Inflammation-associated upregulation of the sulfated steroid transporter Slc10a6 in mouse liver and macrophage cell lines

Astrid Kosters, Emory University
Demesew F. Abebe, Emory University
Julio C. Felix, Baylor College of Medicine
Paul Dawson, Emory University
Saul Karpen, Emory University

Journal Title: Hepatology Research
Volume: Volume 46, Number 8
Publisher: Wiley | 2016-07-01, Pages 794-803
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1111/hepr.12609
Permanent URL: https://pid.emory.edu/ark:/25593/s3sx3

Final published version: http://dx.doi.org/10.1111/hepr.12609

Copyright information:
© 2015 The Japan Society of Hepatology

Accessed December 6, 2019 9:59 PM EST
Inflammation-associated upregulation of the sulfated steroid transporter Slc10a6 in mouse liver and macrophage cell lines

Astrid Kosters¹, Demese F. Abebe¹, Julio C. Felix², Paul A. Dawson¹, and Saul J. Karpen¹

¹Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Emory University School of Medicine, Atlanta GA, 30322.

²Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030

Abstract

**Aim**—Slc10a6, an incompletely characterized member of the SLC10A bile acid transporter family, was one of the most highly-induced RNA transcripts identified in a screen for inflammation responsive genes in mouse liver. This study aimed to elucidate a role for Slc10a6 in hepatic inflammation.

**Methods**—Mice were treated with LPS (2mg/kg) or IL-1β (5mg/kg) for various time points. Cells were treated with LPS (1μg/ml) at various time points, with cell signaling inhibitors, nuclear receptor ligands and Slc10a6 substrates. All mRNA levels were determined by QPCR.

**Results**—Slc10a6 mRNA levels were upregulated in mouse liver at 2h (7-fold), 4h (100-fold), and 16h (50-fold) after LPS treatment, and 35-fold by the cytokine IL-1β (4h). Both absence of the nuclear receptor Fxr and pretreating mice with the synthetic RXRα ligand LG268 attenuated the LPS-upregulation of Slc10a6 mRNA by 60-75%. In vitro, Slc10a6 mRNA was induced 30-fold by LPS in mouse RAW264.7 macrophages in a time-dependent manner (maximum at 8h). The Slc10a6 substrate dehydroxyepiandrosterone sulfate (DHEAS) enhanced LPS-induction of CCL5 mRNA, a pro-inflammatory chemokine, by 50% in RAW264.7 cells. This effect was abrogated in the presence of anti-inflammatory nuclear receptor ligands 9cisRA and Dexamethasone.

**Conclusions**—Dramatic upregulation of Slc10a6 mRNA by LPS combined with enhanced LPS-stimulation of CCL5 expression by the Slc10a6 substrate DHEAS in macrophages, suggests that Slc10a6 function contributes to the hepatic inflammatory response.

Keywords

Inflammation; Liver; Nuclear Receptor; Macrophages; Slc10a6

Introduction

Approximately 1 in 10 Americans suffer from liver disease and the prevalence of diseases such as non-alcoholic fatty liver disease and liver cancer are on the rise¹,². Many forms of
liver diseases (including hepatitis B or C, auto-immune hepatitis, non-alcoholic steatohepatitis) are associated with an inflammatory response, which can contribute to the progression of fibrosis, cirrhosis and liver failure, as well as hepatocellular carcinoma. Aside from its metabolic and detoxification functions, the liver plays a central role in (adaptive) innate immunity as it clears systemic lipopolysaccharides (LPS) derived from the cell wall of gram-negative bacteria directly entering the portal circulation. Moreover, in response to inflammatory stimuli (including LPS), the liver produces and releases various cytokines, chemokines and acute-phase proteins (a reaction collectively known as the “acute phase response”), which involves all cell types in the liver, particularly the sinusoidal resident hepatic macrophages, the Kupffer cells (KC) and hepatocytes. The process is mediated in part by chemokines, which direct leukocyte migration toward sites of inflammation during the immune response. One such a chemokine, CCL5 (Rantes) is upregulated in liver in both acute and chronic liver diseases\(^3\). However, a detailed understanding of the mediators and cellular interplay that forms the initiation and maintenance of the acute phase response is lacking.

Type 2 Nuclear Receptors (NRs) are heterodimeric ligand-activated transcription factors that respond to both endogenous and exogenous compounds, including oxysterols, bile acids, fatty acids, as well as various drugs\(^4\). Retinoid X Receptor α (RXR\(\alpha\)) is known to partner with at least 14 Type 2 NRs in liver\(^5\) and this array of RXR\(\alpha\)-containing heterodimers are among the most prevalent transcription factors in liver chromatin, governing gene expression for many core hepatic functions\(^6\). Hepatic inflammation alters the function of multiple factors associated with chromatin and decreases the DNA-binding capacity of specific NRs, including RXR\(\alpha\), to reduce expression of their target genes\(^7\)-\(^10\). Countering these effects, various ligands for NRs such as FXR\(^11\), RXR\(\alpha\)\(^12\) and GR\(^13\) exhibit anti-inflammatory actions, some of which involve interaction with NFκB, a major downstream mediator of the effects of LPS on hepatic cytokine gene expression.

Slc10a6 is a member of the Slc10 sodium-dependent bile acid (BA) transporter family, which includes the well-characterized carriers, Slc10a1 (Na\(^+\)-taurocholate cotransporting polypeptide; Ntcp) and Slc10a2 (Apical sodium-dependent bile acid transporter; Asbt) that function to maintain the enterohepatic circulation of BA\(^14\)-\(^16\). In contrast to Ntcp and Asbt, Slc10a6 does not transport the major species of unconjugated or glycine/taurine-conjugated BA, but rather functions as a high affinity carrier for sulfated solutes, including steroids such as dehydroepiandrosterone sulfate, pregnenolone sulfate, and estrone-1-sulfate, and sulfated BA such as tauroliothocholate-3-sulfate\(^17,18\). Slc10a6 appears to be specific for sulfated compounds and none of the parent nonsulfated, or glucuronidated forms were identified as substrates. Previous studies focused on the role of Slc10a6 in steroid metabolism by tissues such as testes and placenta\(^17,18\), however little is known regarding its relationship to hepatic inflammation or the in vivo substrates for Slc10a6 in liver under these pathophysiological conditions. A potentially important substrate is dehydroepiandrosterone sulfate (DHEAS), the storage form of DHEA, which is produced the adrenal gland and one of the most abundant hormones in the human systemic circulation and is the precursor for most steroid hormones including testosterone and 17β-estradiol. Steroids such as DHEA and DHEAS are also regulators of gene expression and cell signaling. In the nucleus, DHEA/DHEAS can regulate transcriptional activity of members of the NR superfamily\(^19\)-\(^21\). DHEAS is
converted intracellularly into the biologically more active DHEA by Steroid Sulfatase (STS)\textsuperscript{22}, which itself is down-regulated by inflammation. Several studies have shown beneficial and anti-inflammatory effects of DHEA/DHEAS in rodents, by ameliorating hepatocellular damage in models of obstructive cholestasis\textsuperscript{23, 24}, and delaying mortality in mice subjected to sepsis by cecal ligation and puncture\textsuperscript{25}. However, other studies have found pro-inflammatory or no effects of DHEAS\textsuperscript{26}, and the underlying mechanisms remain to be elucidated. In the current study, we describe the inflammation-associated induction of hepatic Slc10a6 expression and explore the proinflammatory effect of its transport substrate DHEAS on macrophage expression of the chemokine CCL5.

Methods

Animal experiments

8-10 week old male C57BL/6J mice and Fxr−/− mice (Nr1h4\textsuperscript{tm1Gonz/J}), obtained from Jackson labs, were injected intraperitoneally with LPS (Salmonella typhimurium; Sigma) or IL-1β (Biovision) as described before \textsuperscript{9}. Mice were administered LG268 by gavage followed by injection of LPS or saline as described previously\textsuperscript{10}. LG268 was kindly provided by Ligand Pharmaceuticals. Cholic acid feeding experiments were performed as described before\textsuperscript{27}. Obeticholic acid was kindly provided by Intercept. Mice were maintained in a temperature- and humidity-controlled environment and provided with water and rodent chow ad lib. Animal protocols were approved by the Baylor College of Medicine and Emory University Institutional Animal Care and Use Committees.

In vitro experiments

Mouse macrophage RAW264.7 cells were obtained from ATCC and maintained in RPMI containing 10% FBS, complemented with penicillin, streptomycin, glutamine. The human cholangiocyte cell line MzHA1 was a kind gift of Dr. Andrew Feranchak (UT Southwestern, Dallas TX). The human stellate line LX2 was a kind gift of Dr. Scott Friedman (Mount Sinai School of Medicine, New York, NY). The immortalized mouse endothelial cells were obtained from Applied Biological Materials Inc. Cells were treated with LPS (1μg/ml) for the indicated time periods (see figure legends). In addition cells were treated with, IL-6, TNFα, IL-17, IL-12, IFNγ (all from BioLegend), IL-1β (R&D Systems) and IL-4 (BioVision). In other experiments, cells were treated for 24 h with ligands for FXR (50 μM Chenodeoxycholic acid; Sigma), RXRα [1 μM 9-Cis-Retinoic Acid; (9CisRA); Sigma], GR [10 μM Dexamethasone (Dex); Sigma], LXRα (1nM T0901317; Sigma), PPARα (10μM WY-14643; Sigma), PPARβ (10 μM GW501516; Santa Cruz Biotechnology), PPARγ (10 μM Rosiglitazone; Sigma), VDR (1 nM 1alpha,25-Dihydroxyvitamin D3; Santa Cruz Biotechnology), PXR (10 μM pregnenolone-16alpha-carbonitrile (PCN); Sigma) and the Slc10a6 substrates DHEAS (50 μM) [US Biological], Lithocholic acid 3-sulfate, (Santa Cruz Biotechnology), Estrione-1-Sulfate (Santa Cruz Biotechnology), Pregnenolone Sulfate (Spectrum Chemical), Pretreatments with inhibitors for NFκB (3 mM Sulfasalazine; Sigma), JNK (30 μM SP600125; Calbiochem), ERK1/2 (U0126; Cell Signaling), PI3K (LY294002; Cell Signaling), and p38 (SB203580; Cell Signaling) were for either 30 or 60 minutes.
Gene expression analysis

RNA was isolated and gene expression was by measured by quantitative real-time PCR (QPCR) as described previously. Primers and probes were obtained from Sigma Genosys and IDT. All data was normalized to cyclophilin. Sequences are available on request. All data are shown as mean ± standard deviation and were analyzed by ANOVA. P-values< 0.05 were considered significant.

Results

Our previous studies have shown that major changes of gene expression occur after inflammatory signaling addresses the liver involving changes in nuclear localization of the nuclear receptor RXRα. To further examine the hepatic response to inflammation, global gene expression analysis was performed in mice treated with LPS (16h time point; 2 mg/kg LPS), or saline. The mRNA levels for Slc10a6 were found to be one of the top induced by LPS in mouse liver versus saline-treated mice (~500-fold; Fig. 1A; full data set to be published in separate manuscript). QPCR analysis validated these initial findings and showed Slc10a6 mRNA was increased >50-fold in livers of mice administered LPS (16h time point) (Fig. 1B). Time course analysis revealed that hepatic Slc10a6 mRNA was significantly induced by LPS at earlier time points (increased 7-fold at 2h and 100-fold at 4h post-LPS administration) (Fig. 1C). Moreover, hepatic Slc10a6 mRNA expression was induced 35-fold in mice treated with the cytokine IL-1β (Fig. 1D). In agreement with previous studies, only low levels of Slc10a6 mRNA expression were found under basal conditions in mouse liver as compared to intestine, heart, lung, kidney and brown adipose tissue (Fig. 1E). However of the 13 tissues analyzed, liver showed the greatest fold induction of Slc10a6 mRNA expression (~140-fold) after LPS administration compared to approximately 5 to 40-fold in jejunum, kidney, spleen, heart and white adipose tissues (Fig. 1F).

In liver, the related SLC10 family members Ntcp (Slc10a1) and Asbt (Slc10a2) are expressed on the hepatocyte sinusoidal membrane and cholangiocyte apical membrane, respectively. In an effort to determine which liver cell type(s) are responsible for the increased Slc10a6 mRNA expression associated with LPS treatment, representative cell lines were screened for Slc10a6 basal expression and response to inflammatory mediators. Slc10a6 mRNA was not detected in mouse and human hepatocyte cell types (primary mouse hepatocytes, Hepa1c1c7, HepG2, Huh7), under basal conditions or at multiple time points (up to 24h) after administration of LPS (data not shown). Although Slc10a6 mRNA was detected in cell lines derived from human cholangiocytes (MzHA1) and human stellate cells (LX-2), and in mouse sinusoidal endothelial cells, its expression was not induced by LPS treatments during the time points tested (2-24h). All cell lines examined did respond to LPS with induction of standard markers of inflammation, such as TNFα, indicating that LPS signaling was intact (data not shown). As a model for Kupffer cells, Slc10a6 expression was also examined in the mouse macrophage cell line RAW264.7. In those experiments, LPS treatment significantly increased Slc10a6 mRNA expression in a time-dependent fashion, with maximal induction at 8h (Fig 2A). This induction was dependent on the presence of intact JNK and NFκB signaling pathways as demonstrated using the respective pathway-
specific chemical inhibitors SP600125 and sulfasalazine. Additional experiments using inhibitors for other signaling pathways (ERK1/2; p38; PI3K; Gsk3β) were also performed, but these treatments had no effect on the LPS induction of Slc10a6 expression in RAW264.7 cells (data not shown). To determine if the cytokines involved in the hepatic response to inflammation also could induce Slc10a6 expression, RAW264.7 cells were treated with IL-1β, TNFα, IL-6, IL-4, IL-12, IFNγ, and IL-17. However, none of these treatments induced changes in Slc10a6 mRNA levels over the time points tested (2-24h; data not shown).

In order to determine factors involved in transcriptional regulation of Slc10a6 the role of NRs in the transcriptional regulation of Slc10a6 expression was examined in RAW264.7 cells. After treatment of the cells for 24h with various NR ligands, based on similar regulation of Slc10a1 and Slc10a2 by nuclear receptors in their respective cell types, Slc10a6 mRNA expression was found to be significantly down-regulated (80%) by the RXRα ligand 9cisRA and significantly induced (3-fold) by the glucocorticoid receptor (GR) ligand dexamethasone (Dex) (Fig. 2B). There was no evidence for significant regulation of Slc10a6 mRNA expression in RAW264.7 cells by NR ligands for FXR, LXRα, PPARα,β,γ, VDR, or PXR (data not shown). Addition of Dex prevented the down-regulation of Slc10a6 by 9Cis-RA in RAW264.7 cells (Fig 2B).

In silico promoter analysis suggested the likely presence of adjacent binding sites for NFκB and RXRα heterodimers, each of which have been implicated in our in vitro experiments. Several nuclear receptors, including RXRα 10 and FXR 11 have anti-inflammatory actions through interactions with NFκB in mouse liver. Our data showed that pretreatment of mice with LG268 (5 days; 30 mg/kg/day), a synthetic RXRα ligand, attenuated the LPS-mediated increase of liver Slc10a6 mRNA expression by ~60% (Fig 3A). QPCR analysis revealed a ~75% attenuation in the induction of Slc10a6 mRNA in livers of Fxr−/− mice treated with LPS for 16h as compared to wild-type mice (Fig 3B). Though these data implicate RXRα and FXR as regulators and mediators of upregulation of hepatic Slc10a6 mRNA by LPS, the FXR-mediated attenuation did not appear to be due to loss of a direct stimulatory effect of FXR on Slc10a6 expression, since treatment of wild-type mice with LPS plus the synthetic FXR ligand obeticholic acid (OCA) did not further increase hepatic Slc10a6 expression (Fig. 3C). The role of FXR in the regulation of Slc10a6 expression was also explored in wild-type and Fxr−/− mice fed a diet containing 1% Cholic acid. After 5 days of Cholic acid feeding, hepatic Slc10a6 mRNA expression was unchanged in wild-type mice, but induced ~20-fold in Fxr−/− mice (Fig. 3D). However, it was likely that this induction is inflammation-mediated and a secondary effect associated with liver injury, since the Fxr−/− mice showed marked wasting and cholestasis with increased IL-6 mRNA expression after 5 days of cholic acid feeding (Fig 3D).

Next, in order to begin to understand the functional consequences associated with the LPS-induction of Slc10a6 expression, the effects of previously identified Slc10a6 substrates on the LPS-associated inflammatory response was examined in RAW264.7 cells. Addition of the Slc10a6 substrates DHEAS, TLCAS, PREGS, or E1S prior to short-term LPS treatments (2-6h) of RAW264.7 cells did not affect the induction of TNFα, IL-1β, IL-6, iNOS, IFNγ, CCL5, CXCL9, or IL-10 mRNA expression by LPS (data not shown). However during
longer incubations, addition of DHEAS, but not TLCAS, PREGS or E1S, enhanced LPS-associated induction of the neutrophil attractant chemokine CCL5 mRNA by 50% (Fig 4A, and data not shown). LPS-mediated inductions of IL-10, CCL2, iNOS, or IFNγ mRNA levels were not affected by addition of DHEAS (data not shown). Addition of the NR ligands 9cisRA and Dex prevented the DHEAS-mediated enhancement of LPS-induction of CCL5 mRNA (Fig 4B).

Discussion

Many diseases are associated with hepatic inflammation, which often progresses to fibrosis and cirrhosis, and can cause liver failure if left untreated. Identification of pathways and compounds that interfere and counteract these adverse outcomes is an area of active investigation, but has been hampered by the multitude of targets (cellular and molecular) that collectively contribute to the pathogenesis of inflammation. LPS-initiated hepatic inflammation induces the pro-inflammatory cytokines TNFα, IL-1β and IL-6, among others, which subsequently act on hepatocytes7, 29, impairing their normal functioning. A large number of genes important for hepatocyte functions such as lipid, cholesterol, and bile acid metabolism, and the detoxification and elimination of xenobiotics and metabolites are downregulated during inflammation, resulting in local and systemic accumulation of toxic compounds, and eventually in liver damage. In addition to direct alterations of hepatocyte function, liver inflammation affects the function of non-parenchymal cells such as Kupffer cells, cholangiocytes and endothelial cells and the roles played by intercellular molecular communications between these cells types during the inflammatory response is not well understood. In this study we describe the marked upregulation of the nonparenchymal-cell expressed Sodium/sulfated solute cotransporter Slc10a6, in response to LPS and to IL-1β induced inflammation in mouse liver.

Slc10a6 is a member of the SLC10 family of transporters, which includes the well-characterized BA uptake transporters Slc10a1 (Ntcp) and Slc10a2 (Asbt)14, 15. Although the amino acid sequence of Slc10a6 shares 35% identity/56% similarity with Ntcp and 47% identity/66% similarity with Asbt, Slc10a6 has distinct substrate preferences, with highest affinity for sulfated steroids. In liver, Ntcp and Asbt expression is restricted to hepatocytes and cholangiocytes, respectively16. However, our in vitro screen of liver cell types suggests that Slc10a6 functions in non-parenchymal cells, most likely the resident hepatic macrophages, Kupffer cells, which play a central role in the hepatic responses to inflammation and are responsible for the majority of the pro- and anti-inflammatory cytokine production that occurs in the adaptive response to inflammation30. To date, much of the investigation has focused on the potential in vivo functions of Slc10a6 in endocrine tissues17, 18 and a physiological relevant role in hepatic inflammation for Slc10a6 has yet to be identified. We found a marked time-dependent upregulation of Slc10a6 RNA levels following LPS-treatment in the macrophage cell line RAW264.7, which was absent in any of the tested hepatocyte, cholangiocyte, stellate and endothelial cell lines. This cell-type selective expression of the different SLC10 family members likely reflects their different functional roles. It may also minimize potentially deleterious interactions between different SLC10 members since in vitro experiments have shown that Slc10a6 can co-localize and co-immunoprecipitate with Slc10a1 when co-expressed in transfected cells31. In addition, the
Data presented in the current study revealed inflammation-mediated upregulation of Slc10a6 expression is modulated by the NRs RXRα, FXR and GR. Moreover, inhibition of JNK and NF-κB signaling abrogated LPS-mediated induction of Slc10a6.

CCL5 is a pro-inflammatory neutrophil and T-cell attracting chemokine, which is upregulated in liver in both acute and chronic liver diseases. In mouse models, CCL5 and its receptors are essential for NAFLD/NASH development and fibrosis progression32,33, and in humans, patients with chronic liver disease show high levels of CCL534. In our studies, the Slc10a6 substrate DHEAS enhanced the LPS-induced CCL5 mRNA expression in RAW264.7 cells in vitro. As CCL5 plays an important role in the progression of liver disease, the uptake of DHEAS by Slc10a6 may potentiate the inflammatory response.

DHEA, as its storage form DHEAS, is one of the most abundant hormones in plasma, but beyond their role as biosynthetic precursors of other steroid hormones, the physiological functions of these steroids are still unclear. After uptake, DHEAS is intracellularly converted to DHEA by Steroid Sulfatase (STS), which is expressed in multiple cell types, including monocytes and macrophages. Conversely, the synthesis of DHEAS from DHEA is mediated by the sulfotransferase SULT2A1. Interestingly, the circulating levels of DHEAS are decreased during the acute phase response such as following administration of LPS35. Although this decrease was attributed to suppression of SULT2A1 expression, the induction of Slc10a6-mediated DHEAS clearance by liver Kupffer cells and other macrophage cell depots may play an equally important role. Low circulating DHEAS levels were also found in patients with NASH36. Several studies have shown anti-inflammatory effects for DHEA/DHEAS in rodents. For example, DHEA/DHEAS treatment improved cholestasis and ameliorated hepatocellular damage in models of obstructive cholestasis23-25,37. However DHEA could not alleviate sickness behavior induced by LPS in mice26.

Several sulfated steroids show NR agonistic or antagonistic activity, such as 24-Hydroxy-Cholesterol-3-Sulfate38 and 25-Hydroxy-Cholesterol-3-Sulfate39, and can interfere with inflammatory signaling pathways in macrophages. DHEA has been shown to affect NR activity in mouse liver directly by acting as a ligand for PXR and CARβ19,20, or by activating PPARα through an indirect mechanism21. Moreover DHEA/DHEAS has known anti-glucocorticoid actions in other cell types40-42 where it inhibits GR translocation to the nucleus. Indeed addition of an exogenous GR ligand (Dex) attenuated the increase in CCL5 mRNA expression observed with LPS plus DHEAS. In addition, activation of RXRα, another NR with anti-inflammatory actions, down-regulated Slc10a6 mRNA expression in RAW264.7 cells. Our in vivo data also suggests a role for the NRs FXR and RXRα in the upregulation of hepatic Slc10a6 gene expression in response to LPS in mouse liver. GR is a well-known anti-inflammatory nuclear receptor13, whereas FXR and RXRα can act as negative regulators of inflammation through interaction with NFκB11,12 which is downstream in the LPS signaling pathway. In silico promoter analysis suggests the likely presence of adjacent binding sites for NFκB and RXRα heterodimers. We hypothesize that crosstalk between NRs and NFκB mediate the induction of Slc10a6 mRNA expression in response to LPS (Fig 5).

In conclusion, we speculate that increased Slc10a6 mRNA expression in Kupffer cells contributes to the adaptive inflammatory response by increased uptake of Slc10a6 substrates...
with NR regulatory potential, such as DHEAS. The net effect is to modulate induction of pro-inflammatory cytokine and chemokine responses, including the observed enhancement of CCL5 mRNA expression.

**Acknowledgements**

Financial Support was from Emory Egleston Children's Research Center pilot grant (AK) and NIH: DK56239 (SJK). Dr. Dawson has served as a consultant for Lumena Pharmaceuticals in the past. The other authors report no conflict of interest. The authors would like to thank Dr. Andrew Feranchak (UT Southwestern, Dallas TX) and Dr. Scott Friedman (Mount Sinai School of Medicine, New York, NY) for the kind gifts of the human cholangiocyte MzHA1 and stellate line LX2 cell lines respectively, as well as Ligand Pharmaceuticals and Intercept for kindly providing LG268 and Obeticholic acid respectively.

**References**


Figure 1. Time-dependent induction of Slc10a6 mRNA expression by LPS and IL-1β in mouse liver
A) Induction of Slc10a6 mRNA in mouse liver after 16 hrs LPS (2mg/kg) as determined by microarray; n=3. B) Validation of microarray results by QPCR; n=6. Data expressed as mean +SD. * P<0.05 vs control. C) Time-dependent induction of Slc10a6 mRNA expression by LPS (2mg/kg) in mouse liver. D) Time-dependent induction of Slc10a6 mRNA expression by IL-1β (5mg/kg) in mouse liver. E) Distribution of Slc10a6 mRNA expression in male mouse tissues. Testis was used as reference organ. F) Induction of Slc10a6 mRNA expression by LPS (16 hrs; 2 mg/kg) in mouse tissues. For C-F QPCR was performed using cDNA from pooled RNA from n=5-7 mice per time point. All data were normalized to cyclophilin mRNA expression.
Figure 2. LPS-induction of Slc10a6 mRNA expression in the mouse macrophage cell line RAW264.7
A) Time dependent LPS (1 μg/mL) induction of Slc10a6 mRNA and TNFα in mouse macrophages (RAW264.7) incubated in the presence of vehicle (DMSO), or the NFκB and JNK pathway inhibitors Sulfasalazine (60 min pretreatment) and SP600125 (30 min pretreatment), respectively. Data shown is the average of 2 to 4 experiments (+SD) with each time point in triplicate. B) Effect of NR ligands 9cisRA and Dex on Slc10a6 mRNA levels in RAW264.7 cells (24h). Ligands were added directly prior LPS treatment. C) Effect of the RXRα ligand 9cisRA on the LPS-mediated induction of Slc10a6 mRNA. Ligands were added directly prior LPS treatment. Data expressed as mean ± SD. *P<0.05 versus saline. #P<0.05 versus LPS/veh. All data was normalized to cyclophilin mRNA levels.
Figure 3. Modulatory role of nuclear receptors RXRα and FXR in the LPS-mediated induction of hepatic Slc10a6 expression in mice
A) The induction by LPS of hepatic Slc10a6 mRNA was attenuated by activation of RXRα (LG268 treatment for 5 days, 30 mg/kg/day, prior to LPS treatment (2mg/kg for 16h). B) Induction of Slc10a6 mRNA by LPS was attenuated in mice lacking the RXRα partner FXR (fxr/e−). C) Pretreatment with the semi-synthetic FXR ligand OCA did not affect LPS-mediated induction of Slc10a6 mRNA in mouse liver. D) Induction of hepatic Slc10a6 and IL-6 mRNA levels in fxr/e− mice fed a 1% CA diet for 5 days. RNA levels were determined by QPCR (n=6-10). Data expressed as mean±SD. *P<0.05 versus saline. #P<0.05 versus LPS/veh. All data was normalized to cyclophilin mRNA levels.
Figure 4. Effects of Slc10a6 substrate DHEAS on LPS-mediated CCL5 mRNA levels in RAW264.7 cells

A) Enhancement of Slc10a6 substrate DHEAS on LPS-mediated CCL5 mRNA levels in RAW264.7 cells. (1μg/ml LPS for 24h; 50 μM DHEAS for 24h). Representative graph from 6 repeated experiments in triplicate with similar results. B) NR ligands 9CisRA and Dex prevented DHEAS mediated enhancement of LPS-induced CCL5 expression. Data expressed as mean ± SD. *P<0.05 versus saline. #P<0.05 versus LPS/veh. ^ P<0.05 versus LPS/DHEAS. All data was normalized to cyclophilin mRNA levels.
Figure 0005
Figure 5. Schematic representation of working model of Slc10a6 regulation by LPS

A) Early effects of LPS increases Slc10a6 mRNA levels in RAW264.7 macrophages through JNK and NFκB dependent pathways, resulting likely in increased protein on the plasma membrane with increased uptake of Slc10a6 substrate DHEAS. B) Late effects of LPS treatment induces CCL5 mRNA levels. DHEAS imported via Slc10a6, which interferes with nuclear translocation of GR to inhibit NFκB, consequently enhancing CCL5 mRNA expression. CCL5 is a chemokine implied to play a role in pathology and progression of multiple hepatic diseases.