TRYPTOPHAN METABOLITES, INDOLE-3-PYRUVIC ACID, DL-3-INDOLELACTIC ACID, L-KYNURENINE, AND KYNURENIC ACID ACTIVATE Ah RECEPTOR SIGNAL TRANSDUCTION

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Introduction

Many environmental pollutants have been shown to be ligands and activators of the Ah Receptor (AhR), the most potent being the ubiquitous compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin). While this compound is of major consequence, it is becoming evident that there are numerous other chemicals that can bind and activate AhR[1]. However, the endogenous ligand(s) of AhR and the physiological role(s) of AhR remains an enigma. Our work and the work of others have shown that the amino acid tryptophan and several of its metabolites, as well as UV-photoproducts of tryptophan, are activators of the AhR signaling pathway[2,3]. Given the large number of indole-containing compounds and tryptophan metabolites present in biological systems, we have begun a systematic search for other activators of AhR. Utilizing two screening assays we have developed and optimized (gel retardation analysis (GRA) and chemically-activated luciferase gene expression (CALUX)), we have identified several metabolites of tryptophan that are novel activators of the AhR signal transduction pathway.

Materials and Methods

Gel Retardation Analysis (GRA). Male Hartley guinea pig cytosol was used as the source of AhR. The cytosol was incubated with DMSO (20 ul/ml), TCDD (20 nM) of the indicated chemicals or extracts for 2 h at 20^0 C followed by electrophoresis and autoradiography as described previously[4]. Nuclear extracts were obtained by treating the cells for 2 hours with the chemical prior to harvesting the nuclei[2]. Quantification of the amount of protein-DNA complex formed was carried out using a Molecular Dynamics Phosphoimager SI.

Chemically activated luciferase expression (CALUX) bioassay. The CALUX bioassay utilizes recombinant mouse hepatoma (H1L1.1c2) cells developed and maintained as previously described[5]. These cells, derived from the mouse Hepa1c1c7 line, contain a stably integrated DRE-driven firefly luciferase reporter gene whose transcriptional activation occurs as a time and/or dose dependent AhR activated response[5]. For chemical treatment, H1L1.1c2 cells were grown in 6 well tissue culture plates and incubated with DMSO, TCDD (in DMSO) or the test chemical for four hours at 37^0 C. After incubation the cells were lysed and luciferase activity determined using a *Dynatech ML3000* Microplate Luminometer with automatic injection of Promega luciferin[5]. Luciferase activity, normalized to sample protein concentration was determined using the

fluorescamine protein assay and BSA as the protein standard[6]. Data are expressed as means and S.E. of four replicates/treatment. Comparisons between means were performed using ANOVA and the Scheff's F test and considered significant at the $p < 0.05$ level.

Test Chemicals L-kynurenine (L-K) and kynurenic acid (KA) were obtained from from ICN. Indole-3-pyruvic acid (IPA) and DL-3-indolelactic acid (ILA) were obtained from Aldrich. 3-hydroxy-DL-kynurenine (3-OH-K), anthranillic acid (A-A), and kynuramine (K-NH4) were obtained from Sigma. Chemical stocks (100 mM) were prepared as follows: L-K, ILA, and IPA were dissolved in DMSO; KA and A-A were dissolved in glass distilled water; and, 3-OH-K (50 mM) was dissolved 0.5N HCL-Tris pH8.

Results and Discussion

Incubation of the test chemicals with guinea pig cytosol shows that the indoles, ILA and IPA, are capable of activating AhR and, surprisingly, so did L-kynurenine and kynurenic acid (Fig. 1). Kynuramine, anthranillic acid, and 3-OH-K were inactive in this assay. The CALUX bioassay (Fig. 2) shows that the response obtained from treating cells with 100 uM L-K and IPA was not significantly different from 1 nM TCDD. ILA and KA were slightly less efficient at inducing the luciferase reporter *in vivo* than IPA, although in the GRA assay they appeared similar. The differences between the two assays may be attributed to differences in solubility, uptake, and/or metabolism. These problems have been discussed elsewhere[7]. K-NH4 and A-A were weak or inactive as inducers. Further evidence that these chemicals function as *in vivo* activators of AhR was obtained by isolating the nuclei from treated cells. To validate the *in vitro* assays, nuclear extracts of treated cells were examined. Our results from such studies indicate that L-K, IPA, ILA, and kynuruenic acid stimulate AhR nuclear translocation (data not shown), indicating that these chemicals also activate AhR in intact cells. Interestingly, many of these same chemicals, L-K and IPA, are competitive inhibitors of P4501A1-dependent EROD activity (data not shown), suggesting that they may also be substrates for the AhR-induced enzyme.

Tryptophan metabolism has been actively studied because so many of the metabolites have neurogenic activity and some are known carcinogens producing metallurgic oxidative DNA damage[8]. One well characterized tryptophan metabolic pathway results in the formation of serotonin and melatonin. Another branch leads to tryptamine which can easily be converted to the hallucinogenic compound N,N-dimethytryptamine. The kynurenine branch also produces neurogenic compounds, kynurenic acid and quinolinic acid, an antagonist and agonist of the NMDA receptor, respectively[9]. Since many physiological processes can be affected by tryptophan metabolites; vision, well being, sleep cycles, cancer susceptibility, etc., it is not surprising that the enzymatic reactions that determine which metabolic pathway tryptophan will follow are highly regulated. Therefore, a back-up system to help regulate the levels of these chemicals at key branch points might provide an adaptive advantage. Thus, it is possible that AhR may help regulate the levels of neurogenic or cell damaging tryptophan metabolites.

Acknowledgments

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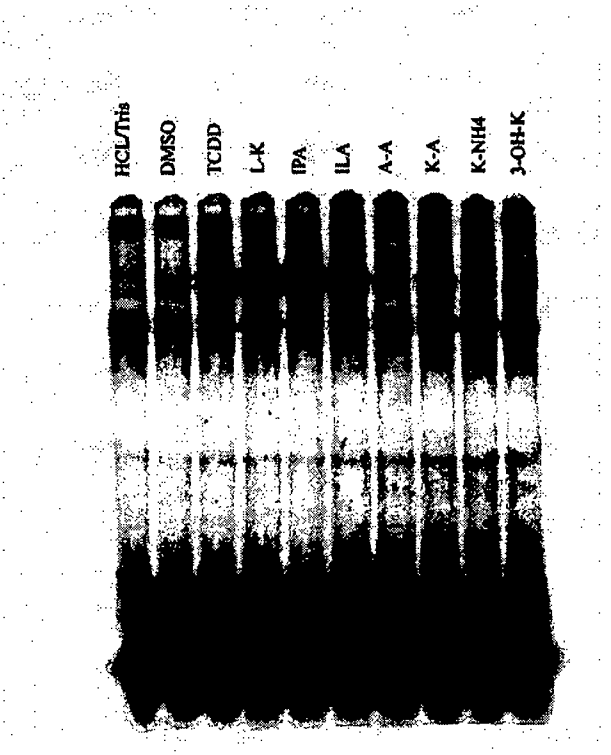

Fig. 1. GRA of tryptophan metabolites.

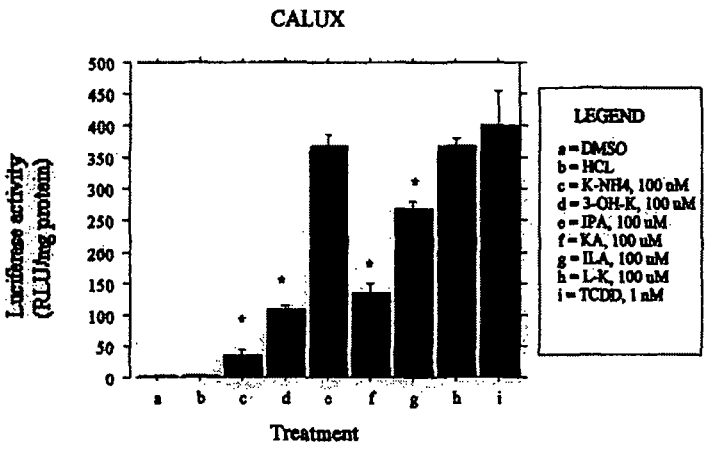

Fig. 2. Relative induction of the luciferase reporter plasmid in Hepa1.1 cells by the indicated tryptophan metabolites following a 4.5 hr treatment. IPA and L-K were not significantly different from TCDD.