

## 8,9- AND 11,12-EETs ARE POTENT ACTIVATORS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR- $\alpha$

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Peroxisome proliferator-activated receptor (PPAR)- $\alpha$  is a fatty acid-activated nuclear receptor with roles regulating lipid metabolism, proliferation and inflammation (Bishop-Bailey, 2000). We have previously shown that CYP2J2, an abundant epoxygenase in human cardiovascular and pulmonary systems, activates PPAR $\alpha$  (Wray *et al.*, 2003). We have therefore, investigated products of CYP2J2 on PPAR $\alpha$  mediated transcriptional activation.

Human embryonic kidney cells (HEK)293 were maintained in DMEM supplemented with antibiotics/ anti-mycotics and 10% FCS (37°C; 5% CO<sub>2</sub>; 95% air). Cells were transfected with combinations (0.5 $\mu$ g of each) of pACO.luc PPAR luciferase reporter gene, mPPAR $\alpha$  or dominant negative PPAR $\alpha$  (h6/29; a gift from Dr. Ruth Roberts; AstraZeneca) using NovaFactor, as described previously (Wray *et al.*, 2003). Cells were then incubated with 1 $\mu$ M of 8,9-, 11,12-, 14,15 epoxyeicosatrienoic acid (EET), or 5,6-dihydroxyeicosatrienoic acid (DiHETE) for 16h. Cells were then lysed, and luciferase activity measured and normalised to protein content (Wray *et al.*, 2003).

Transfection of PPAR $\alpha$  alone induced PPAR transcriptional activation. 8,9-, and 11,12-EET did not effect transcriptional activation alone, but significantly induced PPAR activation in the presence of PPAR $\alpha$ . These effects were abolished when dominant negative h6/29PPAR $\alpha$  was present (figure 1). 14,15-EET or 5,6-DiHETE did not effect PPAR activation under any condition tested (figure 1).

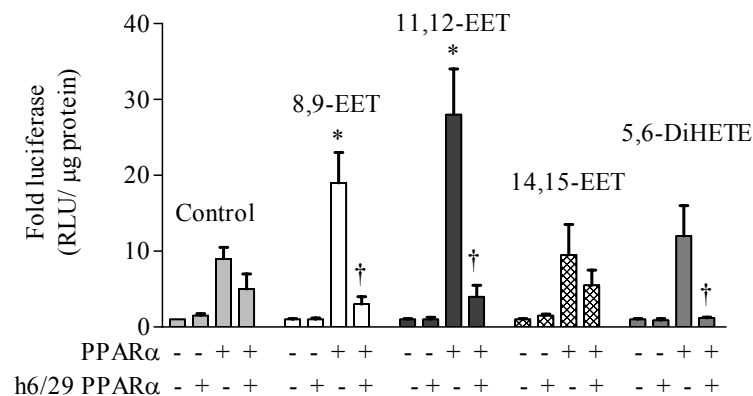


Figure 1. 8,9-EET and 11,12 EET-activate PPAR $\alpha$  mediated reporter gene (fold luciferase/ $\mu$ g protein). HEK293 cells were transfected with pACO.luc, pCMX-mPPAR $\alpha$  and/or h6/29PPAR $\alpha$ . Activation was measured after 16h of incubation with 8,9-, 11,12-, 14,15-EET or 5,6-DiHETE (all 1 $\mu$ M). Data represents mean  $\pm$  SEM of n=9 from 3 experiments. \* indicates p<0.05 by Wilcoxon matched pairs test between PPAR $\alpha$  in the absence and presence of drug treatment and † indicates p<0.05 by Wilcoxon matched pairs test between treatment in the presence or absence of h6/29PPAR $\alpha$ .

The CYP2J2 products 8,9- and 11,12-EET activate PPAR $\alpha$ . CYP2J2 products may represent novel endogenous mediators for PPAR $\alpha$  activation and its subsequent vascular/pulmonary anti-inflammatory and anti-proliferative effects.

Bishop-Bailey, D. (2000). *Br. J. Pharmacol.* **129**, 823-34  
Wray, J.A., et al. (2003). *Br. J. Pharmacol.* **138**, 169P

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