4-Nonylphenol Attenuates Dexamethasone Induction of CYP2B and CYP3A in FVB/NJ Mice.

JUAN HERNANDEZ

Department of Biological Sciences

Approved:

William S. Baldwin, Ph.D., Chair

Rafael de Jesus Cabeza, Ph.D.

Luis E. Martinez Jr., Ph.D.

Charles H. Ambler, Ph.D.
Dean of the Graduate School

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By

JUAN HERNANDEZ

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PREFACE

This thesis is written in part in journal style and organized as introduction, materials and methods, results, discussion, and references. This thesis is intended in part for future submission and publication in *Toxicology and Applied Pharmacology*.

ABSTRACT

4-nonylphenol (4-NP) binds and activates both the estrogen receptor (ER) and the Pregnane X receptor (PXR). Recent work in our lab demonstrated that 7-day exposures of mice to 4-NP induced CYP2B10, but had no effect on CYP3A. In contrast, gestational/chronic treatments of young and chronic treatments of 32 weeks in adults reduced CYP3A levels and attenuated induction of CYP2B10. Therefore, we were interested in determining if 4-NP could reduce the ability of FVB/NJ mice to respond to Constitutive androstane receptor (CAR) or PXR ligands. Mice were treated orally for six weeks with honey as the control or 50 mg/kg/day 4-NP in honey. After six weeks, the control and 4-NP treated mice were further split into groups of six, and were injected for three days with corn oil, dexamethasone (DEX) (PXR activator), or 4-bis [2-(3, 5-dichloropyridyloxy)] benzene also known as TCPOBOP (TC), (CAR activator). Mice were euthanized and RNA and microsomes were prepared from liver for immunoblot analysis of CYP3A and CYP2B, which are transcriptionally activated by PXR and CAR respectively. Q-PCR revealed that DEX induced the co-repressor small heterodimers partner (SHP) and reduced PXR, while 4-NP induced both silencing mediator of retinoid and thyroid receptor (SMRT) and SHP, and reduced PXR. The co-repressors may repress nuclear receptor signaling. Immunoblot analysis showed TC significantly induced CYP2B and CYP3A, but its induction was not significantly reduced when pre-treated with 4-NP. In contrast, DEX induced CYP2B and CYP3A, and 4-NP pre-

treatment significantly attenuated the normal DEX inductive response. Furthermore, testosterone hydroxylation and 7-pentoxyresorufin O-dealkylation (PROD) assays demonstrated that chronic treatment with 4-NP can repress DEX's inductive response of CYP3A and CYP2B activity, respectively. This may indicate that long-term exposure to 4-NP may decrease an organism's ability to respond to specific toxicants or hormones.

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INTRODUCTION

Over 1 billion pounds of nonylphenol ethoxylates are produced annually (Talmage, 1994). Alkylphenols, including 4-nonylphenol (4-NP) are compounds used in the plastic industry as detergents and non-ionic surfactants, and are found in some food products, cosmetics, and drinking water. Human exposure routes are diverse. Oral exposure can occur from contaminated foods and drinking water, but inhalation and dermal absorption after the application of 4-NP containing products and cosmetics is also common (Ahel *et al.*, 1993; Clark *et al.*, 1993).

Previous studies have shown that 4-NP binds to ER, PXR, and the mouse CAR (Tabira et al., 1999; Baldwin et al., 2004; Masuyama et al., 2000). These receptors work with and are dependent on cofactors for activation or repression of transcription. CAR and PXR are especially important in maintaining steroid hormone and bile acid concentrations by controlling the activation of genes important in the clearance of steroids and toxins (Gou *et al*., 2003; Honkakoski and Negishi, 2000; Xie *et al*., 2001). Some of the genes activated by the PXR are known as the cytochrome P-450's (CYP's) that make xenobiotics more water-soluble and provide hydroxyl groups for phase II conjugation and clearance from the cell (Parkinson, 1996).

Two orphan nuclear receptors, CAR and PXR, transactivate the same P-450 genes via the same response element in a xenobiotic-specific manner. The constitutive androstane receptor mediates the barbiturate activation of expression of CYP2B1 and CYP3A1 while PXR activates both genes in response to synthetic steroids (Smirlis *et al.*

2001). P-450's controlled by CAR include CYP2B10 (Takeshi et al., 2000), and CYP's controlled by the PXR include members of the CYP2B and CYP3A families (Wang et al., 2003). CAR and PXR activators include 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TC), phenobarbital for CAR, and dexamethasone and rifampicin for PXR. TC is a relatively potent activator of CAR, with a 50% effective concentration (EC_{50}) of approximately 20 nM (Tzameli *et al*. 2000), while phenobarbital activation of CAR is thought to occur through phosphorylation events that induce translocation of CAR into the nucleus but do not actually involve binding to the receptor (Honkakoski and Negishi, 1998). mRNA levels of several CYP3A family members are markedly increased by DEX but recent work has suggested that CYP3A activation by rifampicin is only observed in humans, but not in mice (Matsunaga *et tal*. 2004). P-450 activation by CAR and PXR ligands is an important first step in an organism's response to toxicants.

An earlier study in our lab demonstrated that acute exposure to 4-NP induced CYP2B levels, but not CYP3A. In contrast, a chronic exposure attenuated CYP2B induction and repressed CYP3A levels (Baldwin et al., 2004). Chronic studies in rats have shown similar effects on CYP3A protein levels (Laurenzana *et al*. 2002). Similarly, fish treated for 1 week with 4-NP demonstrated induction of glutathione S-transferases (GSTs), but those treated for 3 weeks demonstrated repressed GST activity (Uguz *et al.,* 2003). These studies suggest that exposure to 4-NP while inducing P-450s in the short-term, may actually repress P-450 protein levels after long-term exposure and therefore may prevent an organism from responding to toxicants in a normal fashion.

Co-activator recruitment is involved in PXR and CAR's transcriptional activity (Watkins *et al.* 2003; Wei *et al*. 2002). Conversely, co-repressors are involved in transcriptional silencing (Klein *et al.*, 2000). The mechanism by which P-450s are transcriptionally repressed is unknown and may involve increased co-repression, decreased co-activation or another uncharacterized mechanism. 4-NP is known to bind the ER, PXR and CAR. The co-repressor SHP is in part transcriptionally controlled by estrogen receptor activation (Lai *et al*., 2003). SHP is also inducible by Farnesoid X receptor (FXR) activation, and induction of SHP has been shown to have inhibitory physiological effects on FXR (Goodwin *et al*., 2000). Furthermore, SMRT and SHP have been shown to inhibit transcription activation by CAR and PXR in vitro (Bae *et al*., 2004; Takeshita *et al*., 2002). Therefore, it is possible that 4-NP may induce co-repressors and in turn causes P-450 repression.

Recent studies have begun to support the theory that chronic exposure to 4-NP may have damaging effects, which would not allow normal responses to other toxicants. CYP3A is the predominant P-450 involved in Phase I hydroxylation in mice and humans. Recent work has shown decreased levels of CYP3A, and CYP3A activity caused by 4-NP. 4-NP and other compounds initiating similar problems may be cause for concern when responding to endogenous compounds and toxicants. The PXR and CAR receptor are important in metabolism of endogenous steroid hormones as well as toxicants. The bioaccumulation of either steroids or toxicants may lead to long-term physiological problems that may include perturbed toxicant responses, reproductive abnormalities or developmental deformities caused by retention of hormones/toxicants. We hypothesize

that chronic exposure to 4-NP may reduce the expression of cofactors and/or increase the expression of co-repressors, and therefore attenuate the liver's normal response to typical PXR and CAR inducers such as dexamethasone and TC, respectively.