

CHARACTERISATION OF THE HEPATIC EFFECTS OF PHENOBARBITAL (PB) AND PREGNENOLONE 16 α -CARBONITRILE (PCN) IN CONSTITUTIVE ANDROSTANE RECEPTOR (CAR, NR1I3) AND PREGNANE X RECEPTOR (PXR, NR1I2) DOUBLE KNOCKOUT RATS.

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Introduction

The activation of the nuclear receptors constitutive androstane receptor (CAR) and pregnane X receptor (PXR) in rat liver results in hepatocellular hypertrophy (due to the proliferation of smooth endoplasmic reticulum and concomitant induction of enzymes belonging to the CYP2B and 3A families) and hyperplasia (increased semi-conservative DNA synthesis and cell proliferation). This study investigated the effects of the CAR activator phenobarbital sodium salt (PB) and the PXR activator pregnenolone 16 α -carbonitrile (PCN) in CAR/PXR double knockout (CARKO/PXRKO) rats.

Methods

Male Wild Type (WT) and CARKO/PXRKO rats (Horizon Discovery, Boyertown, PA, n = 5 per group), were subcutaneously implanted with osmotic pumps containing BrdU for determination of replicative DNA synthesis (S-phase), and rats were administered either PB (500 ppm) in the diet or PCN (100 mg/kg) p.o. daily for 7 days. Control rats of each strain received diet alone.

After 7 days, the rats were euthanised and livers removed and weighed. Liver was taken for fixation (H&E and BrdU immunohistochemistry), mRNA isolation (Taqman qPCR and microarrays) and the isolation of microsomes (enzyme assays and Western Blotting).

Microsomal monooxygenase activities determined were: ethoxyresorufin-O-deethylation (EROD, CYP1A), pentoxyresorufin-O-depentylation (PROD, CYP2B), benzyloxyresorufin-O-debenzylolation (BROD, CYP2B), benzyloxyquinoline-O-debenzylolation (BQ, CYP3A).

One colour microarray analysis of mRNA isolated from control and treated rat liver samples was performed to facilitate generation of differentially expressed gene signature lists (DEGs) to identify gene changes in response to PB or PCN exposure in WT or CARKO/PXRKO rats. Ingenuity pathway analysis (IPA™) software was used to identify canonical pathways.

Results

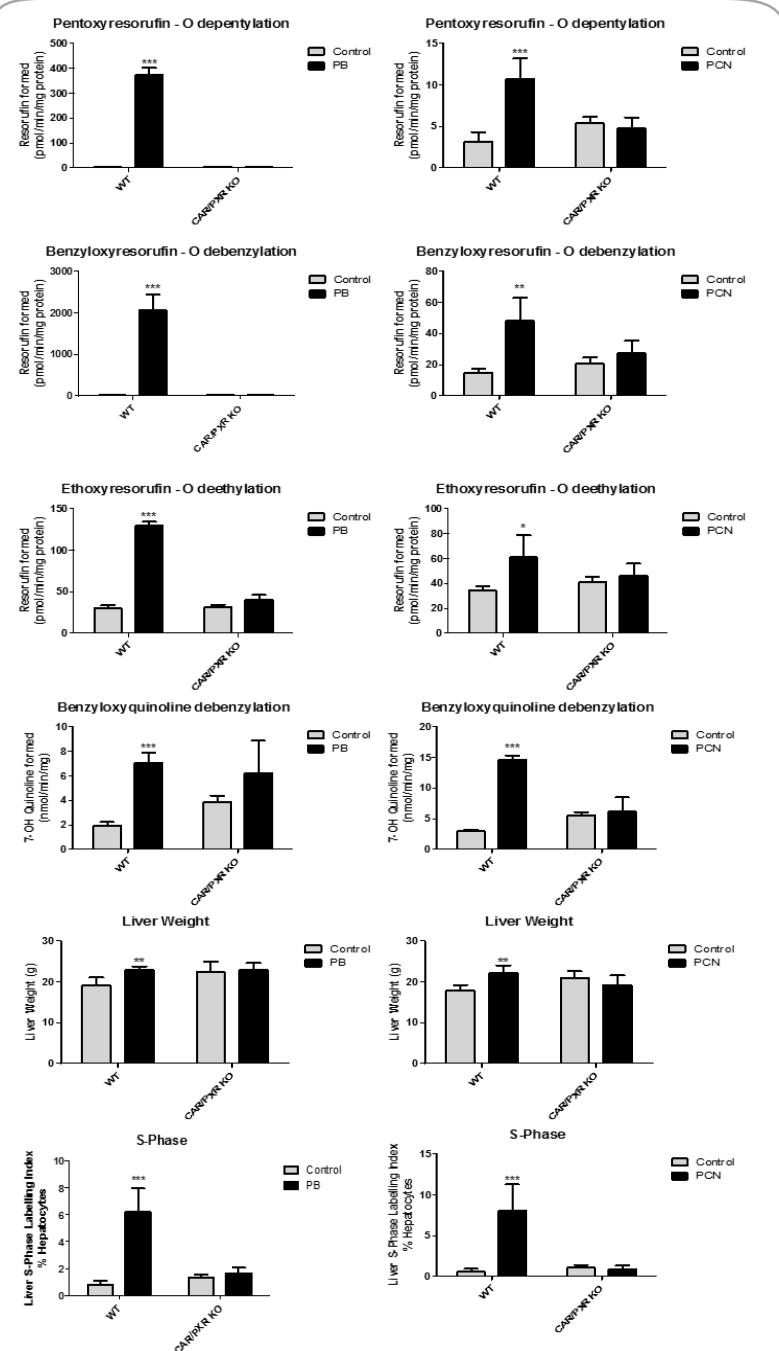
Treatment of WT rats with PB resulted in an increase in liver/body weight ratio (1.2X), induction in hepatocellular S-phase labelling index (7.7X), increase in total microsomal P450 (1.8X), the microsomal CYP2B catalysed reactions PROD and BROD were induced 93- and 145-fold, respectively. Treatment of CARKO/PXRKO rats with PB did not increase any of these parameters.

WT rats treated with PCN resulted in an increase in liver/body weight ratio (1.2X), induction of hepatocellular S-phase labelling index (13.8X) and an increase in total microsomal P450 (1.5X). Treatment of CARKO/PXR KO rats with PCN did not affect any of these parameters.

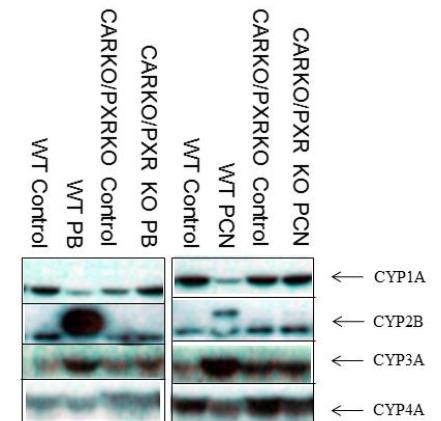
PCN induced the microsomal CYP3A catalysed reaction benzyloxyquinoline debenzylolation (BQ) 4.8-fold in WT rats but not CARKO/PXRKO rats.

Furthermore, CYP2B1, CYP2B2 and CYP3A1 mRNA levels were induced by either PB or PCN in WT but not CARKO/PXRKO rats.

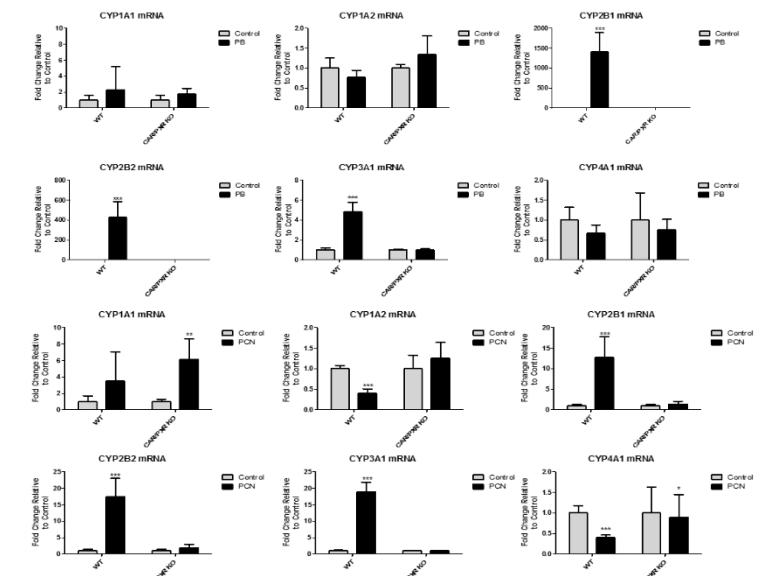
Between 40-50 % of genes induced by PB or PCN appear to be CAR/PXR dependent.



Values are Mean \pm SD (n = 5). A Student's t-test (2-sided) was performed on the results; *statistically different from control p<0.05; **p<0.01, ***p<0.001.



Microsomal samples were pooled for each group (n=5).



Conclusions

- An active CAR and PXR is required for the induction of CYP2B and CYP3A catalysed reactions (PROD, BROD, EROD and BQ) and protein induction.
- Treatment of WT rats with PB or PCN resulted in a significant increase in cell proliferation, however, no increase in cell proliferation was observed in CARKO/PXRKO rats treated with PB or PCN.
- CYP2B1, CYP2B2 and CYP3A1 mRNA levels were induced by either PB or PCN in WT but not CARKO/PXRKO rats.
- In conclusion, an active CAR is required for PB-mediated effects and an active PXR is required for PCN-mediated effects in rats.