NR4A receptors (*Fabp4* and *Lipin1*) in skeletal muscle (49). All three NR4A members are also induced in liver after dietary restriction, highlighting the association of the NR4A subgroup with dietary input in this key metabolic tissue (72).

The role of the NR4A subgroup in the context of pancreatic insulin function has not yet been examined; however, *Nur77* expression is induced by glucose and palmitate in a pancreatic β-cell line *in vitro* (75, 76), and polymorphisms at the Nor-1 locus have been associated with pancreatic insulin function (35).

NR4A Subgroup and Metabolism in Adipose Tissue

The NR4A subgroup has been associated with metabolism in both WAT and BAT. BAT is a well-characterized thermogenic tissue in mammals. Functionally, in BAT thermogenesis is mediated by β-adrenoceptor signaling induced by cold exposure, which in turn activates hormone sensitive lipase activity and uncoupling protein 1 (*Ucp1*) activity and expression (77). In BAT and cultured brown adipocytes (human and mouse), members of the

NR4A subgroup (mRNA and protein) were found to be induced by β-adrenoceptor agonists and cold exposure (65, 66), therefore implicating the NR4A subgroup in nonshivering thermogenesis. Nur77-deficient mice were examined in the context of nonshivering thermogenesis and shown to exhibit normal regulation of this process; however, they did exhibit a superinduction of *Nor-1* expression in BAT, possibly compensating for the loss of Nur77 (65). Supporting the thermogenesis hypothesis, a dominant-negative mutant Nur77 receptor that prevents the transcriptional activity of all NR4A receptors blocked β-adrenoceptor receptor-stimulated *Ucp1* gene transcription *in vitro* (66). Using gel shift, chromatin immunoprecipitation, and lucifease-reporter assay promoter analysis, *Ucp1* appears to be a direct target of Nor-1 in BAT (66).

In WAT, the NR4A subgroup has been associated with glucose metabolism and adipogenesis *in vitro*. First, in terms of insulin and glucose homeostasis in WAT, insulin and the insulin sensitizers thiazolidinediones were shown to induce both *Nur77* and *Nor-1* in 3T3-L1 adipocytes (34). Viral overexpression of *Nor-1* in 3T3-L1 cells increased insulin-stimulated glucose uptake, enhanced protein phosphorylation associated with insulin-stimulated glucose uptake (IRS, AKT), and translocated Glut4 protein to the plasma membrane, whereas conversely, viral Nor-1 siRNA expression did the opposite (34). Supporting this association, WAT expression of *Nur77* and *Nor-1* was also down-regulated in mouse models of obesity and diabetes (34).

Second, the NR4A subgroup is induced during adipogenesis (78). Two studies have implicated the NR4A subgroup as regulators of adipogenesis (79, 80), whereas conversely, one study suggested the NR4A subgroup had no involvement (81). Using *in vitro* models of adipogenesis, it has been demonstrated that mRNA and protein expression for all three members of the NR4A subgroup are highly induced during adipogenesis (79–81). This suggested that the NR4A subgroup could be in involved adipogenesis; however, the NR4A subgroup could also be induced during differentiation for reasons independent of adipogenesis.

To determine whether the induction of the NR4A subgroup is required for adipogenesis, Fumoto *et al.* (79) overexpressed and repressed *Nur77* expression in 3T3-L1 fibroblasts. Both chronic overexpression and chronic repression of *Nur77* inhibited adipogenesis (79). Interestingly, whereas chronic overexpression of Nur77 inhibited adipogenesis, transient overexpression promoted adipogenesis, thus suggesting that only transient induction of *Nur77* may be required for adipogenesis (79). Supporting this study, Chao *et al.* (80) demonstrated that viral transduction of NR4A receptors into preadipocytes inhibits adipogenesis in both 3T3-L1 or 3T3-F442A preadipocytes. NR4A-mediated inhibition of adipogenesis was also not able to be rescued by Pparγ activation (80), a key activator of adipogenesis (82). Opposing these two adipogenesis studies, siRNA suppression of *Nur77* expression during adipogenesis in 3T3-L1 fibroblasts appeared to have no effect on adipogenesis, the induction of adipogenic genes, or lipid accumulation (81). Furthermore, use of a retroviral dominant-negative Nur77 (known to also block the function of Nurr1 and Nor-1) prevented the increased NR4A expression observed during adipogenesis in 3T3-L1 fibroblasts (81). However, this did not affect the expression of genes required for adipocyte differentiation or adipogenesis (81).

Like skeletal muscle, liver, and BAT, WAT is also a target of β-adrenoceptor signaling, with β-adrenoceptor signaling resulting in lipolysis and fatty acid mobilization from this tissue [reviewed in Lafontan and Berlan (83)]. All three NR4A receptors appear transiently induced by the β-adrenoceptor agonist, isoprenaline, in epididymal WAT *in vivo*; however, interestingly, no NR4A induction was observed in inguinal, visceral, or sc fat depots (49). The reason for this differential NR4A induction between fat depots is unclear at this point. Furthermore, acute systemic administration of the β-adrenoceptor agonist initiated widespread changes to metabolic gene expression within epididymal WAT (49). It is likely that the NR4A subgroup may mediate some of these expression changes.

NR4A Subgroup and Satiety

In two mouse models of obesity, hypothalamic *Nor-1* expression was significantly decreased compared with wild-type mice (84). Because the hypothalamus controls appetite, *Nor-1* expression was attenuated in the hypothalamus via local injection of Nor-1 siRNA oligonucleotide in mice (84). Compared with the control siRNA injection, Nor-1 siRNA suppressed food intake and reduced body weight in mice, suggesting that hypothalamic *Nor-1* expression induces feeding behavior and energy accumulation (84). However, because this study used siRNA oligonucleotides, the possibility of nonspecific gene suppression exists. Furthermore, because the expression of the NR4A subgroup is associated with energy usage outside of the hypothalamus (33, 66), it seems paradoxical that the opposing effect of energy accumulation would specifically occur due to hypothalamic expression.

Interestingly, a recent study has demonstrated that α-MSH induces the NR4A subgroup in melanocytes in a melanocortin 1 receptor-dependent manner (36). Given

the role of α-MSH in the regulation of satiety at the hypothalamus (reviewed in Ref. 85) and the apparent peripheral metabolic actions of α-MSH (86), the unexplored possibility exists that regulation of satiety by α-MSH in the hypothalamus and metabolic function in peripheral tissues involve NR4A signaling.

NR4As and Human Disease

In the context of human disease, the NR4A subgroup is induced by: 1) atherogenic stimuli; 2) expressed in atherosclerotic plaques, and lesions; and 3) activated in smooth muscle cells (SMCs) by vascular disease (reviewed in Refs. 87 and 88). Exogenous NR4A expression reduces inflammatory cytokine and scavenger receptor expression (that diminishes low-density lipoprotein accumulation) in macrophages (89). In concordance, siRNA-mediated NR4A loss of function raises low-density lipoprotein absorption and inflammatory cytokine expression (89). Moreover, overexpression of the NR4A subgroup in SMCs attenuates proliferation (and mediates differentiation); concordantly, dominant negative expression of Nur77 augments proliferation (90). Nur77 gain- and loss-of-function mice display reduced arterial lesions and larger lesions, respectively (90). Furthermore, the agonist 6-mercaptopurine attenuated SMC proliferation and lesion formation in mice (25), underscoring the pathophysiological role of these NRs in atherogenic responses.

Recently, population screening was used to examine single-nucleotide polymorphisms within the human Nor-1 locus in the context of insulin function (35). Within the Nor-1 locus, five common single-nucleotide polymorphisms were associated with the control of pancreatic insulin secretion in a population study group of 1495 patients. However, as this population study only examined known common single-nucleotide polymorphisms, less common mutations (which could potentially be more detrimental) may have been missed.

NR4A Agonists

As discussed earlier, currently, no endogenous/native *bona fide* ligands have been identified for the members of the NR4A subgroup (*i.e.* compounds that have specific interactions with the NR ligand-binding cavity and bind the receptors under physiological conditions and concentrations). The NR4A subgroup lacks traditional NR ligand-binding cavities (11–13, 91); however, several synthetic and natural compounds have been identified as agonists (the chemical structures are shown in Fig. 1). It is worth discussing the natural, pharmaceutical, and synthetic compounds that have been reported to modulate the activity of the NR4A subgroup. These compounds serve as pharmacological probes of NR function, allow the identification of new signaling pathways, and complement the genetic gain- and loss-of-function studies. In addition, they provide insights into the potential therapeutic utility of targeting these NRs in human disease.

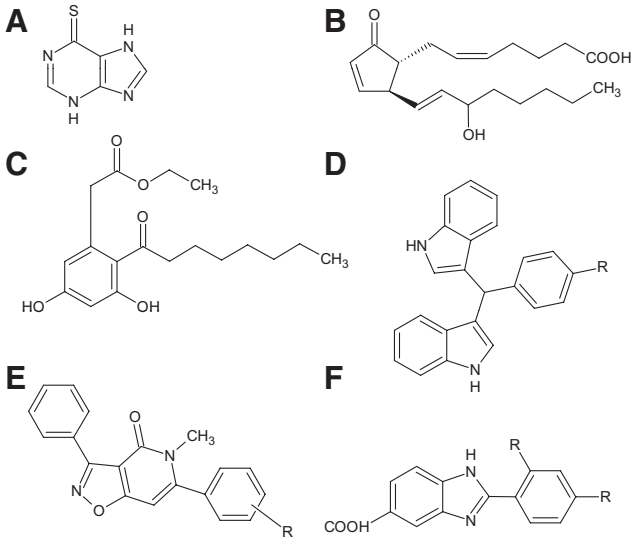

FIG. 1. Skeletal formula of the NR4A agonists. The published compounds that modulate the activity of the NR4 subgroup are: (A) 6-mercaptopurine, (B) prostaglandin A2, (C) cytosporone B, (D) 1,1-di(3′-indolyl)-1-(*p*-substituted phenyl)methane derivatives, (E) isoxazolopyridinone derivatives, and (F) benzimidazole derivatives.

The first NR4A agonist identified was the thiopurine antimetabolite, 6-mercaptopurine, which was identified from high-throughput screening as an agonist of all three NR4A members (11, 92, 93). Furthermore, several related thiopurine compounds were also demonstrated to activate Nor-1 (11). Unlike known ligands for classical NRs that bind and mediate activity via the receptor LBD, deletion studies have shown that activation of Nurr1 and Nor-1 by 6-mercaptopurine involves the modulation of activation function-1 (AF-1) located within the amino-terminal-regulatory (A/B) domain and that this activation is independent of the LBD (11, 92). Furthermore, 6-mercaptopurine was also shown to modulate the activity of the NR4A coactivator TRAP220 (93). Consistent with the role of Nur77 in vascular maintenance (24, 90), local administration of 6-mercaptopurine to arteries protects against excessive vascular SMC proliferation and vascular lesion formation (25).

A number of compounds based on 1,1-di(3′-indolyl)-1-(*p*-substituted phenyl)methanes have also been discovered to be agonists of Nur77 (14, 94). These compounds are derivatives of diindolylmethane, an investigative anticarcinogen compound found in *Brassica* vegetables such as broccoli and cauliflower (14, 94). Through deletion

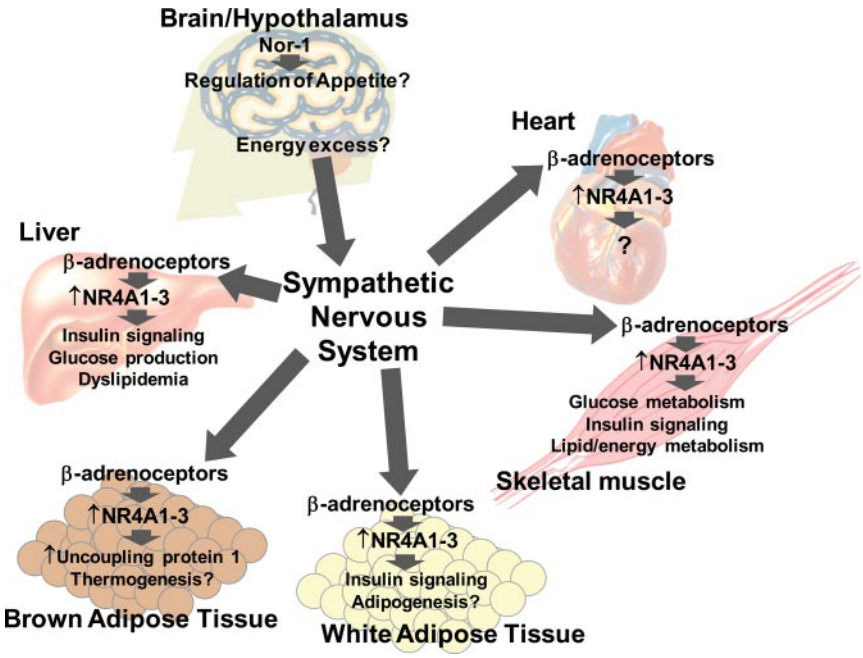

FIG. 2. Summary of the studies describing tissue specific NR4A activity in the context of metabolism.

studies, 1,1-di(3′-indolyl)-1-(*p*-substituted phenyl)methanes were shown to be dependent on the Nur77 LBD for activity, suggesting that these compounds interact with the LBD or interact with a secondary molecule that binds the LBD (14, 94). However, it is not clear whether they bind the ligand-binding cavity directly. To date 1,1-di(3′-indolyl)-1-(*p*-substituted phenyl)methanes have been shown to induce proapoptoic pathways in cancer cells both *in vitro* and *in vivo*, and this appears to be mediated via Nur77 (94). This is consistent with the known proapoptotic effects of Nur77 (26, 95). These compounds also appear to be well tolerated in mice without apparent side effects (94). One drawback of these compounds is that the 1,1-di(3′-indolyl)-1-(*p*-substituted phenyl)methanes display poor selectivity. For example, these compounds also activate the NR Pparγ (96).

Prostaglandin A2 was identified from libraries of bioactive lipid-soluble compounds as a transactivator of Nor-1 (97). Deletion studies revealed that transactivation by prostaglandin A2 was dependent on the Nor-1 LBD for activity. Furthermore, Kagaya *et al.* (97) demonstrated dose-dependent direct binding of prostaglandin A2 to the Nor-1-LBD contained within a glutathione-*S*-transferase-Nor-1-LBD fusion protein using a Biacore system. However, this study did not indicate a specific interaction of prostaglandin A2 to the Nor-1 ligand-binding cavity. Although prostaglandin A2 is an endogenous compound, Nor-1 is not presumed to be the endogenous receptor for this compound because interactions between Nor-1 and prostaglandin A2 *in vitro* appear to be at supraphysiological concentrations, display poor potency, and poorly activate *Nor-1* (97).

Two publications have described synthetic compounds that bind and activate Nurr1. The first paper by Dubois *et al.* (98) describes a series of compounds based on a benzimidazole scaffold that were discovered by virtual screening methods *in silico*. These compounds were subsequently tested *in vitro* and were shown to be very potent agonists of Nurr1 with binding curve EC_{50} values of up to 8 nM. The second paper, by Hintermann *et al.* (99), described a series of isoxazolopyridinone compounds that are even more potent, with EC_{50} values of up to 0.8 nM for Nurr1 *in vitro*. Of interest, these isoxazolopyridinone compounds have high oral bioavailability and can penetrate the blood-brain barrier. Consequently, given the role of Nurr1 in dopamine homeostasis (100–102), these compounds may have therapeutic utility against Parkinson's disease (99). However, interestingly, no *in vivo* experiments have been published with these compounds.

Recently, the octaketide cytosporone B from *Dothiorella* sp. HTF3, a mangrove endophytic fungus, has been identified as an agonist of Nur77 (70). Through the use of fluorescence quenching on Nur77 truncation mutants, cytosporone B was shown to directly bind to the LBD (70). Like the other known NR4A agonists, this study did not indicate a specific interaction to the Nur77 ligand-binding cavity. Cytosporone B was well tolerated in mice and was noted to increase blood glucose levels in fasting mice (70). Furthermore, cytosporone B was also shown to induce apoptosis in cancer cells *in vitro* and inhibit the growth of xenograph tumors *in vivo*.

In summary, *bona fide* endogenous ligands for the NR4A subgroup have not been identified to date; however, several small molecule agonists have been identified. The preliminary studies reviewed suggest that pharmacological manipulation and exploitation of NR4A activity may have therapeutic utility against dyslipidemia, obesity, diabetes, and cardiovascular disease.

Conclusions

Over the past 5 yr, the NR4A subgroup has been implicated in the regulation of lipid/carbohydrate/energy homeostasis in the key metabolic tissues of skeletal muscle,

liver, WAT, and BAT. Because the NR4A subgroup is induced by β-adrenergic signaling in these key tissues, the possibility exists that the NR4A subgroup transcriptionally regulates metabolic function and gene expression in response to β-adrenergic signaling. The importance of β-adrenergic signaling to metabolic homeostasis is highlighted by the increased susceptibility of β-adrenoceptor knockout mice to diet-induced obesity (55). Figure 2 provides a current general summary of tissue-specific involvement of the NR4A subgroup in the metabolic function of various tissues in relation to β-adrenergic signaling.

Whereas future studies should clarify the exact role these receptors play in metabolism, the current data are suggestive that modulation of these receptors could produce useful therapeutic outcomes in the context of metabolic disease, obesity, dyslipidemia, and cardiovascular disease.

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