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INTERACTION OF PHTHALATE ESTERS WITH NUCLEAR RECEPTORS. CROSS-TALK WITH HORMONE-ACTIVATED SIGNALING PATHWAYS

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PPAR and STAT transcription factors activate genes involved in fatty acid metabolism (PPAR α) and adipogenesis (PPAR γ), and mediate hormonal responses important for body growth, liver gene expression and mammary gland development (STAT5a and STAT5b). These seemingly disparate pathways may be subject to mutually inhibitory cross-talk, with growth hormone (GH)-activated STAT5 able to inhibit PPAR-regulated transcription by ~80%, and ligand-activated PPAR able to correspondingly inhibit STAT5-regulated transcription. Given the co-expression of PPAR and STAT5 in multiple tissues, we investigated whether one of these factors dominates the inhibitory cross-talk, in studies using a PPAR-responsive Renilla luciferase reporter to monitor PPAR transcriptional activity in COS-1 cells simultaneously transfected with GH receptor and a STAT5 Firefly luciferase reporter. Dose-response studies revealed that PPAR-STAT5 inhibition proceeds in a mutual and simultaneous fashion. Moreover, the rodent reproductive toxicant and PPAR agonist mono-(2-ethylhexyl)phthalate (MEHP) inhibits STAT5b transcriptional activity at a concentration ($EC_{50} = 1.1 \mu\text{M}$) that is ~10-fold lower than the concentration required for half-maximal PPAR γ activation ($EC_{50} = 10 \mu\text{M}$). Thus, STAT5b transcriptional activity can be inhibited under conditions where only a portion of cellular PPAR γ is activated. Exposure to low levels of MEHP, and potentially other environmental chemical activators of PPAR, may thus lead to inhibition of hormonally-induced, STAT5-regulated responses in tissues such as liver, fat and breast, where both transcription factors are expressed. Conversely, STAT5-activating hormones and cytokines may modulate the responsiveness of PPARs to MEHP and other PPAR-activating environmental chemicals. In other studies, we investigated the potential of phthalate monoesters linked to significant human exposure for activation of PPAR α and PPAR γ ; notably monobenzyl phthalate (MBzP) and mono-sec-butyl phthalate (MBuP), in addition to MEHP. trans-activation studies demonstrated that mouse and human PPAR α were activated by MEHP ($EC_{50} = 0.6-3.2 \mu\text{M}$), MBzP (21-30 μM) and MBuP (60 μM), but not by the monomethyl, mono-n-butyl, dimethyl or diethyl esters of phthalic acid. Mouse and human PPAR γ were activated by MEHP (6-10 μM) and MBzP (75-100 μM). The potential of these phthalates to activate PPAR α was verified in rat liver FAO cells by the induction of endogenous PPAR α target genes, while phthalate activation of endogenous PPAR γ target genes was shown by the induction of PPAR γ -dependent 3T3-L1 fat cell differentiation. Studies of another nuclear receptor, pregnane X receptor (PXR), revealed that MEHP ($EC_{50} = 7-11 \mu\text{M}$) and MBzP both stimulated receptor transcriptional activity, whereas monomethyl phthalate and mono-n-butyl phthalate were inactive. Moreover, the naturally occurring human PXR allelic variants V140M and A370T exhibited patterns of phthalate responsiveness similar to wild-type receptor, whereas a third variant, D163G, was unresponsive to all phthalates tested. PXR-D163G was activated by rifampicin, but only at ~40-fold higher concentrations than wild-type receptor, suggesting that the ligand-binding domain D163G substitution substantially impairs ligand-binding activity. The activation of multiple nuclear receptors by MEHP and other ubiquitous environmental phthalate monoesters, and the cross-talk between these receptors and endogenous hormone-regulated STAT signaling pathways, may help establish a molecular basis for understanding some of the reproductive and developmental toxicities associated with phthalate exposure. The responsiveness of PPAR α and PPAR γ to phthalate monoesters suggests the potential for alteration of PPAR-regulated processes important for development and differentiation, while the activation of PXR raises the possibility that phthalate-induced changes in PXR-regulated steroid hormone metabolism may contribute to the endocrine disruptor activities associated with this class of

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