MICROBIAL METABOLITES DERIVED FROM TRYPTOPHAN AS CYP 1A1-INDUCERS

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Introduction

The physiological ligand(s) of the aryl hydrocarbon receptor (AhR) have not been identified so far. Besides a number of potent halogenated agonists such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a variety of exogenous non-halogenated compounds have been suggested to be likely candidates for physiological ligands. In addition, the existence of (an) endogenous ligand(s) cannot be ruled out.
A number of potent exogenous non-halogenated agonists are indole derivatives probably derived from tryptophan. Interestingly, the findings available so far point to a possible role of microorganisms in the conversion of tryptophan into AhR agonists. One example is the yeast *Candida lipolytica* which forms various tryptanthrins, moderately potent AhR agonist, from the precursors trypthophan and anthranilic acid or its derivatives[1].
Furthermore, malassezin formed by a dermal yeast, *Malassezia furfur*, acts as a potent inducer of cytochrome P450(CYP)1A1[2], possibly resulting from a cyclization of malassezin to indolo[3,2-*b*]carbazole, a highly potent AhR agonist[3].
Incubation of trytophan with rat feces has been reported to lead to the formation of CYP1A1 inducers[4]. Here, we report on the capacity of an extract derived from an incubation of rat feces with tryptophan-enriched medium to form CYP1A1-inducing activity.

Materials and Methods

Fermentations were carried out under anaerobic conditions in an aqueous medium containing 5.5 g Na_2HPO_4 dihydrate, 2.7 g NaH_2PO_4 hydrate, 10 g glucose, and 0.204 g tryptophan per liter. 1 g freshly obtained rat faeces was added and the mixture was incubated under agitation (200 rpm) at 37°C for 24 h in the dark. After filtration, the suspension was extracted with an equal volume of ethyl acetate. After evaporation at 40°C under reduced pressure 92 mg of an oily residue were obtained from a fermentation volume of 10 liter. The residue was dissolved in DMSO at a concentration of 4.6 mg/100 µl (stock solution). 1:10 and 1:100 dilutions of the stock solution were prepared with DMSO. 12.5 µl of the stock solution or the dilutions were added to H4IIE rat hepatoma cells which were cultured as described[5] in a total volume of 4 ml medium per dish. DMSO served as a negative, TCDD (10^{-9}M) as a positive control. The cells were incubated at 37°C in a controlled air/CO_2 (95:5 %) atmosphere. After 48 h cells were washed, harvested, and homogenized, and CYP1A1-catalyzed 7-ethoxyresorufin O-deethylase (EROD) activity was determined as described[5].

Results and Discussion

An overview of the reports on AhR agonists derived from tryptophan/indoles is given in Table. 1. Besides formation of tryptophan photoproducts[6] and the acid-catalyzed conversion of indole-3-carbinol, a natural food constituent, in the stomach into highly potent AhR agonist such as to indolo[3,2-*b*]carbazole[3], a few reports describe the formation of AhR agonists/CYP1A1 inducers by microorganisms. These include members of the residential flora of human skin such as the yeasts *Candida lipolytica* and *Malassezia furfur* as well as the intestinal microflora. In the latter case, no individual microorganisms able to synthesize AhR agonists have been identified so far.
A number of endogenous metabolites of tryptophan have been identified as relatively weak AhR ligands[7].

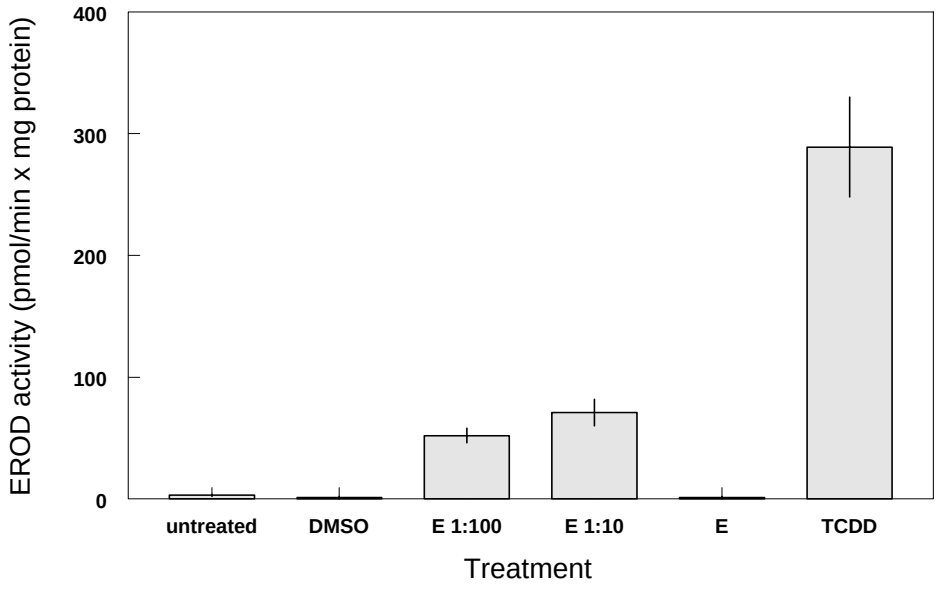
Table 1: AhR agonists derived from tryptophan

Agonist	Effect measured	Source/origin
indolo[3,2-*b*]carbazole	EROD induction in Hepa-1 cells[3]	formed from indole-3-carbinol (acid-catalysed)[3]
6-formylindolo[3,2-*b*]carbazole and 6,12-diformylindolo[3,2-*b*]carbazole	induction of EROD activity and CYP1A1 mRNA in human keratinocytes[6]	formed during irradiation of tryptophan in aqueous solution[6]
tryptamine, indole acetic acid	AhR binding, reporter gene activation[7]	endogenous metabolites
tryptanthrins	EROD induction and gel mobility shift in rat hepatocytes[1]	from tryptophan and anthranilic acid (and derivatives thereof) by *Candida lipolytica*[1]
malassezin	EROD induction in rat hepatocytes[2]	formed by *Malassezia furfur*[2]
unidentified products	EROD induction in Hepa-1 cells[4]	incubation of tryptophan – enriched medium with rat feces[4]

Our findings suggest that the AhR may be part of a defense system aimed at the initiation of metabolic degradation of tryptophan derivatives derived from microorganisms living on human skin and/or in the human intestine. Furthermore, the system should be able to inactivate inducing compounds present in food including contamination by microbial products. The AhR may not only act as a sensor for possibly harmful tryptophan metabolites but also for the producing microorganism itself, thus involved in a defensive effort against those bacteria and/or yeasts.
Induction of CYP1A1-catalyzed EROD activity in H4IIE cells was analyzed with an extract from a fermentation of tryptophan-enriched medium with rat feces. The stock solution was inactive as inducer whereas the 1:10 and 1:100 dilutions led to a significant induction (Fig.

1). Microscopic inspection revealed that addition of the stock solution to the cultures resulted in significant cell death whereas the dilutions led to a minor suppression of cell growth. This effect may explain the lack of EROD induction with the stock solution. The fact that the 1:100 dilution was only slightly less effective than the 1:10 dilution suggests that the extract contains compounds which may interfere with induction and/or general cellular metabolism. If these constituents are identical with or different from the CYP1A1-inducing compounds is currently unknown. Studies with a CYP1A1 promoter-linked reporter gene were inconclusive since no significant effect of the dilutions and/or stock solution on reporter gene activity were obtained (data not shown).

Fig. 1: Effect* of a stock solution (E), 1: 10, and 1:100 dilutions obtained from a fermentation of rat feces with a tryptophan-enriched medium on EROD activity in rat H4IIE hepatoma cells. The solvent (DMSO) served as a negative, TCDD (10^{-9} M) as a positive control.



*means and S.D. from three independent incubations with the same stock solution or dilutions

Conclusions

Incubation of a tryptophan-enriched medium with rat feces leads to the formation of inducers of CYP1A1-catalyzed EROD activity. The identity of these compounds has not been elucidated so far. The finding that dilutions of a dried extract prepared from the fermented medium were more

effective as EROD-inducers than the stock solution indicates the formation of inhibitors of induction and/or catalytic activity. It is unknown if these constituents are identical to the inducers. In summary our findings support the hypothesis that the AhR may be part of a defense system aimed at the inactivation of certain microbial metabolites derived from tryptophan.

References

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