JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

Title Page

Pregnane X receptor activation triggers rapid ATP release in primed macrophages that mediates NLRP3 inflammasome activation

Grace Hudson[1], Kyle L. Flannigan[1], Vivek Krishna Pulakazhi Venu [1], Laurie Alston[1], Christina F. Sandall[2], Justin A. MacDonald[2], Daniel A. Muruve[3], Thomas K. H. Chang[4], Sridhar Mani[5] & Simon A. Hirota[1,6]*

1 – Dept. of Physiology & Pharmacology, University of Calgary, Calgary, AB, Canada
2 – Dept. of Biochemistry & Molecular Biology, University of Calgary, Calgary, AB, Canada
3 – Dept. of Medicine, University of Calgary, Calgary, AB, Canada
4 – Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada
5 – Dept. of Medicine, Albert Einstein College of Medicine, Bronx, NY, USA
6 – Dept. of Immunology, Microbiology & Infectious Diseases, University of Calgary, Calgary, AB, Canada

For individual funding support see Funding Footnote (pg. 26)

Running Title Page

Running Title: The PXR modulates NLRP3 inflammasome activation

* Address correspondence to: Dr. Simon A. Hirota
University of Calgary
3330 Hospital Dr. NW
Health Sciences Room 1845
Calgary, Alberta, Canada
T2N4N1
1-403-210-7888
shirota@ucalgary.ca

Number of pages:	36
Number of tables:	0
Number of figures:	7 main, 1 supplemental
Number of references:	60
Number of words in Abstract:	167
Number of words in Introduction:	747
Number of words in Discussion:	1133

Non-standard abbreviations:

DAMPS	danger-associated molecular patterns
DPI	diphenyleneiodonium chloride
NLRP3	Nucleotide-Binding Oligomerization Domain, Leucine Rich Repeat and Pyrin Domain Containing 3
NOD1	Nucleotide-Binding Oligomerization Domain, Leucine Rich Repeat and CARD Domain Containing 1
NR	nuclear receptor
oATP	oxidized ATP
PAMPs	pathogen-molecular patterns
PCN	pregnenolone 16α-carbonitrile
PMA	phorbol 12-myristate 13-acetate
PXR	pregnane X receptor
ROS	reactive oxygen species
RXR	retinoid X receptor

Section recommendation: Inflammation, Immunopharmacology, and Asthma

Abstract

The pregnane X receptor (PXR) is a ligand-activated nuclear receptor that acts as a xenobiotic sensor, responding to compounds of foreign origin, including pharmaceutical compounds, environmental contaminants and natural products, to induce transcriptional events that regulate drug detoxification and efflux pathways. As such, the PXR is thought to play a key role in the protecting the host from xenobiotic exposure. More recently, the PXR has been reported to regulate the expression of innate immune receptors in the intestine and modulate inflammasome activation in the vasculature. In the current study, we report that activation of the PXR in primed macrophages triggers caspase-1 activation and IL-1β release. Mechanistically, we show that this response is NLRP3-dependent and is driven by the rapid efflux of ATP and P2X7 activation following PXR stimulation, an event that involves pannexin-1 gating, and is sensitive to inhibition of Src-family-kinases. Our findings identify a mechanism whereby the PXR drives innate immune signaling, providing a potential link between xenobiotic exposure and the induction of innate inflammatory responses.

Introduction

The pregnane X receptor (PXR) is a xenobiotic sensor that plays a key role in drug metabolism by regulating the expression of genes that encode enzymes responsible for drug detoxification and efflux (Koutsounas et al., 2013). As a member of the nuclear receptor (NR) superfamily, the PXR acts as a ligand-activated transcription factor, regulating gene expression in concert with its heterodimeric binding partner, the retinoid X receptor (RXR). In contrast to other NRs, the PXR's ligand-binding domain exhibits a large flexible pocket that accommodates the binding of a variety of structurally unique ligands, including rifamycin antibiotics, pharmaceutical compounds, natural compounds and contaminants of environmental origin (e.g. bisphenol A, organochloride pesticides) (Kliewer et al., 2002; Chang and Waxman, 2006; Staudinger et al., 2006; Gupta et al., 2008; Chang, 2009; Shukla et al., 2011).

The PXR is highly expressed in the liver and regions of the small and large intestine, and its role in regulating the host's response to exogenous chemicals at these sites has been well characterized(Koutsounas et al., 2013) given their exposure to high concentration of exogenous ligands and xenobiotics. In addition, the PXR has been shown to regulate tissue inflammation through a reciprocal interaction with nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Shah et al., 2007; Mencarelli et al., 2010; Mencarelli et al., 2011). Indeed, we, and others, have reported that PXR agonists inhibit the release of inflammatory mediators from hepatocytes (Hu et al., 2010; Sun et al., 2015) and intestinal epithelial cells (Shah et al., 2007; Mencarelli et al., 2010; Mencarelli et al., 2011) by inhibiting NF-κB-dependent signaling events, and can afford protection in experimental models of hepatic (Wang et al., 2010) and intestinal inflammation (Ma et al., 2007; Shah et al., 2007; Dou et al., 2012; Dou et al., 2013; Terc et al., 2014; Garg et al., 2016). The PXR can also regulate the expression/function of innate immune pattern recognition receptors within the intestinal

JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

epithelium, an effect that contributes to the proper maintenance of intestinal mucosal homeostasis and barrier function (Venkatesh et al., 2014).

Beyond the gastrointestinal tract, the PXR may contribute to the regulation of inflammation in other cell types (Schote et al., 2007; Zhou et al., 2009; Dubrac et al., 2010; Casey and Blumberg, 2012; Beyer et al., 2013). In the context of innate immunity, in contrast to its reported anti-inflammatory effects, Wang *et al.* found that stimulation of the PXR in cultured vascular endothelial cells enhanced the expression of a number of innate immune receptors, including Toll-like receptors (TLR) 2, 4, & 9, as well as NOD-like receptor family members Nucleotide-Binding Oligomerization Domain, Leucine Rich Repeat and CARD Domain Containing 1 (NOD1) and Nucleotide-Binding Oligomerization Domain, Leucine Rich Repeat and Pyrin Domain Containing 3 (NLRP3) (Wang et al., 2014a). Furthermore, endothelial cells treated with PXR agonists displayed features of NLRP3 inflammasome activation (Wang et al., 2014a).

The NLRP3 inflammasome is an innate immune signaling complex that mediates the responses to a variety of pathogen- (PAMPs) and endogenous danger-associated molecular patterns (DAMPS) (Mangan et al., 2018). The NLRP3 inflammasome's activation following PAMP exposure and the induction of the ensuing innate immune response play key roles in host-defense against viral, bacterial and fungal pathogens (Mangan et al., 2018) while the recognition of DAMPs and subsequent activation of the NLRP3 inflammasome plays an important role in the initiation of sterile inflammatory responses following tissue damage. Interestingly, several studies have reported the independent contributions of the PXR (Wang et al., 2014b) and NLRP3 inflammasome activation (Imaeda et al., 2009) in the pathogenesis of acute liver injury caused by sterile inflammation. In this scenario, the interplay between direct hepatocyte damage, DAMP release and activation of resident macrophages, is thought to contribute to the sterile inflammatory response that propagates acute liver damage

(Hoque et al., 2012). While the response of the hepatocyte has been the focus of much research, the role of PXR signaling and NLRP3 inflammasome within the macrophage, as a driver of inflammation, has not been addressed.

In the current study, we sought to test the hypothesis that the PXR plays a critical role in the macrophage by modulating NLRP3 inflammasome activation. Herein, we report that exposing primed human or mouse macrophages to their respective PXR agonists triggers caspase-1 activation and IL-1β secretion through an NLRP3-dependent mechanism. PXR-induced NLRP3 inflammasome activation was abolished by apyrase and selective inhibition of the P2X7 receptor. Lastly, PXR ligands triggered a rapid and significant release of ATP, an effect that is dependent on pannexin-1 and SRC kinase activation.

Materials & Methods

Reagents

PXR agonists - For experiments in mouse macrophages, the rodent selective PXR agonist pregnenolone 16α-carbonitrile (PCN), (Sigma Aldrich Canada, Oakville, Ontario) was prepared as described above. For experiments in human macrophages, the human selective PXR agonists rifaximin and SR12813 (Sigma Aldrich Canada, Oakville, Ontario) were dissolved in sterile DMSO and added to culture media to attain the final experimental concentrations. As a vehicle control, identical volumes of DMSO were added and did not exceed a concentration of 1% v/v in media. The addition of exogenous ATP (5 mM; Sigma Aldrich Canada, Oakville, Ontario) was used as a positive control in all experiments assessing inflammasome activation, as we have done previously (Ng et al., 2010; Hirota et al., 2011).

Inhibitors – To assess the mechanism(s) involved in PXR agonist-induced NLRP3 inflammasome activation we used the following compounds: to target reactive oxygen species (ROS) we employed the broad-spectrum anti-oxidant diphenyleneiodonium chloride (DPI) (100 μM (Jabaut et al., 2013); Sigma Aldrich Canada, Oakville, Ontario); to chelate intracellular Ca^{2+} and target its effect in NLRP3 inflammasome activation, cells were pretreated with BAPTA-AM (10 μM (Ainscough et al., 2015); Sigma Aldrich Canada, Oakville, Ontario); to assess the role of extracellular ATP and P2X7 receptor activation, we used apyrase (to break down extracellular ATP; 30 units/mL (Iyer et al., 2009); Sigma Aldrich Canada, Oakville, Ontario) or treated cells with oxidized ATP (to selectively inhibit the P2X7 receptor (oATP; 100 μM (Grahames et al., 1999); Sigma Aldrich Canada, Oakville, Ontario). To assess the role of pannexin-1 in PXR-related ATP release, we used the panx-1 mimetic inhibitory peptide ^{10}Panx (400 μM (Grahames et al., 1999); Tocris/Cedarlane, Burlington, Ontario) along with a

scrambled peptide control ^{Sc}Panx (400 μM (Grahames et al., 1999); Tocris/Cedarlane, Burlington, Ontario). To assess the role of Src-family kinases in the induction of ATP release, we used PP2 (10 μM (Chang et al., 2012); Sigma Aldrich Canada, Oakville, Ontario).

Cell culture

Human studies – the human acute monocytic leukemia cell line (THP-1) (ATCC/Cedarlane, Burlington, Ontario) was propagated in RPMI 1640 medium supplemented with 10% FBS and 50 μM 2-mercaptoethanol. To differentiate THP-1 cells into macrophages for inflammasome activation experiments, cells were plated at $5x10^5$ cells/well in a 24-well plate in complete RPMI media and differentiated with phorbol-12-myris- tate-13-acetate (PMA; 50ng/ml; Invivogen/Cedarlane, Burlington, Ontario) for 24 hours, as we have done previously (Ng et al., 2010; Hirota et al., 2011). NLRP3$^{-/-}$ THP-1 cells were generated and propagated as described previously (Lau et al., 2018).

Mouse studies - Peritoneal macrophages were isolated from *Nlrp3-/-* and PXR-/- mice, and their littermate wild-type counterparts (male, 8-10 weeks of age; all bred in house), 48 hours after receiving an intraperitoneal injection of 4% thioglycollate-injected mice (BD Biosciences, San Jose, California), as we have done previously (Ng et al., 2010; Hirota et al., 2011). Isolated macrophages were plated in complete RPMI media at 5 $x10^5$ cells/well of a 24-well plate overnight and stimulated with 100 ng/mL of ultra-pure lipopolysaccharide (LPS; Invivogen, Burlington, ON, Canada) in serum-free Opti-MEM for 30 minutes before challenge. For experiments with knockout mice, littermates were used as the wild-type control group for all experiments to control for potential microbiota-dependent differences in phenotype. All studies were approved by the University of Calgary's Health

Sciences Animal Care Committee (protocol #AC15-0181). All approved activities conform to the guidelines and regulation for laboratory animal use set forth by the Canadian Council for Animal Care.

Assessing inflammasome activation

Western blots - Prior to performing inflammation activation experiments, isolated macrophages were pulsed with ultra-pure lipopolysaccharide (LPS; 100 ng/mL for 30 minutes; Invivogen/Cedarlane, Burlington, Ontario). PMA-differentiated or LPS-pulsed mouse peritoneal macrophages where treated with their respective PXR agonists. Following the designated treatment period, culture supernatants were collected, cells washed with ice-cold PBS, and cell lysates isolated following incubating the cells with lysis buffer (150 mM NaCl, 20 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, and protease inhibitor cocktail; phosphatase inhibitor cocktail - Complete Minitab; Complete PhoStop, Roche/ Sigma Aldrich Canada, Oakville, Ontario). Total protein was quantified using the Precision Red Advanced Protein Assay (Cytoskeleton/Cedarlane, Burlington, Ontario) and sample protein concentration equalized. Culture supernatant and cell lysate samples were resolved and transferred to polyvinylidene difluoride membranes (0.2 µm pores; Bio-Rad Laboratories; Mississauga, Ontario) and blotted with the following antibodies: anti-caspase-1 (Santa Cruz Biotechnology Inc., Dallas, Texas, USA, #sc-622, #sc-56036 and #sc392736). Densitometry was performed using ImageJ, and cleaved caspase-1 expressed as a percentage of pro-caspase-1.

IL-1β secretion - To quantify IL-1β release from PMA-differentiated or LPS-pulsed mouse peritoneal macrophages treated with their respective PXR agonists; culture supernatants were subject to enzyme-linked immunosorbent assay (ELISA; human - #DY201; mouse - #DY401; R&D Systems/ Cedarlane, Burlington, Ontario).

JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

Assessing ATP release

To characterize the mechanism by which PXR agonist triggered inflammasome activation, in some experiments, PMA-differentiated or LPS-pulsed mouse peritoneal macrophages where treated with their respective PXR agonists, culture supernatants collected, and ATP quantified using CellTiter-Glo Luminescent Cell Viability Assay (Promega North America, Madison, Wisconsin), as per the manufacturer's instructions and described previously (Mortimer et al., 2015).

Statistical Analysis

All data were assessed for distribution using D'Agostino-Pearson normality test prior to statistical analysis using GraphPad Prism. Multiple comparisons of parametric data were accomplished using an ANOVA followed by Tukey's post-hoc test. For non-parametric data, or experiments with small samples sizes ($N < 5$), a Kruskal-Wallis test was used, followed by a Mann-Whitney test with a Bonferroni correction for multiple comparisons. In all experiments, N denotes individual experiments performed in different cell passages or cells derived from unique animals.

Results

PXR agonists trigger caspase-1 activation and IL-1β secretion from macrophages in a NLRP3-dependent manner

Recent reports suggest that the PXR can regulate NLRP3 inflammasome activity in cultured vascular endothelial cells (Wang et al., 2014a; Wang et al., 2017), but this has not been assessed in macrophages, the prototypical model for innate immune signaling. To test the hypothesis that stimulation of the PXR triggers NLRP3 inflammasome activation in primed macrophages, we first treated LPS-primed peritoneal macrophages or PMA-differentiated THP-1 cells with their respective species-specific PXR ligands at concentrations previously reported to elicit selective responses in other cell types (Garg et al., 2016). In primed mouse macrophages, treatment with the rodent specific PXR agonist pregnenolone 16α-carbonitrile (PCN; for 6 hrs) was able to trigger the release of IL-1β (Fig. 1 A). In human macrophages, stimulation with two structurally unique selective human PXR agonists (SR12813 or rifaximin; for 6 hrs) also triggered significant IL-1β secretion (Fig. 1B&C).

To test if IL-1β secretion in response to PXR activation in mouse and human macrophages was triggered by NLRP3 inflammasome activation, we next examined the ability of PXR agonists to trigger caspase-1 activation and IL-1β secretion in cells lacking NLRP3. When treated with PCN (1, 10 and 100 μM; for 6 hrs), LPS-primed peritoneal macrophages isolated from *Nlrp3-/-* mice failed to trigger caspase-1 activation and IL-1β secretion (Fig. 2A-C). The human selective PXR agonists (SR12813 – 4 μM; rifaximin; 5 and 10 μM; for 6 hrs) also failed to activate caspase-1 and the subsequent secretion of IL-1β In PMA-differentiated THP-1 cells lacking NLRP3 (Fig. 2D-F).

Since antagonists of the human and mouse PXR are lacking specificity and exhibit off-target effects, we used macrophages cells isolated from PXR-/- mice (Fig. 3A) to validate the role of this receptor in the observed NLRP3 inflammasome responses. In support of our hypothesis, LPS-primed

peritoneal macrophages isolated from PXR-/- mice did not exhibit caspase-1 activation nor secrete IL-1β in response to the selective mouse PXR agonist PCN (10 & 100 μM; Fig. 3B-D), highlighting the role of the PXR in the activation of the inflammasome within macrophages.

PXR-mediated NLRP3 inflammasome activation does not involve ROS or intracellular Ca^{2+}

There are a variety of mechanisms that contribute to NLRP3 inflammasome activation, including, but not limited to, the generation of ROS, mobilization of intracellular Ca^{2+}, and ATP-dependent K^+ efflux via activation of the P2X7 receptor. In a human colon cancer cell line, the expression of the PXR enhanced sensitivity to oxidative stress that was associated with increased agonist-induced ROS production (Gong et al., 2006). To assess the role of ROS in the activation of the NLRP3 inflammasome by the PXR in macrophages, we used the broad-spectrum anti-oxidant DPI treatment (100 μM (Jabaut et al., 2013)). In PMA-differentiated THP-1 cells, DPI pretreatment had no effect on SR12813- or rifaximin-induced IL-1β secretion (Supplemental Fig. 1A), suggesting the ROS production does not mediate the PXR-induced inflammasome activation within macrophages.

Activation of the vitamin D receptor (VDR), a nuclear receptor closely related to the PXR, has been linked with increasing intracellular Ca^{2+} concentrations via an IP_3-dependent process (Tien et al., 1993) in epithelial cells, as well as activation of the NLRP3 inflammasome in macrophages (Tulk et al., 2015). Furthermore, Ainscough *et al.* reported that intracellular Ca^{2+} and its interaction with calmodulin were required for nigericin-induced IL-1β secretion in macrophages (Ainscough et al., 2015). To test whether intracellular Ca^{2+} plays a role in NLRP3 inflammasome activation mediated by the PXR, macrophages were pretreated with BAPTA-AM (10 μM), a cell membrane permeable Ca^{2+} chelating agent. As reported previously, BAPTA-AM significantly reduced ATP-induced IL-1β secretion (Brough et al., 2003), but had no effect on the activation of the inflammasome by PXR agonists

JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

SR12813 and rifaximin (Supplemental Fig. 1B), suggesting mobilization of intracellular Ca^{2+} is not playing a role in PXR-mediated NLRP3 inflammasome activation.

Activation of the PXR triggers ATP release which mediates NLRP3 inflammasome activation

Reports have characterized the activation of the NLRP3 inflammasome through the autocrine/paracrine signaling of ATP released into the extracellular environment via pannexin-1 (Hung et al., 2013; Mortimer et al., 2015). While there is no reported role for the PXR inducing the release of ATP, bile acids, agents known to activate a variety of nuclear receptors, including the PXR, have been shown to induce ATP release in liver cells (Nathanson et al., 2001). Furthermore, activation of the farnesoid X receptor (FXR), a nuclear receptor closely related to the PXR, by bile acids induces rapid release of ATP in pancreatic cell lines (Kowal et al., 2015). Taken together, we next sought to test the hypothesis that PXR-mediated NLRP3 inflammasome activation could be driven by the release of ATP and subsequent activation of P2X7. First, to identify a role for ATP in our system, we co-treated PMA-differentiated THP-1 cells with apyrase (30 units/mL) to breakdown extracellular ATP (Iyer et al., 2009). As in our previous experiments, SR12813 and rifaximin triggered IL-1β secretion, but this response was abolished in macrophages co-treated with apyrase (Fig. 4A). As extracellular ATP is known to activate the NLRP3 inflammasome by triggering the K^+ efflux through the P2X7 receptor [33,39], we next treated macrophages with oxidized ATP (oATP; 100 μM), a selective P2X7 receptor antagonist (Grahames et al., 1999). As with apyrase co-treatment, oATP significantly attenuated IL-1β secretion in response to PXR agonists (Fig. 4B). Importantly, neither apyrase or oATP had any effect on cell viability over the course of our experiments (Supplemental Fig. 2). Taken together, these data suggest that the PXR-mediated activation of the NLRP3 inflammasome involves the release of ATP and its subsequent activation of the P2X7 receptor.

PXR stimulation triggers the rapid release of ATP from macrophages via pannexin-1

To further strengthen the link between the PXR, ATP release and NLRP3 inflammasome activation, we directly quantified ATP release from mouse peritoneal macrophages (WT vs. PXR-/-) and PMA-differentiated THP-1 cells at different time-points. In macrophages isolated from WT mice, the rodent specific PXR agonist PCN triggered rapid and significant extracellular ATP release that could be detected within 15 seconds and was sustained for up to 60 seconds before tapering off after 300s (5mins). ATP release following PCN treatment was completely absent in cells isolated from PXR-/- mice (Fig. 5A). Similar ATP release responses were observed in THP-1 cells treated with either SR12813 or rifaximin (Fig. 5B). Collectively, these data indicate that the activation of the PXR triggers a rapid release of ATP that fits the kinetics reported by other NLRP3 inflammasome activators (Mortimer et al., 2015).

The transmembrane channel pannexin-1 has been implicated in ATP release and NLRP3 inflammasome activation in macrophages [33,39], and thus we sought to determine whether this mechanism was at play following PXR stimulation in our studies. First, to assess the role of pannexin-1 in PXR-driven ATP release, PMA-differentiated THP-1 cells were exposed to pannexin-1-blocking peptide (^{10}Panx; 400 μM (Grahames et al., 1999)) or a scrambled peptide control (^{Sc}Panx; 400 μM) prior to stimulation with SR12813 or rifaximin. PXR activation triggered rapid and significant ATP release in the scrambled peptide-treated cells (Fig. 6A), a response that was significantly attenuated by pannexin-1 channel blockade with ^{10}Panx (Fig. 6B), without affecting cell viability (Supplemental Fig. 2).

While non-genomic roles for other NRs has been described, little is known about how the PXR regulates intracellular signaling processes within the cytosol. Others have reported the involvement of Src-family-kinases (SFK) in the cytosolic effects of NRs (Vertino et al., 2005; Buitrago and Boland,

2010; Peng et al., 2012; Buitrago et al., 2013). Interestingly, receptor-mediated gating of pannexin-1 has been shown to involve SFK-dependent phosphorylation events (Lohman et al., 2015; Weilinger et al., 2016). To assess the role of SFKs in PXR-driven ATP release and NLRP3 inflammasome activation, we pretreated PMA-differentiated THP-1 cells with the Src-kinase inhibitor PP2 (10 μM (Chang et al., 2012)) and exposed them to PXR agonists. Selective inhibition of SFKs abolished the rapid ATP release triggered by SR12813- and rifaximin (Fig. 7E-H). Taken together these data suggest that PXR agonists trigger ATP efflux through an SFK- and pannexin-1-dependent process.

Discussion

In the current study, we found that stimulation of the PXR in primed macrophages triggered the activation of the NLRP3 inflammasome, resulting in caspase-1 activation and IL-1β secretion. Mechanistically, PXR-activation-induced NLRP3 inflammasome signaling was reliant on pannexin-1-dependent ATP release, and subsequent stimulation of the P2X7 receptor, a well-characterized driver of inflammasome activation (Mangan et al., 2018). In this process, PXR-dependent ATP release occurs as early as 15 seconds following receptor stimulation, and involves SFK signaling, suggesting a cytosolic function for the PXR in macrophages. Altogether our data support a novel role for the PXR in trigger a host-defense response in macrophages, linking xenobiotic sensing and innate immunity in a system that may protect against xenobiotics and other chemical contaminants of foreign origin.

The PXR is a member of the nuclear receptor superfamily, which includes such members as the FXR, VDR, glucocorticoid receptor and retinoid X receptor (RXR) (Germain et al., 2006). The PXR, which is highly expressed in the liver and in intestinal epithelial cells, is best characterized for its ability to regulate the expression of enzymes involved in drug metabolism, detoxification and excretion (Koutsounas et al., 2013). The PXR is also expressed in a variety of immune cells, including T cells, macrophages and dendritic cells (Schote et al., 2007), and its signaling has been reported to modulate their function through mechanisms that are less understood (Zhou et al., 2009; Dubrac et al., 2010; Casey and Blumberg, 2012; Beyer et al., 2013). More recently, the PXR's direct and indirect regulation of innate immune signaling has been reported in a number of systems. In a seminal report, Venkatesh *et al.* found that the PXR functions as a negative regulatory of toll-like receptor gene expression in the intestinal epithelium, thereby indirectly modulating innate immune signaling in the intestinal mucosa (Venkatesh et al., 2014). These data build on a body of literature that suggests that

PXR can negatively regulate NF-κB-dependent inflammatory signaling in a variety of cell types (Xie and Tian, 2006; Zhou et al., 2006; Moreau et al., 2008).

In contrast to the notion that the PXR exhibits solely anti-inflammatory effects, Wang *et al.* reported that the PXR could enhance NLRP3 inflammasome activity in vascular endothelial cells. The authors found that PXR stimulation upregulated the expression of NLRP3 and pro-IL-1β, and that prolonged stimulation triggered caspase-1 activation and IL-1β processing indicative of NLRP3 inflammasome activation (Wang et al., 2014a). While the kinetics of activation reported in this study do not conform to the traditional view of the NLRP3 inflammasome as an expeditious effector, our data suggest that PXR activation can trigger immediate ATP efflux from macrophages, a prerequisite for inflammasome activation in response to a variety of stimuli (Mangan et al., 2018). Indeed, the notion that a nuclear receptor can activate intracellular signaling events that culminate in rapid cellular responses is not without precedent. For instance, activation of the FXR by bile acids induces rapid release of ATP in pancreatic cell lines (Kowal et al., 2015). Tulk *et al.* also reported that stimulation of the VDR in primed macrophages triggered rapid NLRP3 inflammasome activation and IL-1β release (Tulk et al., 2015). Our data suggest PXR stimulation involves the rapid gating of pannexin-1 through a SFK-dependent mechanism, resulting in ATP efflux and subsequent P2X7 receptor activation to trigger NLRP3 inflammasome activation.

While our findings support a role for the PXR in initiating cell signaling that activates the NLRP3 inflammasome to induce IL-1β processing and release, others have reported contrasting observations. Sun *et al.* reported that pre-treating hepatocytes with PXR agonists attenuated LPS-induced IL-1β release (Sun et al., 2015). Furthermore, a recent report by Wang *et al.* described a model wherein statins inhibit inflammasome activity in vascular endothelial cells, through the PXR-dependent inhibition of NF-κB-driven *NLRP3* gene transcription (Wang et al., 2017). Some clarity could be

provided to these disparities by interpreting them in context of the temporal nature of inflammasome activation. NLRP3 inflammasome output (i.e. IL-1β processing and release) requires two signals. The first stimulus, often termed "signal 1", often involves TLR-driven NF-κB activation to prime the cells, inducing the expression of the inflammasome components, including NLRP3 and pro-IL-1β (Mangan et al., 2018). In order for a functional NLRP3 inflammasome to elaborate, a second signal (also termed "signal 2") is required, which usually takes the form of a DAMP or PAMP, and results in NLRP3 oligomerization, capsase-1 activation, resulting in IL-1β processing and release (Mangan et al., 2018). The wealth of data implicating the PXR as a negative regulator of NF-κB signaling, suggest its inhibitory effect on IL-1β release may be due to its inhibition of "signal 1" (i.e. inhibiting the induction of NLRP3 and pro-IL-1β expression). Indeed, Luo *et al.* reported that pretreating macrophages with baicalein, an agent reported to activate the PXR (Dou et al., 2012), attenuated LPS-induced NLRP3 and pro-IL-1β expression, through its inhibition of NF-κB signaling (Luo et al., 2017). In this context, baicalein's inhibition of "signal 1" attenuated subsequent ATP-induced IL-1β secretion (Luo et al., 2017). It is important to highlight that in our studies we assessed the impact of PXR activation in primed macrophages that have already received "signal 1". Thus, in the context of a primed cell that exhibits abundant expression of NLRP3 and pro-IL-1β, PXR stimulation acts a "signal 2", gating pannexin-1 to allow the efflux of ATP, which culminates in caspase-1 activation and IL-1β release.

While the functional impact of the PXR's modulation of NLRP3 inflammasome activation has yet to be elucidated, xenobiotic and endobiotic sensing mechanisms are thought to add an additional level of defense in the gastrointestinal tract of multicellular organisms (Dussault and Forman, 2002). For example, *C. elegans* upregulates xenobiotic response genes and initiates avoidance behaviors in the presence of pathogens and/or specific pathogenic factors in a process believed to enhance survival in the context of infection or exposure to environmental contaminants (Melo and Ruvkun,

2012; Jones et al., 2013). Mechanistically, these responses are mediated by a family of nuclear receptors that exhibit functional similarities to the mammalian PXR (Jones et al., 2013). As a ligand-activated receptor, the PXR's flexible binding domain allows a variety of receptor-ligand interactions to occur (Kliewer et al., 2002; Chang and Waxman, 2006; Staudinger et al., 2006; Gupta et al., 2008; Chang, 2009; Shukla et al., 2011). Thus, the interplay between xenobiotics and innate immune signaling through the PXR may be broad reaching. Furthermore, the impact of endogenous PXR ligands of microbial origin and the regulation of intestinal mucosal inflammasome signaling requires further attention. Ultimately, our findings suggest the existence of a complex interplay between xenobiotic sensing mechanisms and innate immunity that may function as a conserved mechanism to protect the host from exposure to chemical agents of foreign origin. Additional work will be required to determine the functional role for this interplay in the context of health and disease.

Acknowledgements

We thank J. Staudinger (University of Kansas, Lawrence, KS) for providing Pxr-/- (Nr1i2-/-) mice.

Author Contributions

The author(s) declare no competing or commercial interests.

Participated in research design: Hirota, Mani, Chang, and Hudson.

Conducted experiments: Hudson, Flannigan, Venu, Alston and Sandall

Contributed new reagents or analytic tools: Sandall, MacDonald and Muruve

Performed data analysis: Hudson, Hirota and Flannigan

Wrote or contributed to the writing of the manuscript: Hirota, Hudson, Flannigan, Chang, Mani and MacDonald

References

Ainscough JS, Gerberick GF, Kimber I and Dearman RJ (2015) Interleukin-1beta Processing Is Dependent on a Calcium-mediated Interaction with Calmodulin. *The Journal of biological chemistry* **290**:31151-31161.

Beyer C, Skapenko A, Distler A, Dees C, Reichert H, Munoz L, Leipe J, Schulze-Koops H, Distler O, Schett G and Distler JH (2013) Activation of pregnane X receptor inhibits experimental dermal fibrosis. *Annals of the rheumatic diseases* **72**:621-625.

Brough D, Le Feuvre RA, Wheeler RD, Solovyova N, Hilfiker S, Rothwell NJ and Verkhratsky A (2003) Ca2+ stores and Ca2+ entry differentially contribute to the release of IL-1 beta and IL-1 alpha from murine macrophages. *Journal of immunology (Baltimore, Md : 1950)* **170**:3029-3036.

Buitrago C and Boland R (2010) Caveolae and caveolin-1 are implicated in 1alpha,25(OH)2-vitamin D3-dependent modulation of Src, MAPK cascades and VDR localization in skeletal muscle cells. *The Journal of steroid biochemistry and molecular biology* **121**:169-175.

Buitrago C, Pardo VG and Boland R (2013) Role of VDR in 1alpha,25-dihydroxyvitamin D3-dependent non-genomic activation of MAPKs, Src and Akt in skeletal muscle cells. *The Journal of steroid biochemistry and molecular biology* **136**:125-130.

Casey SC and Blumberg B (2012) The steroid and xenobiotic receptor negatively regulates B-1 cell development in the fetal liver. *Molecular endocrinology* **26**:916-925.

Chang MY, Huang DY, Ho FM, Huang KC and Lin WW (2012) PKC-dependent human monocyte adhesion requires AMPK and Syk activation. *PloS one* **7**:e40999.

Chang TK (2009) Activation of pregnane X receptor (PXR) and constitutive androstane receptor (CAR) by herbal medicines. *The AAPS journal* **11**:590-601.

Chang TK and Waxman DJ (2006) Synthetic drugs and natural products as modulators of constitutive androstane receptor (CAR) and pregnane X receptor (PXR). *Drug metabolism reviews* **38**:51-73.

Dou W, Mukherjee S, Li H, Venkatesh M, Wang H, Kortagere S, Peleg A, Chilimuri SS, Wang ZT, Feng Y, Fearon ER and Mani S (2012) Alleviation of gut inflammation by Cdx2/Pxr pathway in a mouse model of chemical colitis. *PloS one* **7**:e36075.

Dou W, Zhang J, Zhang E, Sun A, Ding L, Chou G, Wang Z and Mani S (2013) Chrysin ameliorates chemically induced colitis in the mouse through modulation of a PXR/NF-kappaB signaling pathway. *The Journal of pharmacology and experimental therapeutics* **345**:473-482.

Dubrac S, Elentner A, Ebner S, Horejs-Hoeck J and Schmuth M (2010) Modulation of T lymphocyte function by the pregnane X receptor. *Journal of immunology (Baltimore, Md : 1950)* **184**:2949-2957.

Dussault I and Forman BM (2002) The nuclear receptor PXR: a master regulator of "homeland" defense. *Critical reviews in eukaryotic gene expression* **12**:53-64.

Garg A, Zhao A, Erickson SL, Mukherjee S, Lau AJ, Alston L, Chang TK, Mani S and Hirota SA (2016) Pregnane X Receptor Activation Attenuates Inflammation-Associated Intestinal Epithelial Barrier Dysfunction by Inhibiting Cytokine-Induced Myosin Light-Chain Kinase Expression and c-Jun N-Terminal Kinase 1/2 Activation. *The Journal of pharmacology and experimental therapeutics* **359**:91-101.

Germain P, Staels B, Dacquet C, Spedding M and Laudet V (2006) Overview of nomenclature of nuclear receptors. *Pharmacological reviews* **58**:685-704.

Gong H, Singh SV, Singh SP, Mu Y, Lee JH, Saini SP, Toma D, Ren S, Kagan VE, Day BW, Zimniak P and Xie W (2006) Orphan nuclear receptor pregnane X receptor sensitizes oxidative stress responses in transgenic mice and cancerous cells. *Molecular endocrinology* **20**:279-290.

Grahames CB, Michel AD, Chessell IP and Humphrey PP (1999) Pharmacological characterization of ATP- and LPS-induced IL-1beta release in human monocytes. *British journal of pharmacology* **127**:1915-1921.

Gupta A, Mugundu GM, Desai PB, Thummel KE and Unadkat JD (2008) Intestinal human colon adenocarcinoma cell line LS180 is an excellent model to study pregnane X receptor, but not constitutive androstane receptor, mediated CYP3A4 and multidrug resistance transporter 1 induction: studies with anti-human immunodeficiency virus protease inhibitors. *Drug metabolism and disposition: the biological fate of chemicals* **36**:1172-1180.

Hirota SA, Ng J, Lueng A, Khajah M, Parhar K, Li Y, Lam V, Potentier MS, Ng K, Bawa M, McCafferty DM, Rioux KP, Ghosh S, Xavier RJ, Colgan SP, Tschopp J, Muruve D, MacDonald JA and Beck PL (2011) NLRP3 inflammasome plays a key role in the regulation of intestinal homeostasis. *Inflammatory bowel diseases* **17**:1359-1372.

Hoque R, Sohail MA, Salhanick S, Malik AF, Ghani A, Robson SC and Mehal WZ (2012) P2X7 receptor-mediated purinergic signaling promotes liver injury in acetaminophen hepatotoxicity in mice. *Am J Physiol Gastrointest Liver Physiol* **302**:G1171-1179.

Hu G, Xu C and Staudinger JL (2010) Pregnane X receptor is SUMOylated to repress the inflammatory response. *The Journal of pharmacology and experimental therapeutics* **335**:342-350.

Hung SC, Choi CH, Said-Sadier N, Johnson L, Atanasova KR, Sellami H, Yilmaz O and Ojcius DM (2013) P2X4 assembles with P2X7 and pannexin-1 in gingival epithelial cells and modulates ATP-induced reactive oxygen species production and inflammasome activation. *PloS one* **8**:e70210.

Imaeda AB, Watanabe A, Sohail MA, Mahmood S, Mohamadnejad M, Sutterwala FS, Flavell RA and Mehal WZ (2009) Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome. *The Journal of clinical investigation* **119**:305-314.

Iyer SS, Pulskens WP, Sadler JJ, Butter LM, Teske GJ, Ulland TK, Eisenbarth SC, Florquin S, Flavell RA, Leemans JC and Sutterwala FS (2009) Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. *Proc Natl Acad Sci U S A* **106**:20388-20393.

Jabaut J, Ather JL, Taracanova A, Poynter ME and Ckless K (2013) Mitochondria-targeted drugs enhance Nlrp3 inflammasome-dependent IL-1beta secretion in association with alterations in cellular redox and energy status. *Free radical biology & medicine* **60**:233-245.

Jones LM, Rayson SJ, Flemming AJ and Urwin PE (2013) Adaptive and specialised transcriptional responses to xenobiotic stress in Caenorhabditis elegans are regulated by nuclear hormone receptors. *PloS one* **8**:e69956.

Kliewer SA, Goodwin B and Willson TM (2002) The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr Rev* **23**:687-702.

Koutsounas I, Theocharis S, Patsouris E and Giaginis C (2013) Pregnane X receptor (PXR) at the crossroads of human metabolism and disease. *Current drug metabolism* **14**:341-350.

Kowal JM, Haanes KA, Christensen NM and Novak I (2015) Bile acid effects are mediated by ATP release and purinergic signalling in exocrine pancreatic cells. *Cell communication and signaling : CCS* **13**:28.

Lau A, Chung H, Komada T, Platnich JM, Sandall CF, Choudhury SR, Chun J, Naumenko V, Surewaard BG, Nelson MC, Ulke-Lemee A, Beck PL, Benediktsson H, Jevnikar AM, Snelgrove SL, Hickey MJ, Senger DL, James MT, Macdonald JA, Kubes P, Jenne CN and Muruve DA (2018) Renal immune surveillance and dipeptidase-1 contribute to contrast-induced acute kidney injury. *The Journal of clinical investigation* **128**:2894-2913.

Lohman AW, Leskov IL, Butcher JT, Johnstone SR, Stokes TA, Begandt D, DeLalio LJ, Best AK, Penuela S, Leitinger N, Ravichandran KS, Stokes KY and Isakson BE (2015) Pannexin 1 channels regulate

leukocyte emigration through the venous endothelium during acute inflammation. *Nature communications* **6**:7965.

Luo X, Yu Z, Deng C, Zhang J, Ren G, Sun A, Mani S, Wang Z and Dou W (2017) Baicalein ameliorates TNBS-induced colitis by suppressing TLR4/MyD88 signaling cascade and NLRP3 inflammasome activation in mice. *Scientific reports* **7**:16374.

Ma X, Shah YM, Guo GL, Wang T, Krausz KW, Idle JR and Gonzalez FJ (2007) Rifaximin is a gut-specific human pregnane X receptor activator. *The Journal of pharmacology and experimental therapeutics* **322**:391-398.

Mangan MSJ, Olhava EJ, Roush WR, Seidel HM, Glick GD and Latz E (2018) Targeting the NLRP3 inflammasome in inflammatory diseases. *Nature reviews Drug discovery* **17**:588-606.

Melo JA and Ruvkun G (2012) Inactivation of conserved C. elegans genes engages pathogen- and xenobiotic-associated defenses. *Cell* **149**:452-466.

Mencarelli A, Migliorati M, Barbanti M, Cipriani S, Palladino G, Distrutti E, Renga B and Fiorucci S (2010) Pregnane-X-receptor mediates the anti-inflammatory activities of rifaximin on detoxification pathways in intestinal epithelial cells. *Biochemical pharmacology* **80**:1700-1707.

Mencarelli A, Renga B, Palladino G, Claudio D, Ricci P, Distrutti E, Barbanti M, Baldelli F and Fiorucci S (2011) Inhibition of NF-kappaB by a PXR-dependent pathway mediates counter-regulatory activities of rifaximin on innate immunity in intestinal epithelial cells. *Eur J Pharmacol* **668**:317-324.

Moreau A, Vilarem MJ, Maurel P and Pascussi JM (2008) Xenoreceptors CAR and PXR activation and consequences on lipid metabolism, glucose homeostasis, and inflammatory response. *Molecular pharmaceutics* **5**:35-41.

Mortimer L, Moreau F, Cornick S and Chadee K (2015) The NLRP3 Inflammasome Is a Pathogen Sensor for Invasive Entamoeba histolytica via Activation of alpha5beta1 Integrin at the Macrophage-Amebae Intercellular Junction. *PLoS pathogens* **11**:e1004887.

Nathanson MH, Burgstahler AD, Masyuk A and Larusso NF (2001) Stimulation of ATP secretion in the liver by therapeutic bile acids. *The Biochemical journal* **358**:1-5.

Ng J, Hirota SA, Gross O, Li Y, Ulke-Lemee A, Potentier MS, Schenck LP, Vilaysane A, Seamone ME, Feng H, Armstrong GD, Tschopp J, Macdonald JA, Muruve DA and Beck PL (2010) Clostridium difficile toxin-induced inflammation and intestinal injury are mediated by the inflammasome. *Gastroenterology* **139**:542-552, 552 e541-543.

Peng Z, Raufman JP and Xie G (2012) Src-mediated cross-talk between farnesoid X and epidermal growth factor receptors inhibits human intestinal cell proliferation and tumorigenesis. *PloS one* **7**:e48461.

Schote AB, Turner JD, Schiltz J and Muller CP (2007) Nuclear receptors in human immune cells: expression and correlations. *Molecular immunology* **44**:1436-1445.

Shah YM, Ma X, Morimura K, Kim I and Gonzalez FJ (2007) Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-kappaB target gene expression. *Am J Physiol Gastrointest Liver Physiol* **292**:G1114-1122.

Shukla SJ, Sakamuru S, Huang R, Moeller TA, Shinn P, Vanleer D, Auld DS, Austin CP and Xia M (2011) Identification of clinically used drugs that activate pregnane X receptors. *Drug metabolism and disposition: the biological fate of chemicals* **39**:151-159.

Staudinger JL, Ding X and Lichti K (2006) Pregnane X receptor and natural products: beyond drug-drug interactions. *Expert opinion on drug metabolism & toxicology* **2**:847-857.

Sun M, Cui W, Woody SK and Staudinger JL (2015) Pregnane X receptor modulates the inflammatory response in primary cultures of hepatocytes. *Drug metabolism and disposition: the biological fate of chemicals* **43**:335-343.

Terc J, Hansen A, Alston L and Hirota SA (2014) Pregnane X receptor agonists enhance intestinal epithelial wound healing and repair of the intestinal barrier following the induction of experimental colitis. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* **55**:12-19.

Tien XY, Brasitus TA, Qasawa BM, Norman AW and Sitrin MD (1993) Effect of 1,25(OH)2D3 and its analogues on membrane phosphoinositide turnover and [Ca2+]i in Caco-2 cells. *The American journal of physiology* **265**:G143-148.

Tulk SE, Liao KC, Muruve DA, Li Y, Beck PL and MacDonald JA (2015) Vitamin D(3) metabolites enhance the NLRP3-dependent secretion of IL-1beta from human THP-1 monocytic cells. *Journal of cellular biochemistry* **116**:711-720.

Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, Qiu Z, Maher L, Redinbo MR, Phillips RS, Fleet JC, Kortagere S, Mukherjee P, Fasano A, Le Ven J, Nicholson JK, Dumas ME, Khanna KM and Mani S (2014) Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. *Immunity* **41**:296-310.

Vertino AM, Bula CM, Chen JR, Almeida M, Han L, Bellido T, Kousteni S, Norman AW and Manolagas SC (2005) Nongenotropic, anti-apoptotic signaling of 1alpha,25(OH)2-vitamin D3 and analogs through the ligand binding domain of the vitamin D receptor in osteoblasts and osteocytes. Mediation by Src, phosphatidylinositol 3-, and JNK kinases. *The Journal of biological chemistry* **280**:14130-14137.

Wang K, Damjanov I and Wan YJ (2010) The protective role of pregnane X receptor in lipopolysaccharide/D-galactosamine-induced acute liver injury. *Lab Invest* **90**:257-265.

Wang S, Lei T, Zhang K, Zhao W, Fang L, Lai B, Han J, Xiao L and Wang N (2014a) Xenobiotic pregnane X receptor (PXR) regulates innate immunity via activation of NLRP3 inflammasome in vascular endothelial cells. *The Journal of biological chemistry* **289**:30075-30081.

Wang S, Xie X, Lei T, Zhang K, Lai B, Zhang Z, Guan Y, Mao G, Xiao L and Wang N (2017) Statins Attenuate Activation of the NLRP3 Inflammasome by Oxidized LDL or TNFalpha in Vascular Endothelial Cells through a PXR-Dependent Mechanism. *Molecular pharmacology* **92**:256-264.

Wang YM, Chai SC, Brewer CT and Chen T (2014b) Pregnane X receptor and drug-induced liver injury. *Expert opinion on drug metabolism & toxicology* **10**:1521-1532.

Weilinger NL, Lohman AW, Rakai BD, Ma EM, Bialecki J, Maslieieva V, Rilea T, Bandet MV, Ikuta NT, Scott L, Colicos MA, Teskey GC, Winship IR and Thompson RJ (2016) Metabotropic NMDA receptor signaling couples Src family kinases to pannexin-1 during excitotoxicity. *Nature neuroscience* **19**:432-442.

Xie W and Tian Y (2006) Xenobiotic receptor meets NF-kappaB, a collision in the small bowel. *Cell metabolism* **4**:177-178.

Zhou C, King N, Chen KY and Breslow JL (2009) Activation of PXR induces hypercholesterolemia in wild-type and accelerates atherosclerosis in apoE deficient mice. *Journal of lipid research* **50**:2004-2013.

Zhou C, Tabb MM, Nelson EL, Grun F, Verma S, Sadatrafiei A, Lin M, Mallick S, Forman BM, Thummel KE and Blumberg B (2006) Mutual repression between steroid and xenobiotic receptor and NF-kappaB signaling pathways links xenobiotic metabolism and inflammation. *The Journal of clinical investigation* **116**:2280-2289.

Funding Footnote

SAH's salary is supported by the Canadian Institutes of Health Research's (CIHR) Canada Research Chair (CRC) program [Tier II CRC in Host-Microbe Interactions and Chronic Disease]; SAH's lab is supported by an infrastructure grant provided by the Canadian Foundation for Innovation John R. Evans Leaders Fund; operating funds from Crohn's & Colitis Canada (SAH and TKHC co-investigators); the Dr. Lloyd Sutherland Investigatorship in IBD/GI Research; an Natural Sciences and Engineering Research Council (NSERC) Discovery Grant [RGPIN-2016-03842]. SM's lab is supported by NIH grants [CA161879, CA222469], US Department of Defence [W81XWH-17-1-0479] and Broad Medical Research Program - Crohn's & Colitis Foundation (CCFA) Investigator Award [Proposal No. 262520]. JAM and DAM are supported by the CIHR Health Challenges in Chronic Disease Signature Initiative [#THC-13523; to DAM and JAM]. CFS holds scholarship awards from the Natural Sciences and Engineering Research Council and the Cumming School of Medicine. KLF received support from the Beverly Philips postdoctoral fellowship through the Snyder Institute for Chronic Diseases at the University of Calgary and is currently supported by postdoctoral fellowships from Alberta Innovates and the Canadian Association of Gastroenterology/CIHR/Abbvie Inc. TKHC's lab is supported by an NSERC Discovery Grant [RPGIN-2014-03734].

Legends for Figures

Figure 1: Mouse and human PXR agonists trigger IL-1β release from primed macrophages. (A) The rodent-specific PXR agonist pregnenolone 16α-carbonitrile (PCN; 10 and 100 μM for 6 hrs) elicits significant IL-1β release from LPS-primed peritoneal mouse macrophages as measured by ELISA. Human specific PXR agonists (B) SR121813 (SR 0.1, 1, 4, 10 μM; 6 hrs) or (C) rifaximin (rifx 1, 5, 10, 100 μM; 6 hrs) stimulated significant IL-1β release from wild-type PMA-differentiated THP-1 cells. N=3-4; *P<0.05 for indicated comparison generated by ANOVA and Tukey's post-hoc test.

Figure 2: Mouse and human PXR agonists evoke caspase-1 activation and IL-1β release in an NLRP3-dependent manner. ATP (5 mM; positive control) and PCN (1, 10 and 100 μM) trigger caspase-1 activation (as assessed by detecting the cleaved caspase-1 p20 subunit in the supernatant; A-B), along with significant IL-1β release from wild-type (WT) peritoneal macrophages (C), effects at were absent in macrophages isolated from $Nlrp3^{-/-}$ mice (A-C). ATP (5 mM; positive control), SR121813 (SR - 4 μM) and rifaximin (Rifx - 5 and 10 μM) trigger caspase-1 activation (as assessed by detecting the cleaved caspase-1 p10 subunit in the supernatant; D-E), along with significant IL-1β release (F) from wild-type PMA-differentiated THP-1 cells, effects that were absent in NLRP3-deficient THP-1 cells (D-F). CL – cell lysate; SN – supernatant. All outcomes were measured after a 6-hr treatment period. N=3-6; *P<0.05 for indicated comparison generated by ANOVA and Tukey's post-hoc test; western blots are representative of 3 separate experiments.

Figure 3: Mouse PXR agonists fail to evoke caspase-1 activation or IL-1β release in peritoneal macrophages isolated from $PXR^{-/-}$ mice. The PXR is expressed in LPS-pulsed peritoneal macrophages (A) and is required for PCN-induced caspase-1 activation (as assessed by detecting the cleaved

caspase-1 p20 subunit in the supernatant; B-C) and subsequent IL-1β release (D), measured by ELISA. CL – cell lysate; SN – supernatant. All outcomes were measured after a 6-hr treatment period. N=3-6; * denotes P<0.05 for indicated comparison generated by ANOVA and Tukey's post-hoc test; western blots are representative of 3 separate experiments.

Figure 4: PXR-associated NLRP3 inflammasome activation is sensitive to ATP degradation and inhibition of the P2X7 receptor. (A) Apyrase (APY; 30 units/mL) pretreatment, to degrade extracellular ATP, significantly reduces SR12813- (SR 4 μM) and rifaximin- (Rifx 10 μM) induced IL-1β release from PMA-differentiated THP-1 cells. (B) Oxidized-ATP (oATP; 100 μM), a selective P2X7 receptor antagonist, significantly reduces SR12813- (SR 4 μM) and rifaximin- (Rifx 10 μM) induced IL-1β release from PMA-differentiated THP-1 cells. All outcomes were measured after a 6-hr treatment period. N=6; *P<0.05 for indicated comparison generated by ANOVA and Tukey's post-hoc test.

Figure 5: PXR agonists trigger rapid and significant ATP release from mouse and human macrophages. (A) PCN, a mouse PXR agonist, triggers rapid and significant ATP release from LPS-pulsed peritoneal macrophages isolated from wild-type mice, an effect that is absent in cells isolated from $PXR^{-/-}$ mice. N=3; *P<0.05 for wild-type (WT) PCN-treated macrophages compared to naïve and $PXR^{-/-}$ cells generated by ANOVA and Tukey's post-hoc test. (B) Human PXR agonists, rifaximin- (Rifx) and SR12813- (SR), trigger rapid and significant ATP release from PMA-differentiated THP-1 cells. N=3; *P<0.05 for indicated comparison generated by ANOVA and Tukey's post-hoc test.

Figure 6: Blockade of pannexin-1 and inhibition of Src-family-kinases attenuates human PXR agonist-induced ATP and IL-1β release from PMA-differentiated THP-1 cells. (A) Selective inhibition of

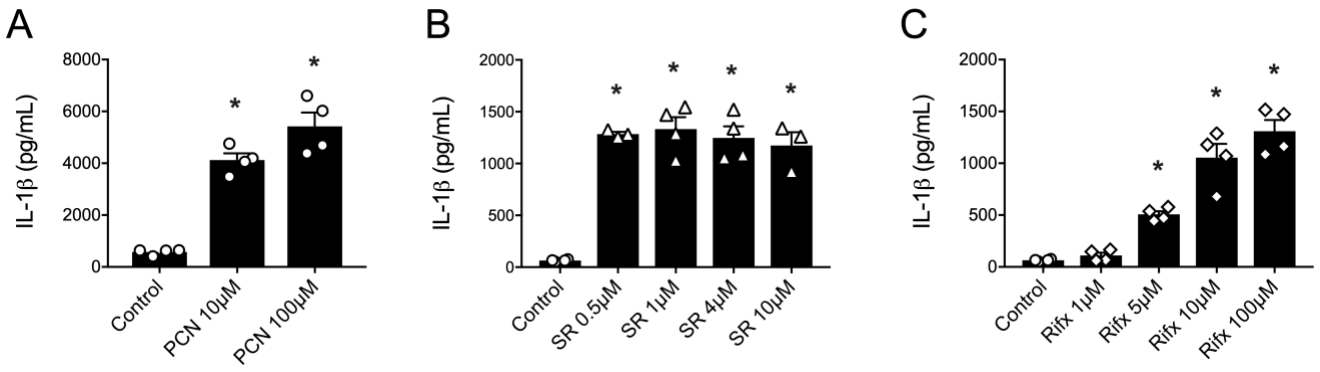
pannexin-1 with a pannexin-1 channel blocking peptide (^{10}Panx, 400 μM; or scrambled control peptide ^{Sc}Panx) abolishes rifaximin- (Rifx) and SR12813- (SR) induced ATP release from PMA-differentiated THP-1 cells. (B) Inhibition of Src-family-kinases with PP2 (10 μM) significantly reduces rifaximin- (Rifx) and SR12813- (SR) induced ATP release from PMA-differentiated THP-1 cells. N=4; *P<0.05 for indicated comparison generated by ANOVA and Tukey's post-hoc test.

Figure 7: Proposed model for the events that link PXR activation to NLRP3 inflammasome activation and IL-1β release. Ligation of the PXR (and heterodimerization with the RXR) triggers the release of intracellular ATP through pannexin-1 channels. Extracellular ATP then binds to the P2X7 receptor, an event that prompts the assembly of the NLRP3 inflammasome and subsequent activation of caspase-1. Caspase-1 then cleaves pro-ILβ into its active, secreted IL-1β.

JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

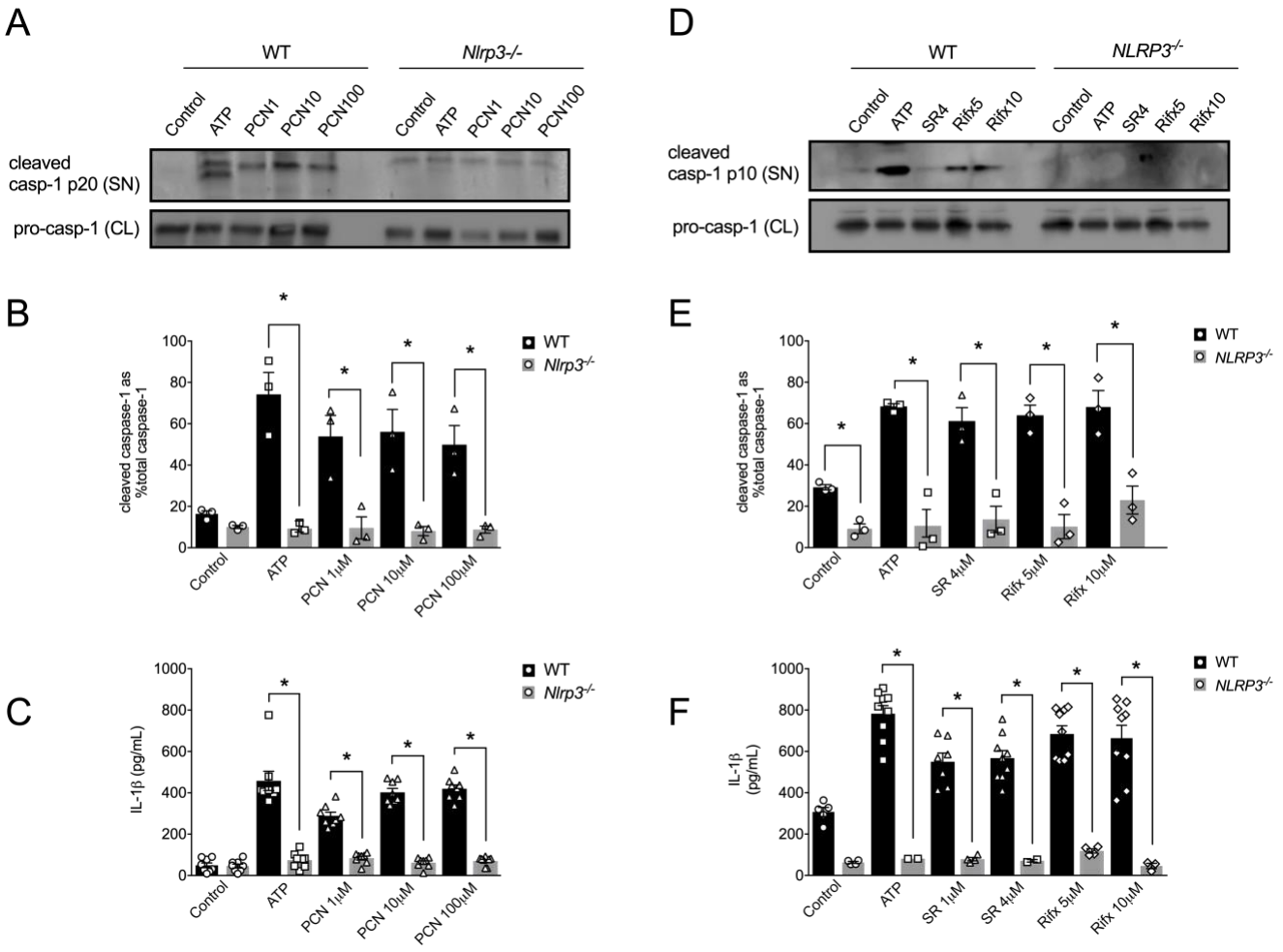
Tables and Figures

Figure 1



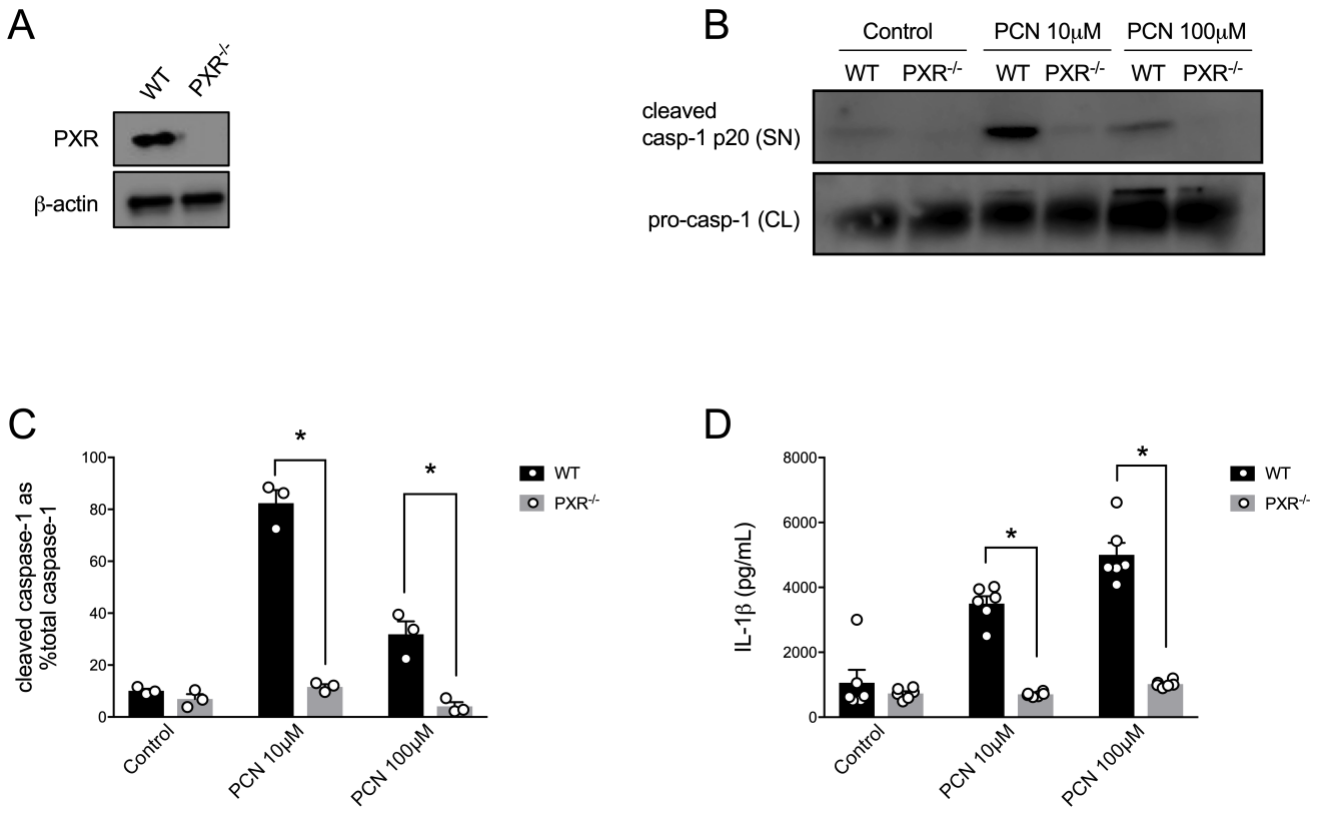
JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 2



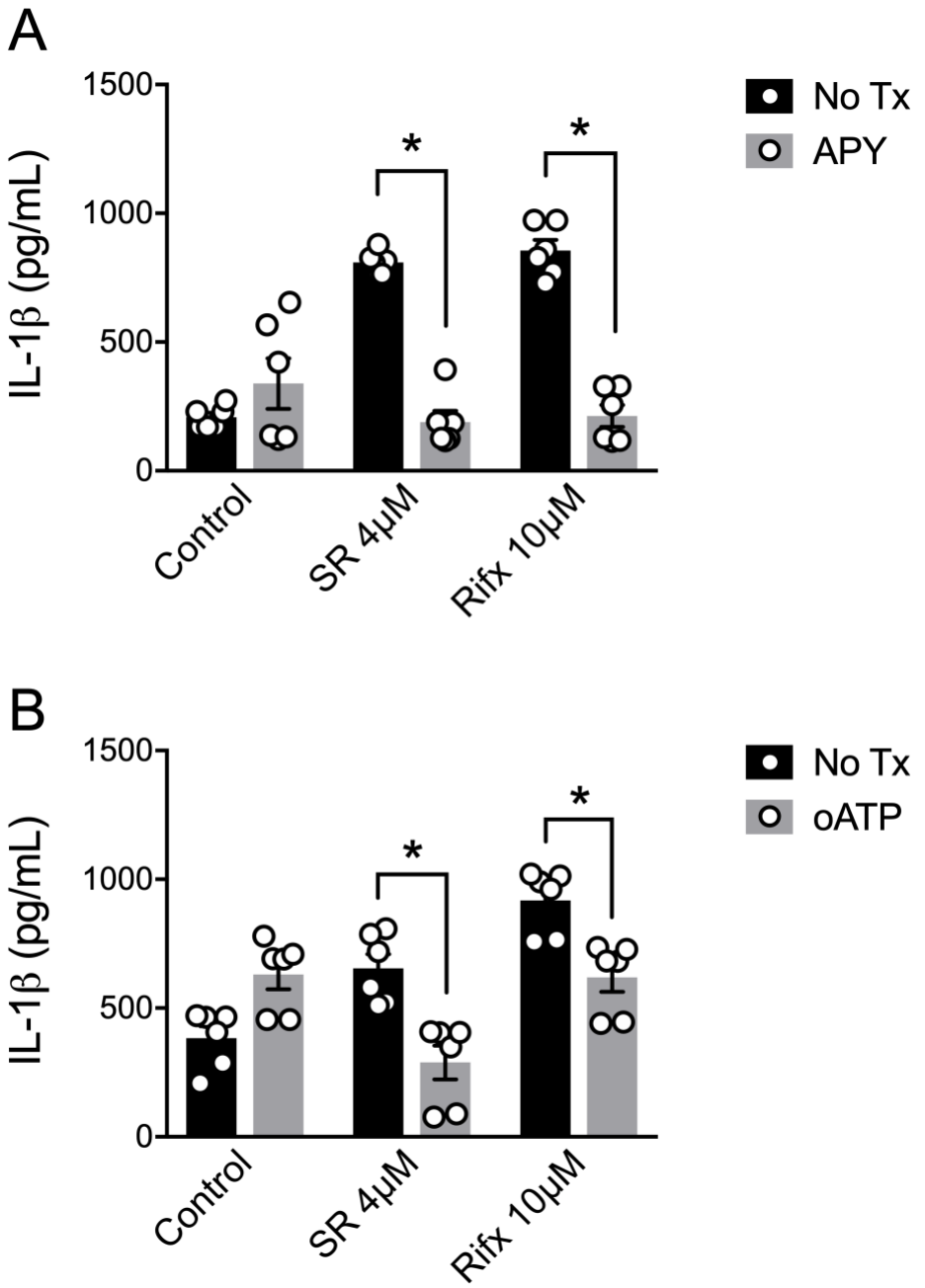
JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 3



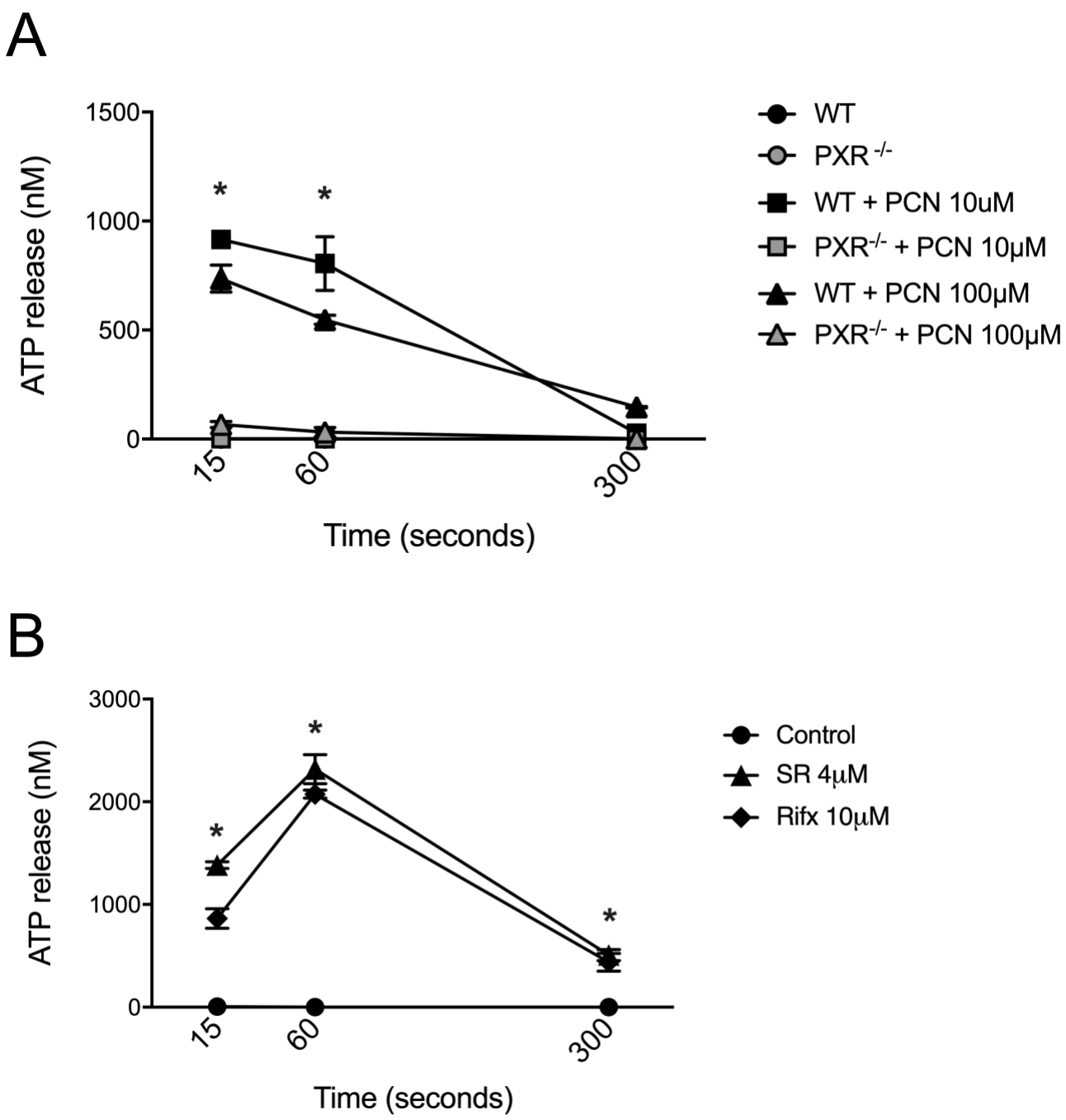
JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 4



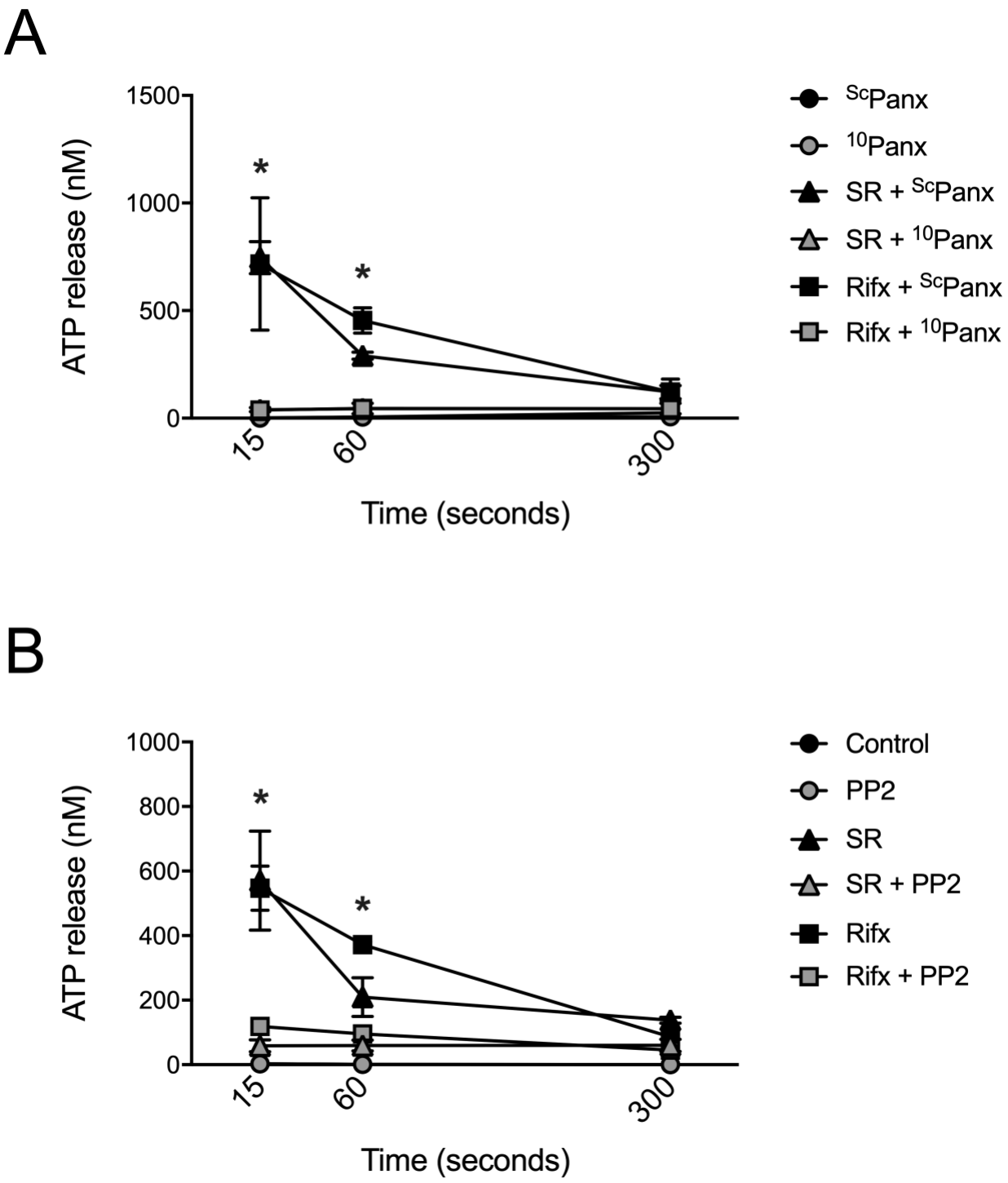
JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 5



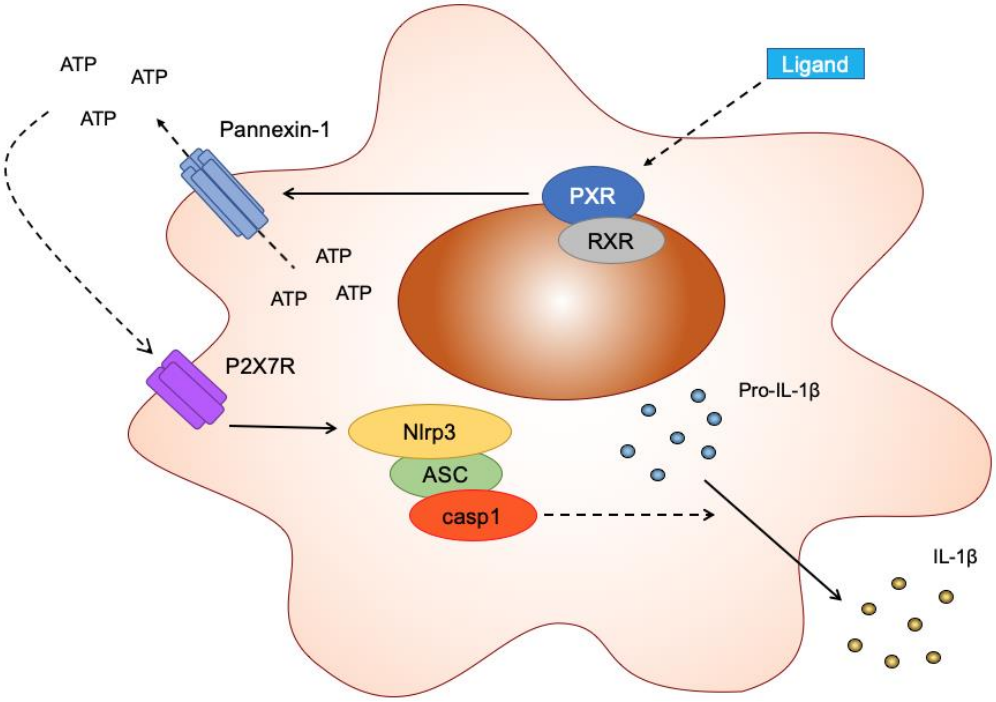
JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 6



JPET Fast Forward. Published on April 19, 2019 as DOI: 10.1124/jpet.118.255679
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 7



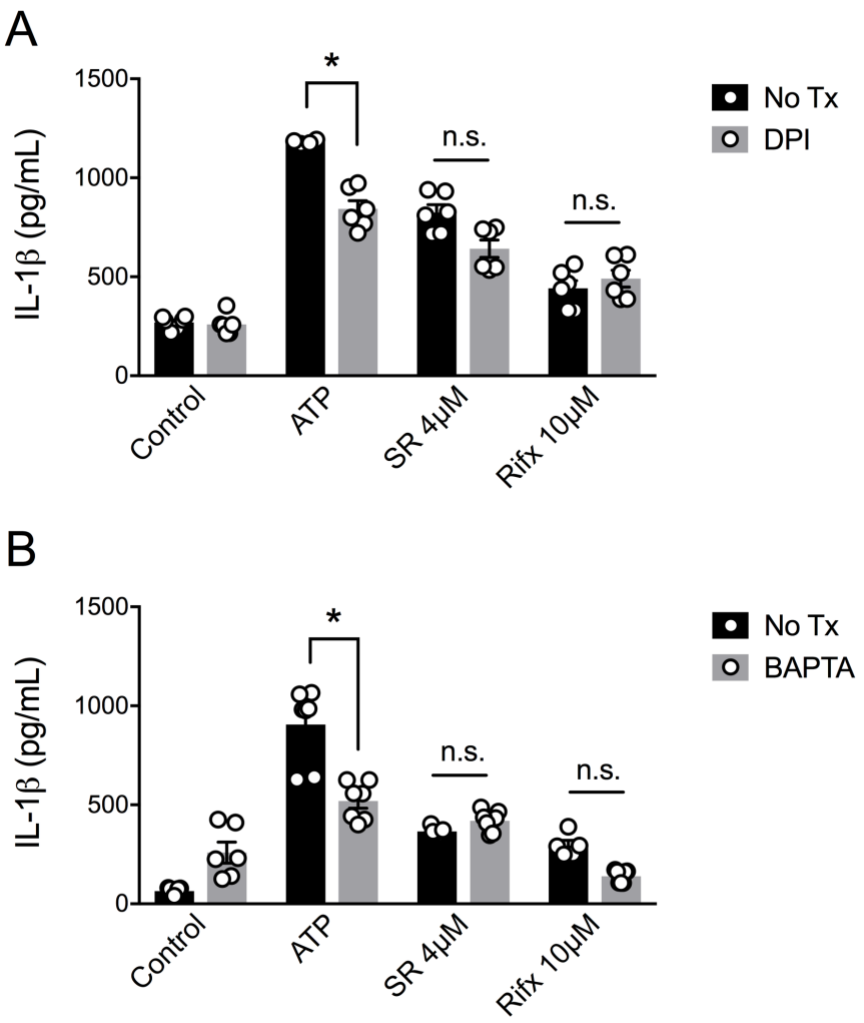
Supplemental Data

Pregnane X receptor activation triggers rapid ATP release in primed macrophages that mediates NLRP3 inflammasome activation

Grace Hudson[1], Kyle L. Flannigan[1], Vivek Krishna Pulakazhi Venu [1], Laurie Alston[1], Christina F. Sandall[2], Justin A. MacDonald[2], Daniel A. Muruve[3], Thomas K. H. Chang[4], Sridhar Mani[5] & Simon A. Hirota[1,6]*

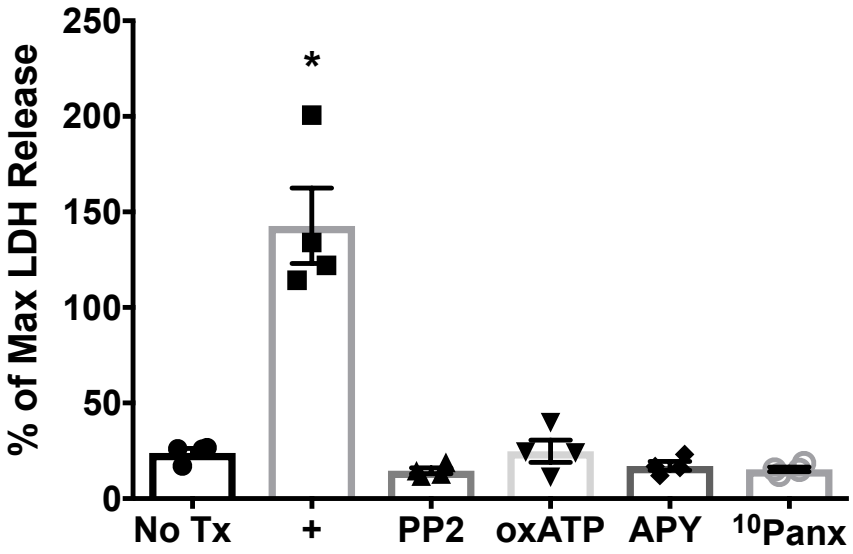
1 – Dept. of Physiology & Pharmacology, University of Calgary, Calgary, AB, Canada
2 – Dept. of Biochemistry & Molecular Biology, University of Calgary, Calgary, AB, Canada
3 – Dept. of Medicine, University of Calgary, Calgary, AB, Canada
4 – Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada
5 – Dept. of Medicine, Albert Einstein College of Medicine, Bronx, NY, USA
6 – Dept. of Immunology, Microbiology & Infectious Diseases, University of Calgary, Calgary, AB, Canada

Supplemental Figure 1



Supplemental Figure 1: Inhibiting ROS production and chelating intracellular calcium do not reduce PXR-associated NLRP3 inflammasome activation. (A) Diphenyleneiodonium (DPI; 100 μM) reduces ATP-induced IL-1β release from PMA-differentiated THP-1 cells but has no effect the responses induced by rifaximin (Rifx) or SR12813 (SR). (B) The membrane permeable calcium chelator BAPTA-AM (10 μM) reduces ATP-induced IL-1β release from PMA-differentiated THP-1 cells but has no effect the responses induced by rifaximin (Rifx) or SR12813 (SR). All outcomes were measured after a 6-hr treatment period. N=6; * denotes $P<0.05$ for indicated comparison generated by ANOVA and Tukey's post-hoc test; ns denotes not statistically significant for the indicated comparison.

Supplemental Figure 2



Supplemental Figure 2: PMA-differentiated THP-1 cells were treated with a variety of pharmacological inhibitors (PP2 – 10 μM; oxATP – 100 μM; APY – 30 units/mL; 10Panx – 400 μM) used in our studies and the release of lactate dehydrogenase (LDH) assessed as an index of alterations in cell viability (as per Thermo Scientific Pierce LDH Cytotoxicity Assay Kit instructions). * denotes $p<0.05$ compared to all groups; No Tx – no treatment; + – positive control; N = 4.