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Tryptanthrins: A Novel Class of Agonists of the Aryl Hydrocarbon Receptor

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ABSTRACT. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related environmental pollutants exert most of their adverse effects such as immunosuppression, induction of endocrine dysfunction, tumor promotion, and teratogenicity via the aryl hydrocarbon or dioxin receptor. While most potent agonists of the aryl hydrocarbon receptor are of synthetic origin, an increasing number of natural compounds are now recognized as receptor agonists. Our findings demonstrated that some tryptanthrin derivatives biosynthesized in incubations of *Candida lipolytica* with tryptophan and anthranilic acid or its derivatives were agonists of the aryl hydrocarbon receptor. The biosynthetic products 8-methyltryptanthrin, 8-chlorotryptanthrin, and 8-bromotryptanthrin induced cytochrome P4501A1 mRNA and protein in rat hepatocytes in primary culture, characteristic features of aryl hydrocarbon receptor agonists. Log-probit analysis of the catalytic activity of cytochrome P4501A1, 7-ethoxyresorufin O-deethylase (EROD), revealed EC_{50} induction values of 1.7, 0.25, and 0.17 μM for 8-methyltryptanthrin, 8-chlorotryptanthrin, and 8-bromotryptanthrin, respectively. Interestingly, the nonsubstituted tryptanthrin molecule, biosynthesized from the common physiological precursors tryptophan and anthranilic acid, was also active as an inducer. The specificity of the inducing effect of tryptanthrins was demonstrated in gel retardation experiments in Hepa-1 mouse hepatoma cells, showing the characteristic interaction of the activated aryl hydrocarbon receptor with an oligonucleotide containing a xenobiotic-responsive element. It is suggested that the receptor may be part of a defense system protecting higher organisms from secondary metabolites formed by the microflora of the host or its environment. BIOCHEM PHARMACOL **54**;1: 165–171, 1997. © 1997 Elsevier Science Inc.

KEY WORDS: aryl hydrocarbon receptor; cytochrome P4501A1; 7-ethoxyreorufin O-deethylase; induction; rat hepatocytes; tryptanthrins

Among the halogenated aromatic hydrocarbons, certain polychlorinated dibenzo-*p*-dioxins (PCDD),§ polychlorinated dibenzofurans (PCDF), and polychlorinated biphenyls (PCB) represent a potential risk to human health because of their toxic properties [1, 2]. The prototype of this subgroup of compounds, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), exhibits a number of adverse effects in laboratory animals such as immunosuppression, induction of endocrine dysfunction, tumor promotion, and teratogenicity [1–5]. Most of these effects are mediated through binding of TCDD or related compounds to the aryl hydrocarbon receptor (AHR). The AHR, which has been cloned in human and mice [6, 7], is a ca. 100 kDa helix-loop-helix protein mainly present in its inactive form in the cytoplasm, bound to the chaperone peptide hsp90. Upon ligand binding, hsp90 is dissociated [8], and the AHR–ligand complex binds to the aryl hydrocarbon receptor nuclear translocator (ARNT) [9, 10]. The AHR–ARNT–ligand complex acts as a nuclear transcription factor that binds specifically to xenobiotic-responsive elements (XREs) in the 5′-flanking region of responsive genes, thereby enhancing their transcription [11, 12]. A number of drug-metabolizing enzymes such as cytochromes P450 (CYP) 1A1 and 1A2, a UDP-glucuronosyltransferase, a glutathione-*S*-transferase, and an NADPH:quinone oxidoreductase [11–15] belong to this group of genes. Their transcriptional activation may be part of an adaptive defense program aimed at enhancing the metabolism and subsequent elimination of the potentially dangerous inducing agent [3].

The best-understood example of a gene regulated under the control of the AHR is CYP1A1 [11, 12], which is transcribed upon binding of the AHR–ARNT–ligand com-

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§*Abbreviations:* AHR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; 8BT, 8-bromotryptanthrin; 8CT, 8-chlorotryptanthrin; CYP, cytochrome P450; DMEM, Dulbecco's modified Eagle's medium; EROD, 7-ethoxyresorufin O-deethylase; LDH, lactate dehydrogenase; 8MT, 8-methylyltryptanthrin; PCB, polychlorinated biphenyls; PCDD, polychlorinated dibenzo-*p*-dioxins; PCDF, polychlorinated dibenzofurans; RT-PCR, reverse transcriptase polymerase chain reaction; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; XRE, xenobiotic-responsive element.

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plex to XREs in its 5′-upstream region. Because the potency of a number of AHR agonists as inducers of CYP1A1 and their toxicity concerning a number of endpoints are in fairly good agreement [2], induction of CYP1A1 mRNA, protein, or catalytic activity, measured as 7-ethoxyresorufin O-deethylase (EROD), has been widely used as a surrogate for the analysis of the toxic potency of the inducer relative to TCDD, the most potent agonist [2, 16, 17].

In addition, measurements of induction of CYP1A1 gene expression serve as a powerful tool for the screening of potential AHR agonists, and a number of biosynthetic compounds with agonistic activity are now recognized. Potent inducers derived from indolo-3-carbinole, a constituent of cruciferous vegetables, are indolo[3,2-*b*]carbazole and a methylated derivative, 3,11-dimethylindolo[3,2-*b*]carbazole [18]. The structural similarities that exist between these compounds and the microbial metabolite tryptanthrin [19] led us to investigate the potential of the class of tryptanthrins as AHR agonists. It was found that among a number of compounds, three tryptanthrin derivatives, formed in incubations of *Candida lipolytica*, exhibited a high agonistic potency.

MATERIALS AND METHODS

Chemicals

[^{32}P]-ATP was from Amersham (Braunschweig, Germany), polyclonal anti-CYP1A antibodies were a gift from Dr. P. Beaune (INSERM U75, CHU Necker, Paris, France), polyclonal anti-AHR antibodies were from Biomol (Hamburg, Germany), poly-ds(dIxdC), one-phor-all PLUS buffer, and polynucleotide kinase T4 from Pharmacia (Freiburg, Germany), and Dulbecco's modified Eagle's medium (DMEM) from Seromed (Berlin, Germany). The deoxynucleotide triphosphates ATP, CTP, GTP, and TTP, and RNAsin® were from Promega (Madison, WI), AMV reverse transcriptase from United States Biochemical Corporation (Cleveland, OH), and oligo(dT)$_{15}$ from Boehringer (Mannheim, Germany). A 32 bp sequence of the 5′-upstream region of the mouse CYP1A1 gene, containing the xenobiotic-responsive element (XRE) DRE3 (5′-GATCTGAGCTCGGAGTTGCGTG AGAAGAGCCG-3′), and an overlapping complementary sequence 3′-ACTCGAGCCTCAACGCACTCTTCTCG GCTAG-5′, according to Denison *et al.* [20], were synthesized by Appligene (Heidelberg, Germany) as coding and complementary sequence.

Tryptanthrin derivatives were synthesized and purified as previously described [19, 21]. Briefly, *Candida lipolytica* CBS 2073 was incubated in minimal medium supplemented with L-tryptophan (Serva, Heidelberg, Germany), and one of the following anthranilic acid derivatives (the products are listed in parentheses): anthranilic acid (trypthantrin), 5-methylanthranilic acid (2-methyltryptanthrin), and 5-chloroanthranilic acid (2-chlorotryptanthrin). Supplementation of anthranilic acid and one of the following tryptophan derivatives led to additional tryptanthrin derivatives (listed in parentheses): 4-methyltryptophan (7-methyltryptanthrin), 5-methyltryptophan (8-methyltryptanthrin), and 5-bromotryptophan (8-bromotryptanthrin). The chemical synthesis of substituted tryptanthrins (3-nitrotryptanthrin, 8-chlorotryptanthrin, and 10-methyltryptanthrin) was performed according to published methods [22, 23]. The identity of the compounds was confirmed by high resolution mass spectrometry. The purity of all preparations used exceeded 98% as revealed by HPLC analysis.

Cell Culture and Treatment

Hepatocytes isolated from adult male Wistar rats (Savo, Kisslegg, Germany) were plated at a density of 100,000 cells/cm^2 on collagen-coated Petri dishes (9 cm in diameter) in DMEM supplemented with 10% calf serum, 10% fetal calf serum, 0.1 μM dexamethasone, 100 units penicillin per mL, and 100 μg streptomycin per mL as previously described [17]. After 2 hr, medium was replaced by fresh medium and TCDD or tryptanthrins were added in DMSO (0.5% final concentration), and cells were harvested after an additional 24 or 48 hr as indicated. Hepa-1 cells were plated and treated under identical conditions. At the time of treatment, the Hepa-1 cell cultures were approximately semiconfluent.

Northern Blotting and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Total RNA from hepatocyte cultures was prepared, submitted to electrophoresis, and blotted as described [24]. Analysis of CYP1A1 mRNA expression was achieved by hybridization to a mouse CYP1A2 cDNA probe [25]. Loading of the gels was controlled using a cDNA probe for the rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene [26]. The probes were labeled by the random primer method using [α-^{32}P] dCTP. Membranes were prehybridized and hybridized, and mRNA was visualized by autoradiography at −80°C using intensifying screens for 1 to 4 days.

Reverse transcription was performed in a final volume of 15 μL containing 50 mM Tris-HCl (pH 8.3), 8 mM $MgCl_2$, 50 mM NaCl, 1 mM dithiothreitol (DTT), 1 mM each of deoxynucleotide triphosphates ATP, CTP, GTP, and TTP, 0.5 units RNAsin®, 10 units AMV reverse transcriptase, 1 μM oligo(dT)$_{15}$, and 0.1 μg total RNA. The samples were incubated at 42°C for 120 min, and reverse transcriptase was inactivated by heating to 95°C for 5 min. 2.5 μL of these complementary DNA samples were used in a polymerase chain reaction, which was performed as described by Vanden Heuvel *et al.* [27].

Immunoblotting and Assay of CYP1A1 Activity

Cells were washed with cold PBS, harvested by scraping off in lysis buffer (10 mM Tris, pH 7.4, 10 mM sodium chloride, 1.5 mM magnesium chloride, 0.05% sodium azide, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiotreitol), centrifuged for 5 min at 1000 × *g*, and homogenized with a Dounce homogenizer in buffered sucrose on ice. The pellet

was suspended in lysis buffer and proteins (50 μg) were separated by SDS-PAGE and electroblotted on Immobilon P membranes (Millipore, Dreieich, Germany). The blots were blocked for 1 hr with Tris-buffered PBS containing 0.05% Tween 20 and 5% bovine serum albumin and CYP1A1 was detected with a 1:100 dilution of rabbit anti-CYP1A antibodies [28]. The immunoreaction was visualized using a horseradish peroxidase-conjugated swine anti-rabbit antibody (DAKO Diagnostica, Hamburg, Germany), followed by enhanced chemoluminescence detection with the ECL technique (Amersham, Braunschweig, Germany).

EROD activity was determined according to Burke and Mayer [29]. Concentration–response curves were calculated using a computerized log-probit procedure (SAS Institute, Cary, NC; technical report P-179), which also allows calculation of EC_{50} values. The variability of the EROD responses in three different cell preparations did not exceed 10%.

Preparation of Nuclear Extracts and Gel Mobility Shift Assay

For hybridization, 50 μg of coding or complementary ds-oligo were dissolved in 1 × TNE buffer (10 mM Tris, 100 mM NaCl, 1 mM EDTA; pH 7.8) at a final volume of 100 μL, and denaturated at 80°C. After cooling to room temperature, the double-stranded (ds) oligo was precipitated with 2 μL 1 M $MgCl_2$, 20 μL 3 M sodium acetate, 78 μL H_2O, and 600 μL ice-cold ethanol. After incubation at −70°C for 30 min, the ds-oligo was sedimented by centrifugation at 10,000 × *g* for 10 min. The pellet was washed with ice-cold ethanol, air dried, dissolved in 1 × TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), and the ds-oligo concentration was adjusted with TE buffer to 1 pmol/μL. Successful hybridization was tested by electrophoresis in a nondenaturating polyacrylamide gel (12% acrylamide in 1 × TBE buffer). The single stranded (ss) oligos and purified ds-oligo were used as standards. For 5′-labeling, 10 μL 10 × one-phor-all buffer PLUS (100 mM Tris/acetate, 100 mM magnesium acetate, 500 mM potassium acetate; pH 7.5), 1.5 μL (9.7 U/μL) polynucleotide kinase T4, 2.5 pmole ds-oligo, 3 μL γ-[^{32}P]-ATP (10 pmol, 1110 MBq), and H_2O (added to a final volume of 100 μL), were incubated at 37°C for 40 min. Nonincorporated ATP was removed using a NICK® Spin Column (Pharmacia, Freiburg, Germany).

Hepa-1 cultures were incubated for 60 min with the solvent (DMSO), 10^{-9} M TCDD, 5 μM 8-methyltryptanthrin (8MT), 2.5 μM 8-bromotryptanthrin (8BT), or 5 μM 8-chlorotryptanthrin (8CT) at 37°C. The preparation of nuclear extracts was performed using the method of Miller *et al.* [30]. For gel retardation experiments, 20 μg nuclear protein were preincubated for 15 min at room temperature in a final volume of 20 μL, containing 25 mM HEPES (pH 7.9), 1 mM EDTA, 150 mM KCl, 0.7 mM dithiotreitol, 10% (v/v) glycerol [31], 1 μg ds-poly(dIxdC), and 0.5 μg denaturated herring sperm DNA. Then, 25 fmol of [^{32}P]-ds-oligo were added and incubated at room temperature.

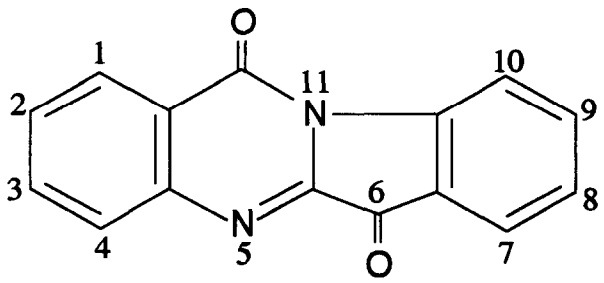

FIG. 1. Structure of tryptanthrin.

After 15 min the incubation mixture was transferred to a 6% polycrylamide gel in TGE buffer (50 μM Tris-HCl, 0.38 mM glycine, 2 mM EDTA; pH 8.5), and proteins were separated at 4°C and 130 V using TGE as electrode buffer. After drying of the gel, bands were visualized by autoradiography. In competition experiments, a 25-, 50-, and 750-fold surplus of unlabeled ds-oligo was added. In another set of experiments, 1 μL of anti-AHR antibodies was preincubated for 20 min at room temperature with nuclear extract and [^{32}P]-ds-oligo. Then, ds-poly(dIxdC) and denaturated herring sperm DNA were added, and the mixture was incubated for another 15 min at room temperature before electrophoresis.

RESULTS

In rat hepatocytes in primary culture, induction of CYP1A-catalyzed EROD activity was tested using the prototype AHR agonist TCDD, and a number of tryptanthrin (Fig. 1) derivatives, biosynthesized in incubations of *Candida lipolytica* with tryptophan and anthranilic acid or anthranilic acid derivatives. Sigmoidal log-probit functions were fitted to the mean values from three independent cell preparations, incubated over 24 hr with each compound. From the obtained concentration–response curves shown in Fig. 2, EC_{50} values of 1.7, 0.25, and 0.17 μM for 8MT, 8CT, and 8BT, respectively, were calculated. For TCDD, an EC_{50} value of 3.7×10^{-11} M (0.000037 μM) was obtained (not shown). The curves showed a typical sigmoidal shape, reaching a maximum EROD activity in the range of 200–300 pmol per mg homogenate per min. The EC_{50} values for a number of additional biosynthetic and synthetic tryptanthrin derivatives, including unsubstituted tryptanthrin, are given in Table 1. With the exception of 3-nitrotryptanthrin, all tryptanthrin derivatives tested were effective as EROD inducers. In particular, EC_{50} values between 1.7 and 6.3 μM were obtained for the unsubstituted tryptanthrin and the derivatives mono-methylated at positions 2, 7, 8, or 10. The EC_{50} values for the potent tryptanthrins did not change significantly when a treatment period of 48 hr was used.

To investigate whether induction by tryptanthrins was influenced by cytotoxicity, leakage of lactate dehydrogenase (LDH) was tested. Using estimates for EC_{50} values obtained from concentration–response plots of the relative

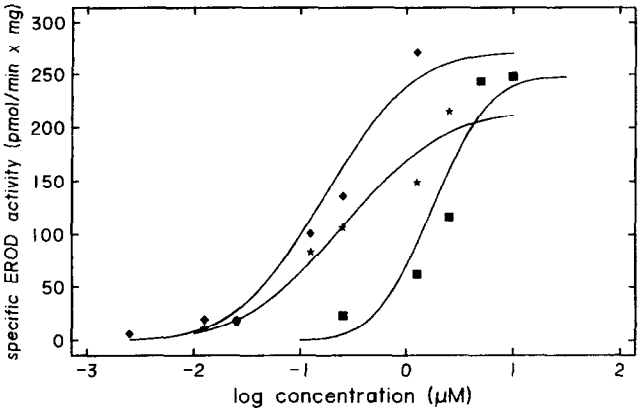

FIG. 2. Induction of EROD activity in rat hepatocytes in primary culture treated with tryptanthrins. Cell cultures were treated for 24 hr with various concentrations of 8-methyltryptanthrin (squares), 8-bromotryptanthrin (rhombuses), or 8-chlorotryptanthrin (asterisks). Symbols represent means of three independent cell preparations, while curves were calculated from these data using a computerized log-probit fitting procedure.

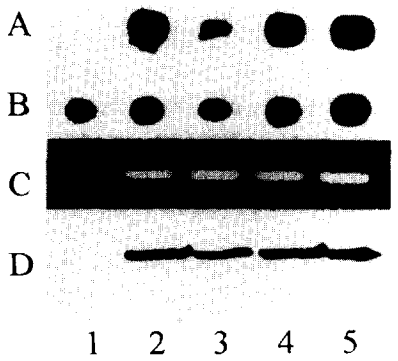

FIG. 3. Effect of tryptanthrins on the levels of CYP1A1 mRNA and CYP1A protein in rat hepatocytes in primary culture. Cell cultures were treated for 24 hr with DMSO (lane 1), 10^{-9} M 2,3,7,8-TCDD (lane 2), 5 μM 8-methyltryptanthrin (lane 3), 2.5 μM 8-bromotryptanthrin (lane 4), or 5 μM 8-chlorotryptanthrin (lane 5). Total RNA was isolated and analyzed by Northern blotting (A) or reverse transcriptase polymerase chain reaction (C). The mRNA of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was determined as a loading control (B) for the Northern blot. For Western blotting (D), cells were treated for 48 hr, harvested, homogenized, and proteins were separated by PAGE and electroblotted. CYP1A1 was detected using rabbit anti-CYP1A antibodies. The patterns are representative of four independent experiments.

leakage of LDH (% of maximum release), it was found (Table 1) that three out of nine derivatives enhanced LDH leakage, namely, those bearing the substituents 3-nitro > 8-bromo > 8-chloro. With all other tryptanthrins, no significantly enhanced LDH leakage was observed at concentrations up to 100 μM.

Total RNA was isolated 24 hr after addition of TCDD or the tryptanthrins 8MT, 8BT, or 8CT, and the level of CYP1A1 mRNA was determined using Northern blotting or RT-PCR. It was found that TCDD led to a strong induction of the CYP1A1 transcript at 10^{-9} M (Fig. 3). In total RNA from TCDD-treated rats, the two transcripts of CYP1A1 and 1A2 were detectable with the same probe (not shown), while the migration properties (molecular weight) of the single transcript from hepatocyte cultures strongly suggest that CYP1A1, but not CYP1A2, was induced to a detectable extent. A marked increase compared to the untreated control was also obtained with 5 μM 8MT, 2.5 μM 8BT, or 5 μM 8CT. On the protein level, the compounds significantly induced CYP1A 48 hr after addition to the cultures, as revealed by Western blot analysis. The antibody used bound to both CYP1A isoforms in liver homogenates from TCDD-treated rats (not shown), while the migration properties (molecular weight) of the single band detected in homogenates from hepatocyte cultures strongly suggest that CYP1A1, but not CYP1A2, was induced to a detectable extent.

TABLE 1. EC_{50} values of tryptanthrin derivatives as inducers of 7-ethoxyresorufin O-deethylase (EROD) activity, and leakage of lactate dehydrogenase (LDH) activity in rat hepatocytes in primary culture

Compound	m.w.	EC_{50} (μM) EROD	EC_{50} (μM) LDH
Tryptanthrin	248	6.1	>100
10-Methyltryptanthrin	262	5.2	>100
8-Methyltryptanthrin	262	1.7	>100
7-Methyltryptanthrin	262	2.3	>100
2-Methyltryptanthrin	262	6.3	>100
8-Bromotryptanthrin	327	0.17	26
8-Chlorotryptanthrin	282	0.25	85
2-Chlorotryptanthrin	282	2.2	>100
3-Nitrotryptanthrin	293	n.d.*	18

*Not detectable.

In another set of experiments, the hypothesis stating that the induction of CYP1A1 catalytic activity, mRNA, and protein might be due to a specific AHR activation resulting in binding of the AHR to an XRE-comprising ds-oligo was tested. In cultured rat hepatocytes, TCDD and tryptanthrins led to an increased binding of the XRE-containing ds-oligo to more than one peptide (not shown). In TCDD-treated Hepa-1 cells, widely used for the analysis of AHR activation in gel retardation experiments, a single retarded band, presumably comprising the activated AHR, was found. Addition of 5 μM 8MT, 2.5 μM 8BT, or 5 μM 8CT resulted in a pronounced binding of AHR to the XRE-ds-oligo as visualized by gel retardation (Fig. 4). TCDD served as a positive control, causing a strong binding at a concentration of 10^{-9} M. Finally, the specificity of the retardation was confirmed in competition experiments with 10^{-9} M TCDD plus unlabeled double-stranded XRE oligonucleotide, or in incubations with anti-AHR antibodies, showing the expected reduced interaction between the labeled oligonucleotide and the ligand-activated AHR.

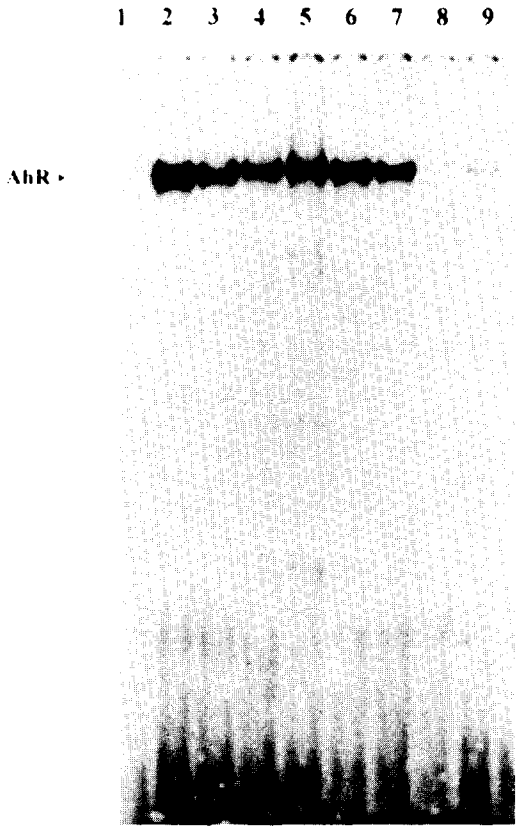

FIG. 4. Gel retardation of nuclear extract proteins from tryptanthrin-treated Hepa-1 cells using a [^{32}P]-labeled double-stranded XRE oligonucleotide. Incubations contained nuclear extract from untreated Hepa-1 cells (lane 1), or after 24 hr of treatment with 10^{-9} M TCDD (lane 2), 5 μM 8-methyltryptanthrin (lane 3), 2.5 μM 8-bromotryptanthrin (lane 4), 5 μM 8-chlorotryptanthrin (lane 5), 10^{-9} M TCDD plus 625 fmol (lane 6), 1.25 pmol (lane 7), or 18.75 pmol (lane 8) unlabeled double-stranded XRE oligonucleotide, or 10^{-9} M TCDD after preincubation with 1 μL of anti-AHR antibodies (lane 9).

DISCUSSION

In this study we have shown for the first time that tryptanthrin and a number of tryptanthrin derivatives are agonists of the rat AHR. Analysis of induction of CYP1A1 activity, a widely used method for the determination of the relative potency of a given AHR agonist [2, 16, 17], showed that the EC_{50} values of the most potent tryptanthrins were higher by ca. three orders of magnitude than that of TCDD. It should be taken into account, however, that hepatocytes exhibiting a high capacity for drug metabolism may metabolize tryptanthrins and may thus lower the effective intracellular concentration of the ligands, in particular that of the nonsubstitued tryptanthrin. In the case of the potent halogenated tryptanthrins, however, EC_{50} values of EROD induction did not change significantly when the incubation period was extended to 48 hr, suggesting that these compounds are relatively resistant to metabolic degradation.

Interestingly, substitution at lateral position 8, either by a methyl group or a halogen substituent, led to the most potent inducers found in our study. Introduction of a nitro group at position 3 completely abrogated the inducing potency of the molecule. This may reflect a structural restriction for AHR binding resulting from the bulky nitro substituent. Furthermore, the 3-nitrotryptanthrin, being the most cytotoxic derivative tested, may inhibit synthesis of CYP1A1.

Analysis of CYP1A1 mRNA and protein by Northern or Western blotting, respectively, was in agreement with the results of the EROD assay. It was found that both TCDD and the three most potent tryptanthrins strongly induced CYP1A1 mRNA and protein, respectively, 24 or 48 hr after addition to the cultures. RT-PCR of total RNA, though not quantitative, led to the same result.

Gel retardation experiments in Hepa-1 cells demonstrated that the three most potent tryptanthrins, 8MT, 8BT, and 8CT, transform the AHR into an XRE-binding form, which is in agreement with previous findings on other synthetic and biosynthetic inducers [10, 11, 18].

The question of the physiological ligand(s) of the AHR remains controversial. Besides the synthetic ligands, a number of natural ligands are now recognized, such as indolo-3-carbinol and 3,3-diindodolylmethane in cruciferous vegetables [32, 33]. The highly potent AHR ligand indole[3,2-*b*]carbazole [18] was formed *in vitro* from indolo-3-carbinol under acidic conditions [34], in fecal suspensions [35], and in the intestinal tract of mice treated orally with indolo-3-carbinol [33]. Moreover, incubation of rat fecal suspensions with tryptophan led to the formation of AHR agonists of unknown structure [35]. The structural similarities between tryptanthrins [19] and indole[3,2-*b*]carbazole are evident. Indeed, the potency of 8BT, the most potent tryptanthrin, is in the range reported for EROD induction in Hepa-1 cells with indole[3,2-*b*]carbazole and 3,11-dimethylindole[3,2-*b*]carbazole [36]. It remains to be elucidated if tryptanthrins are more potent in gel shift or reporter gene transcription experiments than as EROD inducers, as can be concluded for indolo[3,2-*b*]carbazole from different reports [18, 36]. The most interesting feature of tryptanthrins, however, is their biosynthesis from tryptophan and anthranilic acid derivatives by *Candida lipolytica*, a yeast found in human food [37]. Thus, the nonsubstituted tryptanthrin is the first identified microbial metabolite derived from ubiquitous physiological precursor molecules, which acts as a potent AHR agonist. Substituted tryptanthrins may be formed from anthranilic acid derivatives present in plants. However, no information is available in the literature on the possible occurrence of halogenated precursors of tryptanthrin biosynthesis in plants or microorganisms. Our findings indicate that the AHR may be part of a defense system protecting the organism from permanent challenge by microbial metabolites such as tryptanthrins. The role of other metabolites formed by microorganisms playing a more prominent role in the gastrointestinal tract is currently being investigated in our laboratory.

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