Hepatic expression of PPARα, a molecular target of fibrates, is regulated during inflammation in a gender-specific manner

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Abstract Dyslipidemia, inflammation and gender are major risk factors in cardiovascular disease. Here we show that hepatic expression of Peroxisome proliferator-activated receptor α (PPARα), a nuclear receptor that regulates lipid metabolism and inflammation, is regulated in a gender-specific manner during lipopolysaccharide (LPS)-induced systemic inflammation. Immediately following LPS-induced systemic inflammation, hepatic PPARα mRNA level decreased dramatically in mice. It was restored to baseline within 24 h in females but remained below baseline for >72 h in male mice. In gonadectomized mice of both sexes, PPARα mRNA level was restored to baseline within 48 h after the initial decrease.

Key words: Peroxisome proliferator-activated receptor α; Inflammation; Gender; mRNA; Cardiovascular disease

1. Introduction

Peroxisome proliferator-activated receptor α (PPARα) is a member of the large steroid/thyroid/retinoid nuclear receptor superfamily, distinguished by its hallmark DNA binding domain of two zinc finger-like motifs that recognizes DNA response elements consisting of two copies of a core motif. The canonical DNA response element that binds PPARα is known as peroxisome proliferator response element (PPRE) and consists of two hexanucleotides with the consensus sequence AGGTCA separated by a single nucleotide spacer. When activated by ligand binding and/or phosphorylation, PPARα undergoes conformational changes to recruit coactivator complexes for transcription of target genes. Like other PPARs, activated PPARα heterodimerizes with retinoid X receptor for binding to PPREs (reviewed in [1,2]).

PPARα plays a significant role in lipid metabolism by regulating expression of catabolic enzymes, structural proteins and transport proteins that are involved in mitochondrial and peroxisomal β-oxidation, microsomal ω-oxidation, cellular fatty acid uptake and efflux, lipoprotein metabolism etc. PPARα can be activated by a wide variety of naturally occurring fatty acids and eicosanoids, endogenous agonists composed of polyunsaturated fatty acids (e.g. α-linoleic, γ-linolenic, arachidonic and eicosapentaenoic acids) and medium-chain saturated and monounsaturated fatty acids (e.g. palmitic and oleic acids, leukotriene B_4 (LTB_4) and 8(S)HETE)) (reviewed in [1–6]).

PPARα is also implicated in the development and intensity of inflammatory responses. This role appears to be multi-faceted and intimately linked to its role as a regulator of lipid metabolism and its activation by inflammatory ligands such as LTB_4 (reviewed in [7]). Consistent with this, PPARα null mice have prolonged inflammatory responses than their wild-type littermates in response to LTB_4 [8]. PPARα agonists such as fenofibrates have been shown to modulate mediators of inflammation such as NFκB, IκBa, plasma interleukin (IL)-6 concentration and plasma tumor necrosis factor-α (TNF-α) level [9].

As a key regulator of lipid metabolism, PPARα is a major molecular target for treating hypertriglyceridemia as part of a therapeutic strategy to reduce cardiovascular risk. PPARα agonists such as the fibrates have proven to be therapeutically effective in managing hypertriglyceridemia and reducing cardiovascular risk [10–12]. The role of PPARα in the molecular pathogenesis of cardiovascular disease is likely to be effected through a multi-factorial mechanism that includes its role in lipid metabolism, and both systemic and local inflammation. Incidentally, cardiovascular disease itself is a disease of complex etiology, and dyslipidemia and inflammation are two major causative factors [13].

To better understand the role of PPARα in cardiovascular disease, we examined the regulation of hepatic PPARα expression during systemic inflammation in male and female mice. A previous report has indicated that PPARα is a limiting factor and that PPARα expression levels have a direct impact on the regulation of its target genes [14]. Therefore, regulating PPARα expression level is as critical as activating the receptor itself. The examination of hepatic PPARα expression during systemic inflammation in male and female would provide insights into gender-specific lipid regulation during inflammation in the major organ of lipid metabolism and a major response organ in systemic inflammation. Like dyslipidemia and inflammation, the male gender is also an impor-

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Abbreviations: PPARα, peroxisome proliferator-activated receptor α; PPRE, peroxisome proliferator response element

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tant risk factor in cardiovascular disease [13]. Indeed, we have previously shown that expression of several genes known to be important in cardiovascular disease, such as angiotensin converting enzyme (ACE), high density lipoprotein-associated antioxidant and paraoxonase-1, is regulated in a gender-specific manner [15,16].

2. Materials and methods

2.1. Animals

All animal experimentations were carried out in accordance with the guiding principles for animal research as specified by the Animal Ethics Committee of the National University of Singapore and have been previously described [15]. Briefly, systemic inflammation was induced in intact and gonadectomized C57BL/6J mice with lipopolysaccharide (LPS), and liver RNA was prepared at 0, 2, 4, 6, 24, 48 and 72 h.

2.2. Northern analysis

Total RNA was prepared by homogenizing tissues in guanidine isothiocyanate buffer and purification over a CsCl gradient as previously described [17]. The RNA was analyzed by Northern blot analysis. Briefly, 10 mg of total RNA was denatured by glyoxal, separated on an agarose gel, transferred onto a nylon membrane and probed with random-primed, ^{32}P-labelled DNA fragments as previously described [17]. Signal intensity was analyzed by phosphorimaging. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalizing variations in RNA loading between samples. The probes used were reverse transcription (RT)-PCR products that were generated using the following sets of primers: (1) PPARα 5′-ACGGCAATGGCTTTATCACAC-3′ and 5′-CTACGCTCAGCCCTCTTCATC-3′ (420 bp), (2) GAPDH 5′-GGCAAATTCAACGGCACAGTC-3′ and 5′-CTGTTATTATGGGGGTCTGGG-3′ (967 bp), (3) serum amyloid A protein (SAA) 5′-ATCACGTGATGCAAGAGAGAGC-3′ and 5′-ATTACCCTCTCCTCCTCAAGC-3′ (269 bp), (4) serum amyloid P protein (SAP) 5′-TGTTTGTCTTCACCAGCCTTC-3′ and 5′-GTGAACTCTGCCGCCGCCTTGACC-3′ (406 bp), (5) IL-1β 5′-AAGATGTCCAACTTCACCTTCAAGGAGAGCCG-3′ and 5′-AGGTCGGTCTCACTACCTGTGATGAGTTTTGG-3′ (491 bp), (6) IL-6 5′-ATGAAGTTCCTCTCTGCAAGAGAC-3′ and 5′-CACTAGGTTTGCCGAGTAGATCTC-3′ (638 bp), (7) TNF-α 5′-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3′ and 5′-GTATGAGATAGCAAATCGGCTGACGGTGTGGG-3′ (354 bp). RT-PCR was carried out as previously described [18].

2.3. Hepa cell culture

Hepa cells (a gift from Heinz Baumann) were cultured in Dulbecco minimal essential media with 10% fetal calf serum. The cells were treated with (a) 10 μg/ml LPS, (b) 1 mM dexamethasone or (c) 10 μg/ml LPS and 1 mM dexamethasone for 0, 2, 4, 6, 24 and 48 h. Total RNA was prepared and analyzed by Northern blot hybridization.

3. Results

3.1. Regulation of PPARα expression in a gender-specific manner during systemic inflammation

PPARα mRNA was easily detectable in the livers of male and female mice (Fig. 1a,b). Within 2 h of inducing systemic inflammation by LPS, PPARα mRNA levels declined more than 50% in both male and female mice. In female mice, PPARα mRNA level recovered to baseline level within 24 h. It remained slightly but not significantly elevated above base-

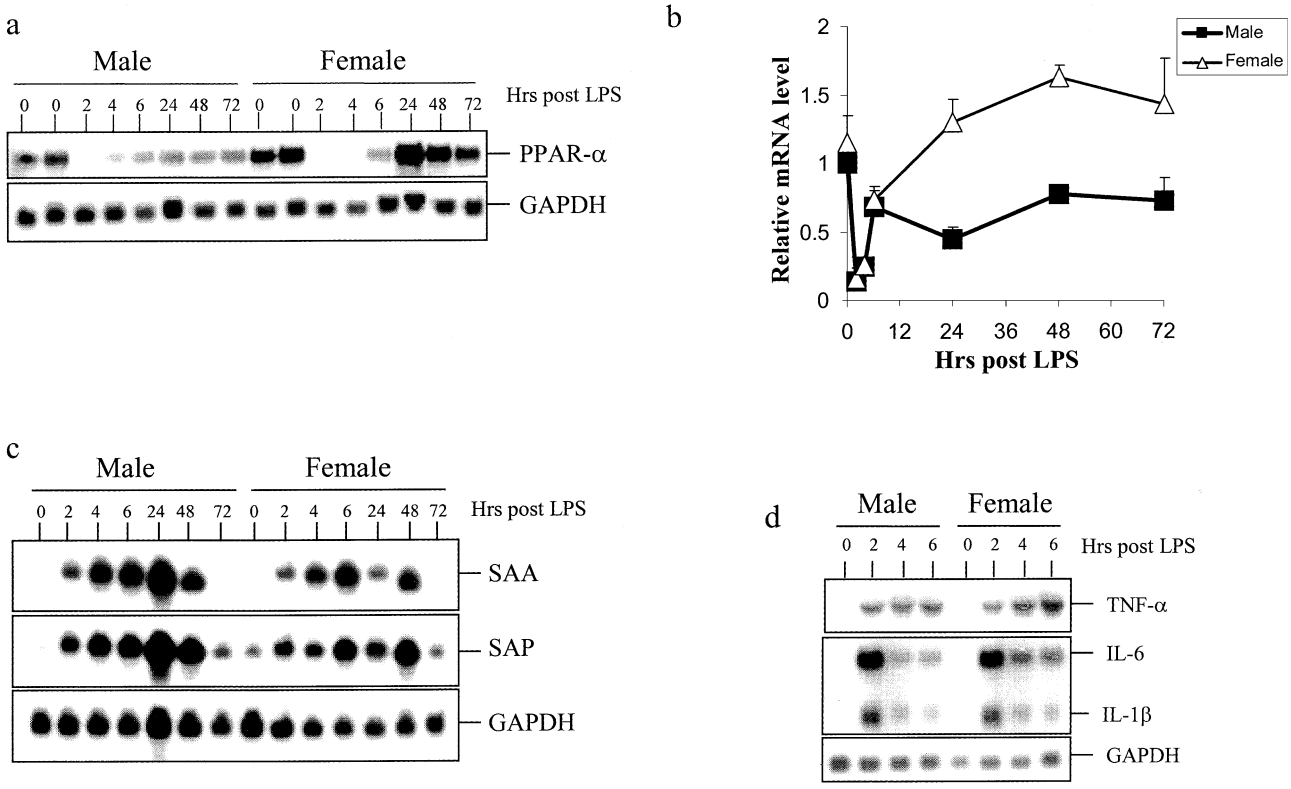

Fig. 1. Analysis of hepatic RNA from LPS-treated mice. a: Northern analysis for PPARα mRNA. The blot shown was representative of four independent experiments. Each Northern blot membrane was probed with ^{32}P-labelled PPARα and GAPDH-specific probes. b: Relative PPARα mRNA levels. PPARα signals were quantitated by phosphorimaging and normalized to that of GAPDH. The normalized PPARα mRNA level in male mice at 0 h was designated 1. Data represent means of four independent experiments ± S.E.M.. c,d: Assessment of systemic inflammation by Northern blot analysis. The hepatic RNA was also analyzed for acute phase reactants such as SAA, SAP and cytokines such as TNF-α, IL-1β and IL-2.

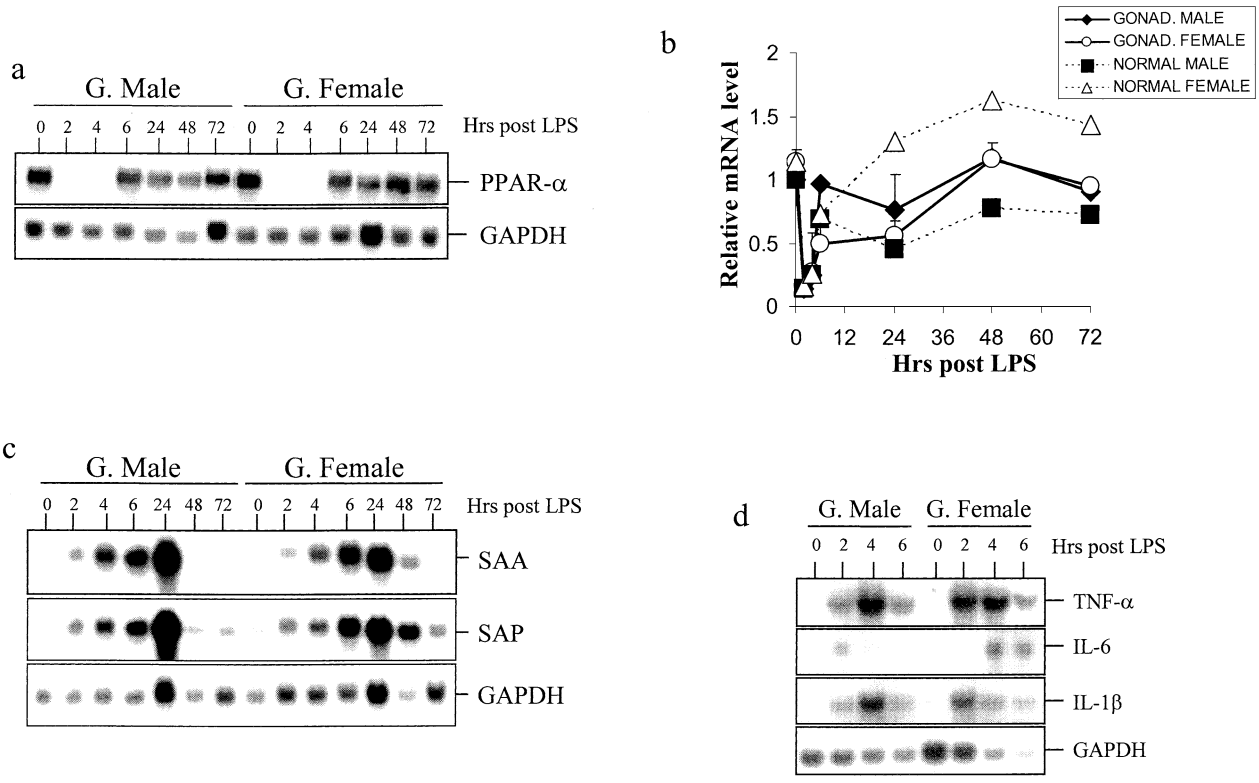

Fig. 2. Analysis of hepatic RNA from LPS-treated gonadectomized mice. a: Northern analysis for PPARα mRNA. The blot shown was representative of four independent experiments. Each Northern blot membrane was probed with ^{32}P-labelled PPARα and GAPDH-specific probes. b: Relative PPARα mRNA levels. PPARα signals were quantitated by phosphorimaging and normalized to that of GAPDH. The normalized PPARα mRNA level in male mice at 0 h was designated 1. Data represent means of four independent experiments ± S.E.M. c,d: Assessment of systemic inflammation by Northern blot analysis. The hepatic RNA was also analyzed for acute phase reactants such as SAA, SAP and cytokines such as TNF-α, IL-1β and IL-2.

line for up to 72 h. In contrast, PPARα mRNA level in male mice failed to recover to baseline level 72 h after induction of systemic inflammation. To ensure that the magnitude of the induced systemic inflammation in both sexes was equivalent, IL-1, IL-6, TNF-α, SAA and SAP mRNA levels were monitored (Fig. 1c,d). When normalized against GAPDH mRNA level, there was no difference in the induction between male and female mice.

3.2. Gonadectomy attenuates regulation of PPARα mRNA during systemic inflammation

The above experiment was repeated in gonadectomized mice. Like the intact animals, PPARα mRNA in gonadectomized animals also decreased within 4 h after LPS challenge (Fig. 2a,b). However, PPARα mRNA levels in both male and female gonadectomized mice were restored to baseline within 48 h and were not significantly different between gonadectomized male and female animals. Gonadectomy not only abolished the sexual dimorphism seen in intact animals, it also moderated LPS-induced changes in PPARα mRNA level in both male and female gonadectomized mice to that intermediate between those in intact males and females (Fig. 2b). Unlike PPARα mRNA, the induction of SAA, SAP, IL-1β, IL-6 and TNF-α mRNA levels during LPS challenge was not altered by gonadectomy (Fig. 2c,d).

3.3. LPS did not affect PPARα expression in Hepa cells

To determine if LPS exerted a direct effect on PPARα gene expression, Hepa cells, a mouse hepatoma cell line, were treated with LPS. When treated with dexamethasone, PPARα mRNA level increased 10-fold elevation, as expected [14,19,20]. However, LPS alone had no effect on PPARα mRNA in Hepa cells but it had a synergistic effect on dexamethasone-mediated increase in PPARα mRNA level (Fig. 3).

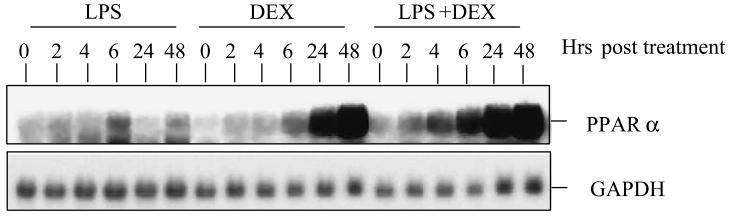

Fig. 3. Effects of LPS and dexamethasone on PPARα mRNA level in Hepa cells. The cells were treated with 10 μg/ml LPS, 1 mM dexamethasone or both for 0, 2, 4, 6, 24 and 48 h. Total RNA was prepared and analyzed on Northern blot assay.

4. Discussion

Our study demonstrated that PPARα expression in the liver was regulated in a gender-specific manner during LPS-induced inflammation. Gonadectomy abolished the gender differences. Gonadectomized male and female mice exhibited an attenuated female gene expression pattern during inflammation, suggesting that the female gene expression pattern is the default pattern and that the male gonads had a dominant effect on the expression pattern while the female gonads may enhance expression. We have previously demonstrated that in the conventional housing system of five adult male mice to a cage, serum testosterone in all the male mice except the dominant male is equivalent to that in the female mice and the dominant mice have a serum testosterone level that is at least an order higher [15,16]. Therefore, the male gonadal effect on PPARα expression was not likely to be mediated by serum testosterone level.

PPARα expression in liver has previously been reported to be regulated by physiological situations such as the diurnal surge of glucocorticoids affects [14], and the secretion of glucocorticoids by the hypothalamus-pituitary-adrenal axis during inflammation has long been recognized as gender-specific with females generally secreting higher levels of glucocorticoids during inflammation including LPS-induced inflammation ([21]; reviewed in [22]). We also demonstrated that LPS accentuated upregulation of PPARα expression by dexamethasone. Together this suggested that the more rapid recovery of PPAR mRNA level to baseline in female mice during inflammation was likely to be mediated by the higher secretion of glucocorticoids in female mice.

In summary, our study demonstrated that PPAR expression in the liver is regulated in a gender-specific manner during inflammation. Since the liver is the major organ for lipid metabolism, and PPARα is limiting with ligand binding activity directly correlated to its mRNA level [14], the immediate implication of our study is that lipid metabolism during inflammation is regulated in a gender-specific manner during inflammation. In addition, we have identified a candidate molecule that could provide a common underlying molecular mechanism for three major risk factors in cardiovascular disease, namely gender, inflammation and dyslipidemia [13].

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