Liver X receptor in the cardiovascular system

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LXR-α and the RAAS

Activation of liver X receptor-α reduces activation of the renal and cardiac renin-angiotensin-aldosterone system

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ABSTRACT

Liver X receptor (LXR)-α is a pivotal player in reverse cholesterol metabolism. Recently, LXR-a was implicated as an immediate regulator of renin expression in a cAMP-responsive manner. To determine whether long-term LXR-α activation affects activation of the renal and cardiac renin–angiotensin–aldosterone system (RAAS), we treated mice with T0901317 (T09, a specific synthetic LXR agonist) in combination with the RAAS inducer isoproterenol (ISO). LXR-α-deficient (LXR-$\alpha^{-/-}$) and wildtype (WT) C57Bl/6J mice were treated with ISO, T09 or both for 7 days. Low-dose ISO treatment, not associated with an increase in blood pressure, caused an increase in renal renin mRNA, renin protein and ACE protein in WT mice. WT mice treated with both ISO and T09 had decreased renal renin, ACE and AT_1R mRNA expression compared with mice treated with ISO only. Cardiac ACE mRNA expression was also reduced in the hearts of WT mice treated with ISO and T09 compared with those treated with ISO alone. The transcriptional changes of renin, ACE and AT_1R were mostly absent in mice deficient for LXR-α, suggesting that these effects are importantly conferred through LXR-α. In conclusion, LXR-α activation blunts ISO-induced increases in mRNA expression of renin, AT_1R and ACE in the heart and kidney. These findings suggest a role for LXR-α in RAAS regulation.

INTRODUCTION

Liver X receptors (LXRs) are nuclear hormone receptors that act as transcription factors. As such, LXRs regulate the expression of genes involved in cholesterol and fatty acid metabolism.[1] In the liver, LXRs regulate expression of genes involved in bile acid and cholesterol metabolism, similar to sterol regulatory element binding protein (SREBP)-1c, which stimulates lipogenesis, and many other downstream genes.[2] In the macrophages, gut and other cell types and tissues, LXRs have a crucial role in 'reverse cholesterol transport,[3] thereby stimulating the efflux of cholesterol from the peripheral tissue to the liver. Two different, yet highly homologous isoforms of LXR have been described, namely LXR-α (NR1H3) and LXR-β (NR1H2). Both LXRs heterodimerize with the retinoid X receptor (RXR) and bind to a DR-4 response element in the promoter of target genes.[4] Natural ligands for LXRs are oxysterols, but strong synthetic agonists such as T0901317 (T09) and GW3965 have been developed. Although LXR-b is ubiquitously expressed, LXR-α is expressed mainly in the liver, adipose tissue, macrophages, intestine, spleen, kidney and heart.[5,6]

In the kidney, LXRs are specifically expressed in renin-producing juxtaglomerular (JG) cells.[7] In JG cells, renin transcriptional and translational control is meticulously regulated at multiple levels.[8] The hormone renin is the rate limiting step in the renin–angiotensin–aldosterone system (RAAS), which is the most critical regulator of blood pressure and salt-volume homeostasis in physiological and pathophysiological conditions. LXRs were shown to regulate renin expression *in vivo*, suggesting a cross talk between the RAAS and lipid metabolism.[7] It has been reported that LXRs regulate renin gene expression in a ligand-independent manner by interacting with a specific responsive element in the renin promoter, known as cAMP-negative response element (CNRE).[7] However, it has also been shown that treatment of rats with the synthetic LXR agonist GW3965 interferes with angiotensin II-mediated pressor responses.[9] These two observations suggest that a cross talk between (ligand-dependent) LXR activation and (ligand-independent?) RAAS activation exists, but it remains unknown whether long-term LXR stimulation modulates RAAS activity, and if so, what enzymes/peptides of the RAAS are affected by LXR stimulation. To determine which enzymes/peptides of the RAAS are affected by LXR activation, and whether LXR exerts stimulatory or rather inhibitory effects on the RAAS, we treated LXR-α deficient (LXR-$a^{-/-}$) and wildtype (WT) mice with the cAMP releasing β_1-adrenergic agonist isoproterenol (ISO) in the absence or presence of the synthetic LXR agonist T09.

METHODS

Animals and housing conditions

All experiments were approved by the local Committee on Animal Experimentation and were conducted under international guidelines on animal experimentation. Male C57BL/6J WT mice were obtained from Harlan at the age of 10 weeks. LXR-$\alpha^{-/-}$ mice on C57BL/6J background were generated by Deltagen (Redwood City, CA, USA), as described previously.[10] During the entire experiment, animals were kept on a 12 h light:12 h dark cycle with ad libitum access to food and water.

Experimental procedures

Mice (groups consisted of n=7–9) were fed either standard laboratory chow or chow supplemented with the synthetic LXR agonist T09 (50mg/kg per day, Cayman Chemicals, Ann Arbor, MI, USA). This dose was previously shown to cause a strong, sustained activation of LXRs *in vivo*.[11] ISO (10mg/kg per day) was administered as a continuous infusion through an osmotic pump (Alzet, Maastricht, The Netherlands; Model 1002). This dose was chosen because it causes a strong increase in renal renin expression and plasma renin activity.[7] Pumps were inserted subcutaneously in the left flank under sterile conditions, while the mouse was anesthetized with isoflurane (2% in O2). Control animals and animals treated with only T09 received a saline-filled (NaCl 0.09%, vehicle) pump. After 7 days, body weight (BW) was determined, mice were anesthetized (as described) and a microtip pressure transducer (Millar Instr., Houston, TX, USA) was inserted into the right carotid artery. After a 5-min period of stabilization, heart rate (HR) and arterial systolic blood pressure (SBP)/diastolic blood pressure were recorded. Subsequently, animals were killed by cardiac puncture to aspirate blood. The heart (left ventricle), kidneys and liver were excised. All collected tissues were snap frozen in liquid nitrogen and stored at -80°C for RNA and protein isolation and biochemical analysis.

RNA analysis

All disposable products were purchased from Greiner Bio-One B.V., Alphen a/d Rijn, The Netherlands. Total RNA from cells or tissues was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA synthesis was performed using standard methods. Briefly, first strand cDNA was prepared using random primer mix and subsequently used (50 ng per reaction) as a template for quantitative real-time reverse-transcriptase PCR (qRT-PCR). mRNA levels were expressed in relative units on the basis of a standard curve obtained from serial dilutions of a calibrator cDNA mixture. All PCR results were normalized to β-actin mRNA levels. qRT-PCR primers used for β-actin were as follows: sense CGAGCGTGGCTACAGCTTCA, antisense AGGAAGAGGATGCGGCAGTG; for renin: sense ACCTTCAGTCTCCCAACACG, antisense CTTTGTGAATACGCCCC ATT; for ACE: sense TGAGAAAAGCACGGAGGTATCC, antisense AGAGTTTTGAAAGTTGCTCACATCA; and for AT_1R: sense CCATTGTCCACCCGATGAAG, antisense TGCAGGTGACTTTGGCCAC.

Western blot

All disposable products were purchased from Greiner Bio-One B.V., Alphen a/d Rijn, The Netherlands. Protein was isolated by homogenizing ~100 mg of frozen tissue in 1ml of radioimmunoprecipitation assay buffer (0.5% sodium deoxycholate, 0.1% SDS, 1% Igepal ca-630 in 1xPBS) supplemented with protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA). After centrifugation at 14 000 r.p.m. for 20 min at 4°C, the supernatant was collected and protein concentrations were determined using the DC assay (Bio-Rad, Veenendaal, The Netherlands) with a bovine albumin standard. SDS sample buffer was added to 20 mg of protein and samples were denaturized by heat at 99°C for 5 min. Proteins were separated by SDS-PAGE (Thermo Scientific, Breda, The Netherlands), and transferred to PVDF membranes (Bio-Rad). Blots were incubated overnight in antibodies for renin (1:750, kindly provided by Dr. Tadashi Inagami, Vanderbilt University School of Medicine, Nashville, TN, USA) and ACE (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted in milk. All western blot results were normalized to β-actin protein levels (1:20.000 Sigma-Aldrich, Zwijndrecht, The Netherlands).

Cell culture of Calu-6 cells

All disposable products were purchased from Greiner Bio-One B.V., Alphen a/d Rijn, The Netherlands. The human renin-expressing Calu-6 cell line was obtained from the American Type Culture Collection (ATCC no. HTB- 56) and grown at 37°C in humidified air with 5% CO_2 in Eagle's minimal essential medium (EMEM), supplemented with 2mM L-glutamine, nonessential amino acids and 10% fetal bovine serum (FBS). After overnight starvation (EMEM containing 0.1% FBS), cells were incubated with either solvent (1% DMSO), 8-Br-cAMP (1 mM, Sigma-Aldrich), T09 (3 mM, Cayman Chemicals) or both cAMP and T09 for 6 h.

Electrophoretic mobility shift assays

All disposable products were purchased from Greiner Bio-One B.V., Alphen a/d Rijn, The Netherlands. Electrophoretic mobility shift assays (EMSAs) were performed with ^{32}P-labeled double-stranded oligonucleotide probes (Amersham Biosciences, Roosendaal, The Netherlands). Binding reactions were performed for 30 min at room temperature in Gel Shift Assay Core Systems (Promega, Leiden, The Netherlands), using 10 mg nuclear protein extracts. Nuclear extracts were isolated from human renin expressing Calu-6 cells treated with cAMP, T09 or both (as described) and from WT mice and LXR-a$^{-/-}$ mice. For neutralization studies, samples were coincubated with a 100-fold molar excess of unlabeled probe and anti-LXR-a mouse monoclonal antibody (Perseus Proteomics, Tokyo, Japan) for an additional 15 min. After incubation, samples were separated on a 6% polyacrylamide gel and electrophoresed in 1x trisborate EDTA buffer for 3 h at 180 V. After electrophoresis, the gel was dried and exposed to autoradiography film overnight at -80°C. The sense oligonucleotide sequence is: CTAACTTGGTCTCACAGGCTAGAA (CNRE).

Plasma cholesterol

Plasma cholesterol was determined using a commercial kit (Human Diagnostics Worldwide).

Statistical analysis

Measured values are presented as means±s.e.m., unless stated otherwise. Statistical analysis was performed using an analysis of variance with *post hoc* comparisons (Tukey's test). A *P*-value of <0.05 was considered statistically significant.

Table 1 Animal characteristics and plasma cholesterol concentrations in control

	Wildtype				LXR-α-/-			
Characteristics	**Control**	**ISO**	**T09**	**ISO + T09**	**control**	**ISO**	**T09**	**ISO + T09**
Heart rate (bpm)	481 ± 23	535 ± 41	484 ± 19	446 ± 56	417 ± 25‡	535 ± 23	524 ± 34	511 ± 15
HW/BW	5.77 ± 0.1	6.71 ± 0.1*6	.07 ± 0.1	7.44 ± 0.1*,†	4.02 ± 0.2‡4	.76 ± 0.1*,‡	4.44 ± 0.1‡5	.27 ± 0.1*,†,‡
KW/BW	13.12 ± 0.1	12.68 ± 0.1	13.71 ± 0.1	13.28 ± 0.1†	15.47 ± 0.5‡	14.40 ± 0.3‡	16.31 ± 0.4‡	13.80 ± 0.2*
LW/BW	56.05 ± 0.9	53.85 ± 1.3	84.06 ± 1.0*	74.09 ± 1.6*	48.53 ± 1.6‡	49.14 ± 1.7‡	57.53 ± 2.6*,‡	57.78 ± 1.2*,†,‡
Cholesterol (mg/dl)	81.85 ± 6.0	90.60 ± 4.9	190.33 ± 8.1*	214.32 ± 6.7*,†	91.50 ± 7.0	122.20 ± 15.0	141.55 ± 29.8*	178.37 ± 14.6*,†,‡

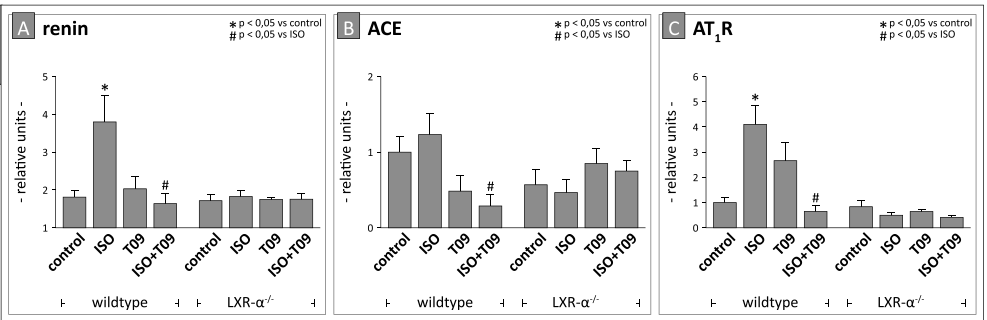
Animal characteristics and plasma cholesterol concentrations in control, ISO, T09 and ISO+T09 treated mice (* p<0.05 vs control, † p<0.05 vs ISO, ‡ p<0.05 vs WT). Bpm: beats per minute; HW/BW: heart weight/body weight; KW/BW: kidney weight/body weight; LW/BW: liver weight/body weight

RESULTS

Body and organ weights, effects of T09

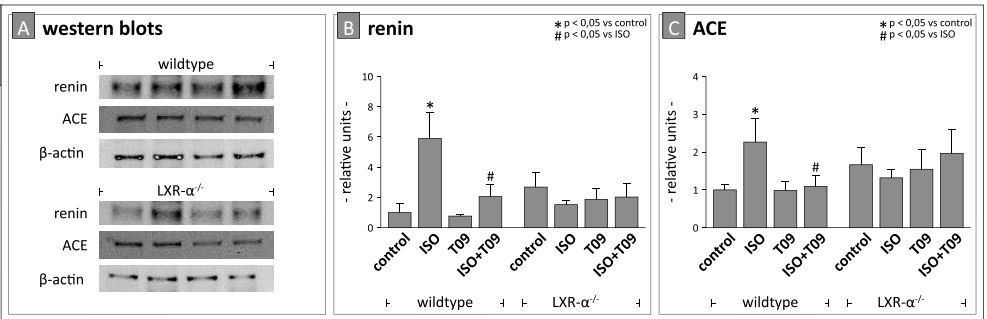
Mice were treated with ISO, T09 or both for 7 days. We chose the dose of ISO, so that it would not increase HR or blood pressure. In WT mice, SBP was 103±4mmHg with saline infusion and 114±11mmHg with ISO infusion. HR was identical between groups. In WT mice, HW/BW ratios were significantly increased in mice treated with ISO or ISO+T09 (table 1). As expected, T09 treatment caused a significant increase in liver weights (LWs) (LW/BW ratios), plasma cholesterol levels and hepatic SREBP-1c mRNA levels (data not shown). These data show that the dosing of T09 indeed resulted in LXR activation. In LXR-$\alpha^{-/-}$ mice, the HW/BW ratio was lower compared with that in WT mice. However, in both genotypes, a significant increase was found in the HW/BW ratio after treatment with either ISO alone or ISO+T09. The LW/BW ratio was also significantly lower in LXR-$\alpha^{-/-}$ mice, but both genotypes showed an increase in LW after T09 (T09 alone or ISO±T09) treatment. As expected, this increase was more pronounced in WT mice than in LXR-$\alpha^{-/-}$ mice (142 and 135%; *P*=0.000 and *P*=0.000, respectively, in WT as opposed to 119 and 119%; *P*=0.010 and *P*=0.006 in LXR-$\alpha^{-/-}$ mice, T09 and ISO+T09, respectively). Increases in cholesterol levels after treatment with either T09 alone or both ISO+T09 were also more pronounced in WT mice than in LXR-$\alpha^{-/-}$ mice. On the other hand, KW/BW ratios were significantly

Figure 1



ISO treatment induced increased renal expression of renin (A) and AT1R (C) mRNA in WT mice, but not in LXR-α-/- mice. Co-treatment with T09 blunted the ISO-induced increase of renin, ACE (B) and AT1R mRNA expression in WT mice. Relative gene expression was measured by real-time qRT-PCR. Expression values were normalized to β-actin levels.

Figure 2



(A) Shows western blots of renal renin and ACE protein, with β-actin as control. Relative protein expression was calculated by densitometric analysis of bands from all blots and expression values were normalized to β-actin levels. ISO treatment induced increased renal expression of renin (B) and ACE (C) protein in WT mice, but not in LXR-$\alpha^{-/-}$ mice. Co-treatment with T09 blunted the ISO-induced increase of renin and ACE protein expression in WT mice.

higher in LXR-$\alpha^{-/-}$ mice than in their WT littermates, but not in ISO+T09-treated animals.

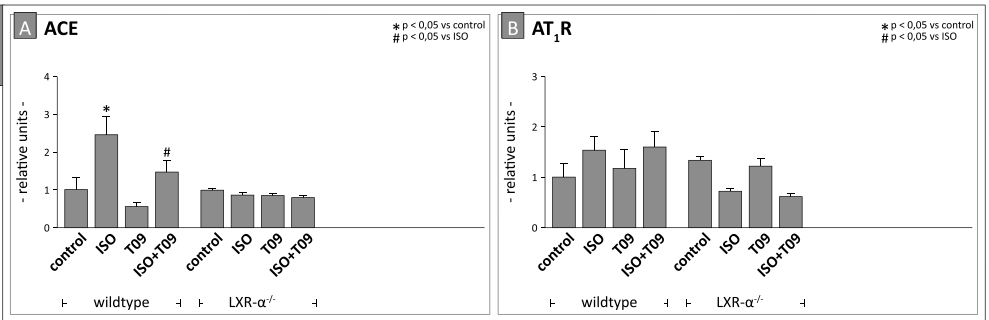
T09 decreases renal renin, ACE and AT_1R mRNA in WT mice, but not in LXR-$\alpha^{-/-}$ mice

Renal mRNA was isolated and gene expression of renin, ACE and the AT_1R was assessed by qRT-PCR. Renal renin mRNA expression was increased in WT mice treated with ISO compared with saline-infused controls (control: 0.74±0.17 *vs* ISO: 3.36±0.97, *P*=0.018; figure 1A). Cotreatment with both ISO and T09 showed that the ISO-induced increase in renin gene expression could be completely abolished by T09 treatment (0.59±0.28, *P*=0.012 *vs* ISO), whereas treatment with T09 alone did not affect renin mRNA expression (0.96±0.30, *P*=0.993 *vs* control). Interestingly, in LXR-$\alpha^{-/-}$ mice, no effect of ISO treatment was found on renal renin expression. Renin mRNA levels were significantly lower in ISO-treated LXR-$\alpha^{-/-}$ mice than in ISO-treated WT mice. Similar findings were seen in renal ACE and AT_1R mRNA and expression (see figure 1B and C). In WT mice, ISO+T09 treatment resulted in lower ACE and AT_1R mRNA expression than did ISO treatment. Again, this was not observed in LXR-$\alpha^{-/-}$ ice. In fact, ACE mRNA levels were higher in LXR-$\alpha^{-/-}$ mice treated with ISO+T09 than in WT mice treated with ISO+T09 (figure 1B). AT_1R mRNA levels were lower in LXR-$\alpha^{-/-}$ mice treated with ISO or T09 compared with the expression in WT mice after the same treatment (figure 1C). The transcriptional changes were paralleled by changes in protein expression of renal renin and ACE, which also remained unaffected by ISO treatment in LXR-$\alpha^{-/-}$ mice, whereas in WT mice, ISO treatment significantly increased both renal renin and ACE protein levels (figure 2). Again, in WT mice, ISO induced renal protein expression of renin and ACE was blunted by cotreatment with T09.

T09 decreases cardiac ACE mRNA in WT mice, but not in LXR-$\alpha^{-/-}$ mice

In WT mice, cardiac ACE mRNA levels were significantly lower in ISO+T09-treated animals compared with animals treated with ISO alone (figure 3A). In LXR-$\alpha^{-/-}$ mice, treatment with ISO and/or T09 did not affect ACE mRNA expression and ACE mRNA expression was lower in ISO-treated LXR-$\alpha^{-/-}$ mice than in WT mice. Cardiac AT_1R mRNA expression was not altered in WT mice after ISO and/or T09 treatment, but again, AT_1R mRNA expression was lower in ISO- and ISO+T09-treated LXR-$\alpha^{-/-}$ mice compared with WT mice treated with ISO or ISO+T09 (figure 3B).

Figure 3



Cardiac ACE mRNA (A) was significantly lower in hearts of mice treated with ISO+T09 compared to hearts of mice treated with ISO alone in WT mice, but no effect was found in LXR-$\alpha^{-/-}$ mice. ISO, T09 or ISO+T09 treatment showed no effect on cardiac AT1R mRNA levels (B). Relative gene expression was measured by real-time qRT-PCR. Expression values were normalized to β-actin levels.

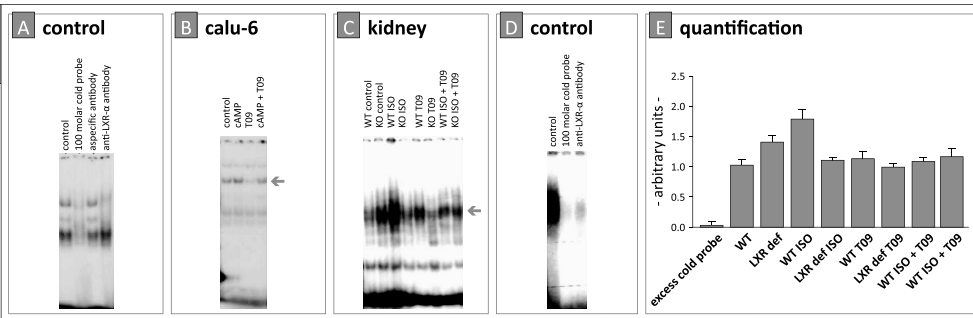
T09 blunts affinity of LXR to CNRE

To corroborate our observations that LXR-α is responsible for the ISO-induced activation of the RAAS, we performed an EMSA to assess the affinity of LXR-α to the CNRE (a responsive element in the renin promoter that was previously shown to bind LXR-α[12]). We used (1) nuclear protein extractions of human renin-expressing Calu-6 cells, treated with cAMP, T09 or both and (2) renal nuclear protein extracts of all experimental groups to compare WT with LXR-$\alpha^{-/-}$ mice. figure 4A shows a specific shift, indicative of LXR-a binding to the CNRE. This shift is largely abolished by coincubation with excess unlabeled CNRE (100M cold probe, lane 2) or by a specific anti-LXR-α antibody (anti-LXR-α antibody, lane 4) but not by an aspecific antibody (IgG, lane 3). In figure 4B, it is shown that affinity of LXR to CNRE was increased when Calu-6 cells were treated with cAMP (lane 2). On the other hand, T09 caused a decrease in binding affinity to CNRE (lane 3), whereas coincubation with both cAMP and T09 restored the shift to control values (figure 4B, lane 4). In figure 4C, EMSA results from renal nuclear lysates are shown. In WT mice, an increase in LXR-α-CNRE affinity was found under ISO treatment (lane 3 compared with lane 1); it was observed that this increase was blunted after additional treatment with T09 (lane 7). However, in LXR-$\alpha^{-/-}$ mice, no differences were found between the experimental groups (lanes 2, 4, 6 and 8). LXR-α-CNRE affinity was found to be more pronounced in WT mice as compared with LXR-$\alpha^{-/-}$ mice. In figure 4D, it is shown that the shift in renal lysates is abolished with excess unlabeled probe and neutralizing antibody. figure 4E shows morphometric analysis of the results in figure 4C.

DISCUSSION

We report that activation of LXR with the specific agonist T09 leads to a decrease in renal and cardiac RAAS activation due to ISO infusion. The LXR-α agonist T09 blunts ISO induced increases in renin, ACE and AT1R expression *in vitro* and *in vivo*, and competes with the binding of LXR-α to the CNRE in the renin promoter. The observed effects are completely absent in LXR-$\alpha^{-/-}$ mice, suggesting LXR-α dependency. On the basis of these data, we postulate that LXR agonists may serve as inhibitors of the RAAS.

Figure 4



EMSA of LXR-a binding to a 32P-labeled CNRE probe. Anti-LXR-α monoclonal antibody abolished the complexes formed by LXR-a with CNRE (A). Calu-6 cells were treated with cAMP, T09 or both. cAMP caused an increased affinity of LXR-α to the CNRE probe, whereas T09 blunted CNRE affinity of LXR-α cAMP induced increased CNRE-LXR-α affinity is blunted to control levels by cotreatment with cAMP and T09 (B). In WT animals, ISO treatment causes an increased affinity of LXR-α to the CNRE probe. This affinity is diminished to control levels in mice treated with both ISO+T09. In LXR-$\alpha^{-/-}$ mice, no effect of ISO, T09 or cotreatment with ISO+T09 was seen (C). Arrows indicate height of LXR-α-CNRE complex. The shift in renal lysates is abolished by coincubation with excess (100 molar) unlabeled probe (CNRE) or neutralising anti-LXR-α antibody (D). Densitometric analysis of (C) is displayed in (E).

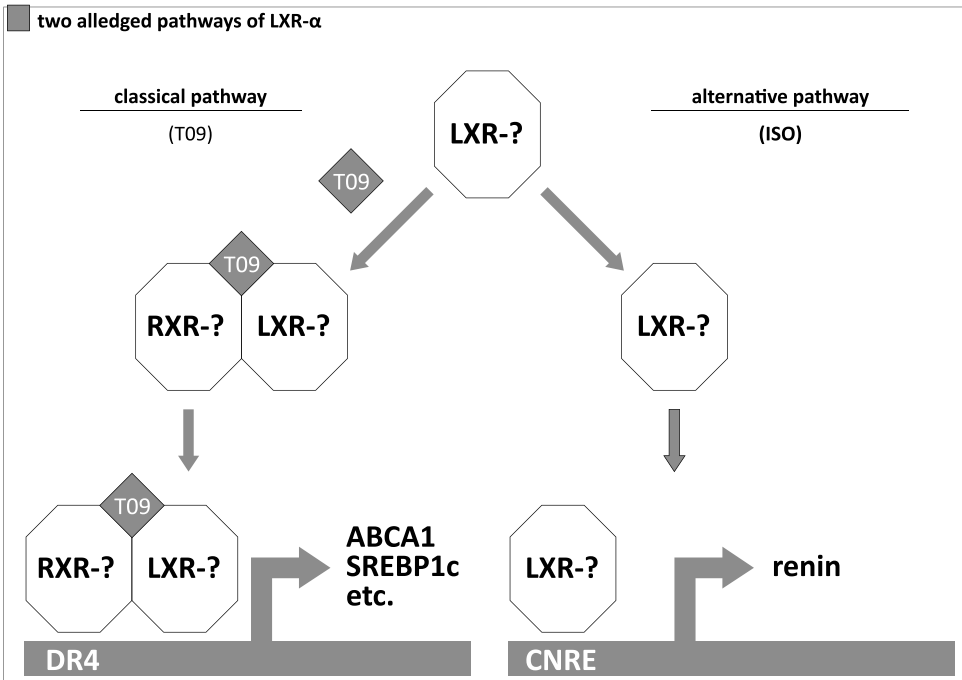
We recently discussed the potential profound modulatory effects of nuclear hormone receptors on the RAAS.[13] We now extent this hypothesis by showing that LXR activation inhibits transcription of RAAS components in the kidney and the heart. The ISO-induced activation of the RAAS was observed at a transcriptional level by the increase in renal renin and ACE mRNA. Activation of LXR by T09 treatment resulted in decreased transcriptional levels of renin, ACE and AT_1R mRNA. Recently, LXR-α was denoted as a cAMP-dependent regulator of renin.[12] Morello *et al.*[7] showed that LXR-α regulates renin transcription *in vivo*. LXR-$\alpha^{-/-}$ mice (and LXR-α/β double knock-out mice) were unable to regulate renin transcription upon ISO stimulation in contrast to LXR-$\beta^{-/-}$ mice. This renders LXR-α as a cAMP-dependent regulator of renin, whereas LXR-β seems to act more as a constitutive regulator of renin. This study portends that long-term treatment with T09 rather inhibits renin expression, and expression of other enzymes/proteins of the RAAS in the heart and kidneys. Our finding that T09 competes with the binding of LXR-α to the CNRE provides evidence for a transcriptional regulation. The transcriptional regulation of ACE is largely unknown, whereas regulation of AT_1R is tight, complex and well described.[14] Our findings of decreased renal AT_1R mRNA expression are in line with those of Imayama *et al.*,[15] who demonstrated LXR to be a negative regulator of AT_1R in cultured vascular smooth muscle cells. On the other hand, Leik *et al.* showed that LXR activation by GW3965 leads to a transient increase in vascular gene expression of the AT_1R, which decreased over time (measured over an 8-h period). These findings may resemble the reported initial increase in renin transcription single dose of T09 (reported by Morello *et al.*[7]) and the decrease in renin gene expression that we found after a long-term period of LXR activation. Therefore, for both AT_1R and renin transcription, it seems that LXR activation results in a transient increase, followed by gradual and sustained decrease over time at the transcriptional and translational levels.

If long-term activation of LXR results in decreased transcription levels of RAAS components, it is striking that LXR-$\alpha^{-/-}$ mice do not show increased mRNA levels of these RAAS components, neither on a basal level nor after ISO treatment. To explain this apparent discrepancy, in this study, we propose a model of a 'classical' and an 'alternative' LXR-α pathway (figure 5). The classical pathway is entered upon binding of a specific ligand (oxysterols, T09), subsequent heterodimerization with RXR and binding of a DR4 element, initiating transcription of genes, such as SREBP-1c and ATP-binding-cassette (ABC) transporters. In the alternative pathway, LXR-α is activated in a different manner, in this study with cAMP, which may lead to phosphorylation. However, we do not know what specific mechanism is operative herein. The long-term treatment with T09 may 'push' all LXR-α toward the classical pathway, leaving no LXR-α to enter the alternative route. In support of this observation, we observed that with all of our perturbations (ISO, T09, combination), the transcript levels of LXR-α remain stable (data not shown).

This study is the first to report the regulatory role of LXR on long-term RAAS gene and protein expression. T09 treatment led to sustained suppression of RAAS genes, leading to decreased protein production of both renin and ACE, as compared with protein levels after ISO treatment alone. Although we found effects of LXR activation on both mRNA and protein levels, these changes in RAAS activation were not translated into significant changes in blood pressure. As we initially intended to study the role of LXR on a transcriptional level, we chose a dose of the $\beta_1$adrenergic agonist ISO that does acti-

vate renin transcription, yet reportedly does not cause hypertension. As we were in the normotensive range, this potentially explains why we did not observe any effects of LXR treatment on blood pressure. In experiments conducted by Leik *et al.,*[9] changes in blood pressure were only found in animals treated with doses of angiotensin II known to cause hypertension. It remains to be proven whether LXR activation has a role in blood pressure regulation, but given the observed effects on (renin, ACE, AT1R) gene expression, we hypothesize LXR may have a role in this. We show that LXR activation leads to decreased RAAS activation, suggesting a cross talk between the classical and an alternative pathway of LXR signaling. Activation of LXR leads to marked and sustained inhibition of ISO induced RAAS activity in the heart and kidney. On the basis of our observations, we hypothesize that LXR activation might provide a novel strategy in the treatment approach for several disorders, including metabolic disorders and cardiovascular disease.

Figure 5



On the left, the "classical" pathway is depicted, in which LXR-α is activated by a specific ligand (T09, but also GW3965 and oxysterols). The complex that is formed in this pathway (LXR-α, RXR-α, and ligand) binds to a DR4 element, initiating transcription of genes such as SREBP-1c and ATP-binding-cassette (ABC) transporters. On the right, the "alternative" LXR-α pathway is shown, in which LXR-α is activated in a different manner, e.g. cAMP which may lead to phosphorylation. From our data, we postulate that if classically activated, all LXR-α may be diverted into the classical route and no longer be available to bind to the renin promoter.

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