8,9- AND 11,12-EETs ARE POTENT ACTIVATORS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-α

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Peroxisome proliferator-activated receptor (PPAR)-α 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 PPARα (Wray *et al.*, 2003). We have therefore, investigated products of CYP2J2 on PPARα 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_2; 95% air). Cells were transfected with combinations (0.5μg of each) of pACO.luc PPAR luciferase reporter gene, mPPARα or dominant negative PPARα (h6/29; a gift from Dr. Ruth Roberts; AstraZeneca) using NovaFector, as described previously (Wray *et al.*, 2003). Cells were then incubated with 1μM 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 PPARα 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 PPARα. These effects were abolished when dominant negative h6/29PPARα was present (figure 1). 14,15-EET or 5,6-DiHETE did not effect PPAR activation under any condition tested (figure 1).

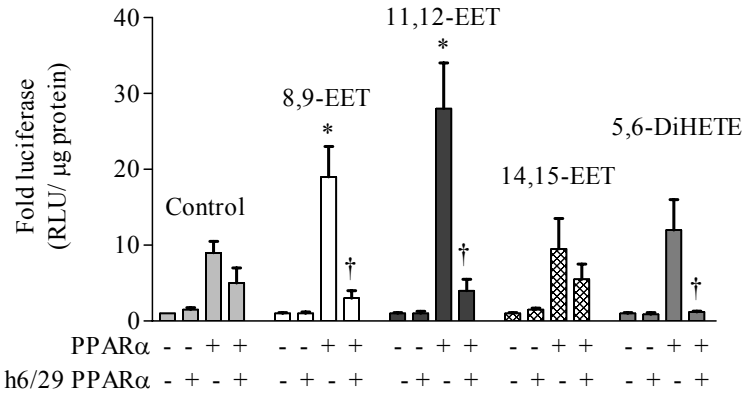

*Figure 1. 8,9-EET and 11,12 EET-activate PPARα mediated reporter gene (fold luciferase/μg protein). HEK293 cells were transfected with pACO.luc, pCMX-mPPARα and/or h6/29PPARα. Activation was measured after 16h of incubation with 8,9-, 11,12-, 14,15-EET or 5,6-DiHETE (all 1μM). Data represents mean ± SEM of n=9 from 3 experiments. * indicates p<0.05 by Wilcoxon matched pairs test between PPARα 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 h6/29PPARα.*

The CYP2J2 products 8,9- and 11,12-EET activate PPARα. CYP2J2 products may represent novel endogenous mediators for PPARα 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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