1730 SMRT INTERACTS WITH THE AHR, BUT NOT AS A TRANSCRIPTIONAL SILENCER.

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Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or related chemicals such as polycyclic and/or halogenated aromatic compounds leads to a variety of tissue and species specific toxic and biological effects, the majority of which are mediated by the aryl hydrocarbon receptor (AhR). The AhR complex is cytosolic until ligand binding occurs, whereupon it translocates to the nucleus, dimerizes with the AhR nuclear translocator (ARNT), binds to its specific DNA recognition site and activates gene transcription. As part of an overall effort to characterize transcriptional cofactors regulating AhR signaling, we have examined the ability of the nuclear corepressor, Silencing Mediator for Retinoic acid and Thyroid hormone receptors (SMRT), to interact with and mediate transcriptional repression of AhR. Using a GST pull-down binding assay, we have mapped the major interaction between these factors to the silencing domain of SMRT and the PAS domain of AhR and this major interaction is unaffected by the addition of an AhR ligand. Pull-down and gel retardation assays reveal that, although SMRT does not associate with or interfere in formation of the AhR/ARNT/DNA complex, the binding of SMRT to AhR blocks its ability to bind ARNT or DNA. In contrast to the protein-protein interaction results, transient co-transfection experiments reveal that SMRT does not repress TCDD-dependent induction of a DRE-driven luciferase reporter gene in mammalian cells (Hepa1c1c7, MCF-7, and BG-1), but may actually enhance it. Thus, although SMRT can clearly interact with the AhR prior to its dimerization with ARNT and DNA binding, SMRT does not appear to repress AhR-dependent signaling in these cells. This work was supported by NIEHS grants ES07072 and ES05707.

1731 A LABILE FACTOR REGULATES INDUCTION OF NQOR BY TCDD AND PHENOLIC ANTIOXIDANTS.

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NAD(P)H:quinone reductase (NQOR, DT-diaphorase) catalyzes the two electron reduction of quinones and quinoid chemicals. Induction of NQOR constitutes a major mechanism of defense against toxicity of quinones, azo chemicals, and other redox-cycling chemicals. Prototypical inducers of NQOR include TCDD, a potent agonist of the Ah receptor, and tert-butylhydroquinone (tBHQ), a phenolic antioxidant. Induction of NQOR by TCDD involves binding of TCDD to AhR, AhR/Arnt dimer formation, and binding of the dimer to a dioxin response element (DRE) in the upstream region of NQOR. The antioxidants induce NQOR through a pathway involving activation of the Nrf2 transcription factor, which dimerizes with a Maf protein and binds to an antioxidant response element (ARE). However, the molecular mechanism of the induction by these inducers is not well understood. To gain new insights into the molecular steps of the induction, we examined the effect of cycloheximide (CHX), a potent inhibitor of protein synthesis, on the transcriptional regulation of NQOR by TCDD and tBHQ. Northern blot analyses reveal that CHX blocks induction of the enzyme by both TCDD and tBHQ. The inhibition occurs in a dose- and time- dependent manner, and does not affect the mRNA stability of NQOR. These results demonstrate that CHX inhibits both DRE- and ARE-mediated transcriptional control of NQOR. The blocking involves inhibition of protein synthesis. Time course analyses of the inhibition implicate a labile factor in the induction. Taken together, our results demonstrate that a novel labile factor is required for the transcriptional regulation of NQOR by TCDD and phenolic antioxidants. These findings provide new insights into the regulation of phase II enzymes by chemical inducers.

1732 EVIDENCE OF A BIPHASIC NF-κB RESPONSE TO AN EXCITOTOXIN IN HIPPOCAMPUS.

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Nuclear factor κB (NFκB), a transcription factor shown to have both protective and proinflammatory roles (Trends Biochem Sci 25:434, 2000), was evaluated in organotypic slice cultures from hippocampus, a region that is selectively vulnerable to stroke-type excitotoxicity. Slices were exposed to the excitotoxin NMDA (200 μM) for 20 min, followed by rapid quenching with NMDA receptor antagonists. NFκB activation determined by EMSA was increased 3-fold ($p<0.0001$; n=13), an increase found to be steady 1-5 h post-insult. Following a 24-h recovery period, a further increase of 73% ($p<0.01$; n=13) was observed, suggesting a biphasic nature in NFκB's response during post-insult recovery. In an attempt to discriminate between protective and proinflammatory response components, additive effects were tested with a potent Ampakine (Cortex Pharm.), a neuroprotectant that appears to act through MAP kinase pathways to reduce excitotoxin-induced cytoskeletal damage ($p<0.01$) and pre- and postsynaptic decay. Ampakine alone (10-30 μM) indeed caused an approximate 2-fold increase in NFκB activation after 1-5 h incubation ($p=0.02$), and a 6-fold increase after 24 h ($p<0.0001$). When applied after the 20-min NMDA treatment, however, Ampakine only produced a small additive effect on NFκB activity at 1-5 h post-insult (31 ± 20%) which was not significant. Interestingly, Ampakine did have an additive influence on the NMDA-mediated response (108 ± 41%, $p=0.01$; n=13) at 24 h post-insult. These data provide evidence that the early NFκB response to an excitotoxic episode is primarily acting as a protective compensatory mechanism, as indicated by the inability of a neuroprotectant to contribute additively. Conversely, a second phase of NFκB activation occurring late after the excitotoxic event appears to be pathogenic, perhaps proinflammatory. The latter is indicated by the fact that the delayed NFκB response to the excitotoxin is acting through a separate mechanism from that produced by the neuroprotectant. (Supported by USAMRMC grant DAMD17-99-C9090, NIH grant 1R43NS38404-01).

1733 ANDROGEN RECEPTOR ANTAGONISM BY THE ORGANOPHOSPHATE INSECTICIDE FENITROTHION.

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Organophosphate insecticides are a widely used class of pesticides with high potential for human exposure in both rural and residential environments. We investigated the interaction of the organophosphothioate pesticide fenitrothion [O,O-dimethyl O-(4-nitro-m-tolyl) phosphorothioate] with the androgen receptor (AR). Fenitrothion blocked dihydrotestosterone-dependent AR activity in a dose-responsive and competitive manner in HepG2 human hepatoma liver cells transiently transfected with human AR and an AR-dependent luciferase reporter gene. To determine the antiandrogenic potential of fenitrothion in vivo, seven-week old castrated Sprague-Dawley rats, were dosed once a day for 7 days with testosterone propionate (50 μg/day, sc) plus gavage doses of either corn oil vehicle or fenitrothion (15 or 30 mg/kg/day). An additional group of rats was given testosterone propionate and flutamide (50 mg/kg/day). Motor activity and acetylcholinesterase activity in whole blood and brain were also assessed. Fenitrothion caused significant dose-dependent decreases in the ventral prostate, seminal vesicle, and levator ani plus bulbocavernosus muscles tissue weights. In contrast, blood acetylcholinesterase activity, a standard biomarker of organophosphate poisoning, was only inhibited at the higher dose of fenitrothion (30 mg/kg). Our results demonstrate that fenitrothion is a competitive AR antagonist comparable in potency to the pharmaceutical antiandrogen flutamide and more potent, based on *in vitro* assays, than the known environmental antiandrogens linuron and p,p′-DDE.

1734 DIVERSE SKIN TUMOR PROMOTERS DIFFERENTIALLY ACTIVATE MAPKS IN MOUSE KERATINOCYTES.

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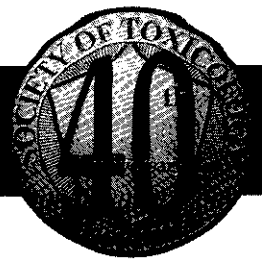
Palytoxin is classified as a non-12-O-tetradecanoylphorbol-13-acetate (TPA)-type tumor promoter; unlike the prototypical skin tumor promoter TPA, palytoxin does not activate protein kinase C. Accordingly, previous studies showed that palytoxin stimulates signal transduction pathways that differ significantly from those stimulated by TPA. The mitogen-activated protein kinase (MAPK) family of serine/threonine kinases plays a key role in coordinating the transduction of intracellular signals to the nucleus. To further investigate the signaling pathways that may be important in the biochemical mechanisms of action of palytoxin, we compared the activation of three major members of the MAPK family by palytoxin and TPA in a keratinocyte cell line derived from initiated mouse skin (308 cells). TPA stimulates robust activation of extracellular signal-regulated kinase (ERK), which is typically activated by mitogens, but does not stimulate significant activation of c-Jun N-terminal kinase (JNK) or p38, which are typically activated by stress. Palytoxin stimulates robust activation of JNK and p38. Surprisingly, palytoxin also stimulates activation of ERK in 308 cells. The two tumor promoters stimulate ERK activation with markedly different kinetics, however. TPA stimulates rapid, transient ERK activation, which peaks within 5 minutes of exposure. By contrast, palytoxin-stimulated ERK activation is detected only after prolonged exposure (120 minutes) and remains elevated through at least 4 hours of exposure. Finally, palytoxin and TPA



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Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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