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NR4A nuclear receptors mediate carnitine palmitoyltransferase 1A gene expression by the rexinoid HX600

Michiyasu Ishizawa [a], Hiroyuki Kagechika [b], Makoto Makishima [a,*]

[a] Division of Biochemistry, Department of Biomedical Sciences, Nihon University School of Medicine, 30-1 Oyaguchi-kamicho, Itabashi-ku, Tokyo 173-8610, Japan
[b] Graduate School of Biomedical Science, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan

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ABSTRACT

Retinoid X receptors (RXRs) are members of the nuclear receptor superfamily and can be activated by 9-cis retinoic acid (9CRA). RXRs form homodimers and heterodimers with other nuclear receptors such as the retinoic acid receptor and NR4 subfamily nuclear receptors, Nur77 and NURR1. Potential physiological roles of the Nur77-RXR and NURR1-RXR heterodimers have not been elucidated. In this study, we identified a gene regulated by these heterodimers utilizing HX600, a selective RXR agonist for Nur77-RXR and NURR1-RXR. While 9CRA induced many genes, including RAR-target genes, HX600 effectively induced only carnitine palmitoyltransferase 1A (CPT1A) in human teratocarcinoma NT2/D1 cells, which express RXRα, Nur77 and NURR1. HX600 also increased CPT1A expression in human embryonic kidney (HEK) 293 cells and hepatocyte-derived HepG2 cells. Although HX600 induced CPT1A less effectively than 9CRA, overexpression of Nur77 or NURR1 increased the HX600 response to levels similar to 9CRA in NT2/D1 and HEK293 cells. A dominant-negative form of Nur77 or NURR1 repressed the induction of CPT1A by HX600. A protein synthesis inhibitor did not alter HX600-dependent CPT1A induction. Thus, the rexinoid HX600 directly induces expression of CPT1A through a Nur77 or NURR1-mediated mechanism. CPT1A, a gene involved in fatty acid β-oxidation, could be a target of RXR-NR4 receptor heterodimers.

1. Introduction

Retinoid X receptor α (RXRα; NR2B1), RXRβ (NR2B2), and RXRγ (NR2B3) are receptors for 9-cis retinoic acid (9CRA) and regulate many biological processes by forming heterodimers with other members of the nuclear receptor superfamily, including retinoic acid receptor (RAR; NR1B), vitamin D receptor (NR1I1), thyroid hormone receptor (NR1A), peroxisome proliferator-activated receptor (PPAR; NR1C), liver X receptor (LXR; NR1H2/3), and farnesoid X receptor (NR1H4) [1,2]. Heterodimers composed of RXR and permissive partners, such as PPAR, LXR and farnesoid X receptor, can be activated by agonists for both RXR and the partner receptor [2,3]. In contrast, RXR heterodimers with nonpermissive partners, such as vitamin D receptor and thyroid hormone receptor, can be activated only by the partner receptor's agonist but not by an RXR agonist. Although several lines of evidence indicate that RXR plays an important role in multiple nuclear receptor functions, an understanding of RXR ligand signaling in RXR heterodimers is only recently emerging [2,3].

The orphan nuclear receptors growth factor-inducible immediate early gene nur/77-like receptor (Nur77; NR4A1, also known as NGFI-B and TR3) and Nur-related protein 1 (NURR1; NR4A2) belong to the NR4A nuclear receptor subfamily, which also includes neuron-derived orphan receptor 1 (NOR1; NR4A3) and the Drosophila ortholog hormone receptor-like 38 (NR4A4) [4,5]. Structural studies reveal that the NR4A subfamily receptors lack a cavity for ligand binding due to the presence of bulky hydrophobic amino acid residue side chains [6–8]. NR4A receptors bind to a specific sequence as monomers or homodimers and promote constitutive activation of transactivation [4]. The activity of NR4A receptors is determined by their expression level, subcellular localization, and posttranslational modifications [4,9]. Nur77 and NURR1, but not NOR1, can heterodimerize with RXR. RXR ligands can induce transactivation of Nur77-RXR and NURR1-RXR heterodimers [3,10], indicating that Nur77 and NURR1 are permissive heterodimerization partners for

Abbreviations: ABCA1, ATP-binding cassette, sub-family A (ABC1), member 1; AF2, activation function 2; ATRA, all-trans retinoic acid; CPT1A, carnitine palmitoyltransferase 1A; 9CRA, 9-cis retinoic acid; CRABP2, cellular retinoic acid binding protein 2; DHRS3, dehydrogenase/reductase (SDR family) member 3; HEK, human embryonic kidney; LEFTY, left-right determination factor; LXR, liver X receptor; NOR1, neuron-derived orphan receptor 1; Nur77, growth factor-inducible immediate early gene nur/77-like receptor; NURR1, Nur-related protein 1; PCR, polymerase chain reaction; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; RT-qPCR, real-time quantitative PCR; RXR, retinoid X receptor.

* Corresponding author. Fax: +81 3 3972 8199.
E-mail address: makishima.makoto@nihon-u.ac.jp (M. Makishima).

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RXR signaling. In addition to transcriptional regulation, Nur77 plays a role in apoptosis by interacting with Bcl-2 in mitochondria [11]. While unliganded RXR is required for export of Nur77 from nuclei to mitochondria, RXR ligand suppresses nuclear export of the Nur77-RXR heterodimer and inhibits apoptosis [12]. These findings suggest that RXR ligand regulates the transactivation of Nur77- and NURR1-containing RXR heterodimers on specific target genes. Although NR4A response elements have been identified in several genes, including proopiomelanocortin and tyrosine hydroxylase, which are targets for monomers, homodimers, or heterodimers of NR4A receptors [13], endogenous target genes of the Nur77-RXR and NURR1-RXR heterodimers have not been established.

We previously reported that the RXR heterodimers of Nur77 and NURR1 are selectively activated by the dibenzodiazepine RXR ligand HX600 [14]. In contrast to 9CRA, which can activate permissive RXR heterodimers, HX600 induces activation of Nur77-RXR and NURR1-RXR as well as RXR homodimers, but not RXR heterodimers with LXR, farnesoid X receptor or PPARγ [14]. In this study, we identified carnitine palmitoyltransferase 1A (CPT1A) as an HX600-inducible gene. Induction of CPT1A by HX600 was enhanced by overexpression of Nur77 or NURR1 and suppressed by cotransfection of a dominant-negative form of Nur77 or NURR1. Thus, CPT1A could be a target of RXR-NR4A receptor heterodimers.

2. Materials and methods

2.1. Chemicals

HX600 (Fig. 1A) was synthesized as reported previously [15]. All-trans retinoic acid (ATRA) and 9CRA were purchased from Wako Pure Chemical Industries (Osaka, Japan), and cycloheximide was from Nacalai Tesque (Kyoto, Japan).

2.2. Cell lines and cell cultures

Human embryonic kidney (HEK) 293 cells (RIKEN Cell Bank, Tsukuba, Japan) were cultured in Dulbecco's modified Eagle's medium containing 5% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Human teratocarcinoma NT2/D1 cells (American Type Culture Collection, Rockville, MD) were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum.

2.3. Reverse transcription, polymerase chain reaction (PCR) and real-time quantitative PCR (RT-qPCR)

Total RNAs from samples were prepared by an acid guanidine thiocyanate-phenol/chloroform method [16]. cDNAs were synthesized using an ImProm-II Reverse Transcription system (Promega Corporation, Madison, WI). Conventional PCR was performed using GoTaq Master Mix (Promega Corporation). RT-qPCR was performed on an ABI PRISM 7000 Sequence Detection System (Life Technologies Corporation, Rockville, MD, USA) with Power SYBR Green PCR Master Mix (Life Technologies Corporation). Primer sequences were as follows: RXRα (GenBank ID: NM_002957), 5′-GGG AAG GTT CGC TAA GCT CTT-3′ and 5′-TAA GTC ATT TGG TGC GGC G-3′; Nur77 (GenBank ID: NM_002135), 5′-ACA ACG CTT CAT GCC AGC A-3′ and 5′-GGA CAA CTT CCT TCA CCA TGC-3′; NURR1 (GenBank ID: NM_006186) 5′-GAA TGA AGA GAG ACG CGG AGA A-3′ and 5′-GAA CAC AAG GCA TGG CTT CAG-3′; NOR1 (GenBank ID: NM_006981), 5′-TTG GAG CTG TTT GTC CTC AGA C-3′ and 5′-TCG AGC CAC TCC CCA AAT CCA C-3′; RARβ (GenBank ID: NM_000965), 5′-CAT GAC TTT CTC AGA CGG CCT T-3′ and 5′-GCG GTC TCC ACA GAT TAA GCA-3′; cellular retinoic acid binding protein 2 (CRABP2; GenBank ID: NM_001878), 5′-CGA TCG GAA AAC TTC GAG GA-3′ and 5′-GGT GGT GGA GGT TTT GAT GTA-3′; CPT1A (GenBank ID: NM_001876), 5′-CTG CTT TAC AGG CGC AAA CTG-3′ and 5′-TGT GCT GGA TGG TGT CTG TCT C-3′; dehydrogenase/reductase (SDR family) member 3 (DHRS3; GenBank ID: NM_004753), 5′-TCC CAT GGA CAA TGC ATG C-3′ and 5′-TCC TGT CTC TAT GTC CGC CCT-3′; left–right determination factor 1 (LEFTY1; GenBank ID: NM_020997), 5′-CAG CTG GAG CTG CAC ACC CT-3′ and 5′-TCC TGG TAC CCT CGA ACA CT-3′; LEFTY2 (GenBank ID: NM_003240), 5′-CAG CTG GAG CTG CAC ACC CT-3′ and 5′-ACC TCT ATG CAC ACG TTC AG-3′. Primers for ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1; GenBank ID: NM_005502)

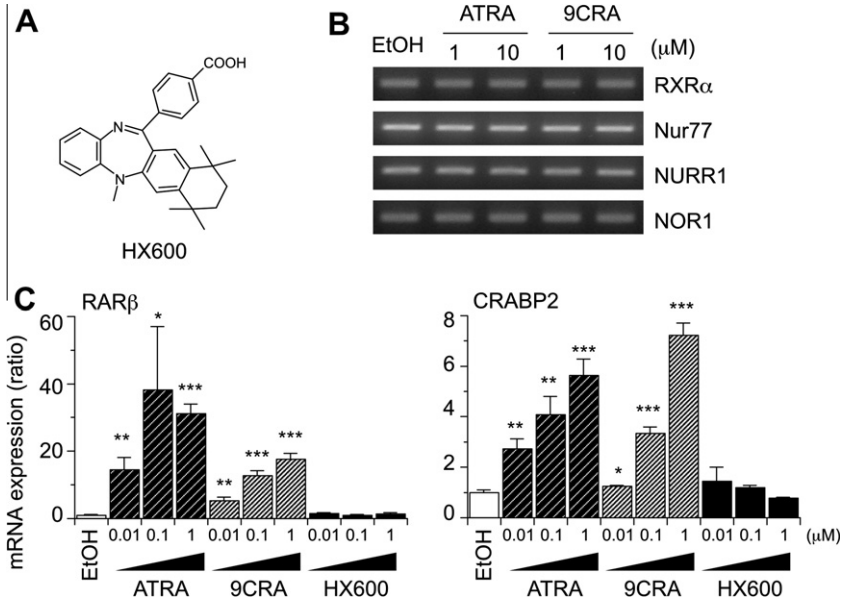

Fig. 1. Retinoic acids, but not HX600, induce the expression of RARβ and CRABP2. (A) Chemical structure of HX600. (B) mRNA expression of RXRα, Nur77, NURR1 and NOR1 in NT2/D1 cells. (C) Concentration-dependent induction of RARβ and CRABP2 by ATRA and 9CRA but not by HX600. NT2/D1 cells were treated with ethanol control (EtOH) or the indicated concentrations of ATRA, 9CRA or HX600 for 24 h, and mRNA levels were evaluated by conventional PCR (B) and RT-qPCR (C). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, compared to ethanol control.