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Aryl hydrocarbon receptor agonists trigger avoidance of novel food in rats

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HIGHLIGHTS

- Avoidance of novel food in rats is a behavioural change triggered by AHR agonists.
- The duration of the avoidance appears to depend on the AHR agonist.
- The behaviour seems to be specifically linked to induction of AHR activity.
- The avoidance response may be retained longer than the agonist itself.
- Findings point to the stomach or the upper GI-tract as a possible key target tissue.

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ABSTRACT

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the toxicity of dioxins, but also plays important physiological roles, which are only beginning to unfold. Previous studies have surprisingly unveiled that low doses of the potent AHR agonist TCDD induce a strong and persistent avoidance of novel food items in rats. Here, we further examined the involvement of the AHR in the avoidance response in Sprague-Dawley rats with three established AHR agonists: 6-formylindolo(3,2-b)carbazole (FICZ), β-naphthoflavone (BNF) and benzo[*a*]pyrene (BaP); with a novel selective AHR modulator (C2); and with an activator of another nuclear receptor, CAR: 2,4,6-tryphenyldioxane-1,3 (TPD). As sensitive indices of AHR or CAR activity, we used *Cyp1a1* and *Cyp2b1* gene expression, as they are, respectively, the drug-metabolizing enzymes specifically regulated by them. We further attempted to address the roles played by enhanced neophobia and conditioned taste aversion (CTA) in the avoidance behaviour. All AHR agonists triggered practically total avoidance of novel chocolate, but the durations varied. Likewise, acutely subtoxic doses of C2, differing by 25-fold, all elicited a similar outcome. In contrast, TPD did not influence chocolate consumption at all. If rats were initially accustomed to chocolate for 6 h after single FICZ or BNF exposure, avoidance was still clearly present two weeks later when chocolate was offered again. Hence, the avoidance response appears to specifically involve the AHR instead of being triggered by induction of intestinal or hepatic nuclear receptor signalling in general. It is also shared by both endogenous and exogenous AHR activators. Moreover, this behavioural change in rats seems to contain elements of both CTA and enhanced neophobia, but further clarification of this is still required.

1. Introduction

The aryl hydrocarbon receptor (AHR) is an evolutionarily ancient, evidently over 600 million-year-old protein. It is a ligand-activated transcription factor that is present in most cell types across all vertebrates, including humans [reviewed in [21]]. Evidence is rapidly accumulating to show that the AHR is involved in numerous physiological phenomena, even if its role is currently often incompletely understood. The endogenous functions of the AHR elucidated so far include participation in the metabolism of xenobiotics, regulation of reproduction, development, cell growth and differentiation, and autoimmunity [15,16,39]. Recent studies have further revealed a major role for the AHR in the control of intestinal microbiota and innate immunity [25,30,40], rendering it thus a promising new target for pharmacological research in several fields.

The molecular mechanism of AHR action has been revealed in detail for transcriptional induction of a drug-metabolizing enzyme, CYP1A1, but it is believed to represent a more general pattern. In inactive state, the AHR is located in the cytosol in association with the chaperone proteins HSP90, XAP2 and p23. Binding of a ligand such as 2,3,7,8-

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tetrachlorodibenzo-*p*-dioxin (TCDD) triggers transformation in the protein structure causing the AHR to translocate into the nucleus. There it sheds the cytosolic protein partners and dimerizes with a structurally related protein, ARNT. The AHR-ARNT dimer then binds to the DNA at specific enhancer sites called dioxin response elements (DREs) in the promoter region of the *Cyp1a1* gene, eventually leading to induced transcription of CYP1A1 mRNA [37]. This is an adaptive, chiefly beneficial physiological response that leads to augmented detoxification capacity. Induction of *Cyp1a1* is also a rapid and highly sensitive marker for AHR activation [1].

While the AHR is notably promiscuous with an extensive array of both endogenous and exogenous ligands, no single endogenous substance has stood out as its primary physiological activator in all tissues to date. Therefore, the best-known and most studied role of the AHR so far is its indispensable involvement in the mechanism of toxicity of a large group of environmental contaminants encompassing halogenated and polycyclic aromatic hydrocarbons, of which dioxins are particularly important. Dioxins (polychlorinated dibenzo-*p*-dioxins, dibenzofurans and co-planar PCBs) are mostly by-products of industrial thermal processes and incomplete combustion [60,74]. They are chemically highly persistent and hydrophobic, which leads to their accumulation in the food chain, and eventually in humans [26]. The most toxic dioxin is TCDD [70]. It causes a multitude of adverse effects in laboratory animals including hypophagia, wasting syndrome, developmental toxicity, endocrine disruption, carcinogenicity and immunotoxicity [47]. The current consensus is that these result from inappropriate and untimely activation of the AHR [8,11].

In the course of the evolution, two related but distinct behavioural mechanisms have evolved to protect animals from eating potentially toxic novel food items: taste neophobia and conditioned taste or food aversion (CTA). Neophobia is considered an innate, protective behaviour that can be experienced towards food, but also novel objects or environments. It is a novelty-induced fear response, which typically subdues rapidly when novel food is deemed safe and becomes familiar. CTA, on the other hand, is a behavioural change seen in both humans and animals, where aversion to the taste or odour of a specific foodstuff (conditioned stimulus) develops due to nausea or gastrointestinal malaise (unconditioned stimulus) that is experienced in conjunction to or relatively soon after consuming the food, regardless of whether the two events are causally related [reviewed e.g. in [34,71,73]]. It is considered a special form of classical conditioning, where the trigger and effect can be even several hours apart. CTA has been interpreted as a mechanism serving to protect the animal from ingesting harmful chemicals and microbes. As such, it protects from foods that, based on a previous encounter, might be harmful, and may persist even for weeks or months [34,71]. It can occur with familiar food, but is usually more pronounced and persistent with unfamiliar foodstuffs. Interestingly, studies in laboratory animals suggest that the feeling of nausea or gastrointestinal discomfort may not always be required for the effect to take place, and it can even be instigated when the animals are unconscious while exposed to CTA-inducing compounds [reviewed e.g. in [18,34,71]]. Although CTA has, as a peculiar form of learning behaviour, been extensively studied for decades, its molecular basis has remained elusive [reviewed e.g. in [5,20,43]]. As proposed by Lin et al. [33], taste neophobia and CTA may be intertwined so that the former primes the CTA mechanism to become engaged when suspicions of toxicity are aroused, thereby enhancing CTA.

Based on an originally fortuitous and unexpected finding, it was previously shown that low doses of TCDD that do not cause overt acute toxicity, induce strong avoidance of novel food items in rats, and this phenomenon seemed to correlate with induction of *Cyp1a1* drug-metabolizing enzyme in the liver [31,32,67]. Unlike many other TCDD-induced effects, the avoidance emerges rapidly, within hours of a single TCDD exposure that is coupled with presentation of a previously unfamiliar food. This response has been shown to be induced by TCDD towards such novel foods as milk chocolate and cheese, as well as sucrose (10%) and saccharin (0.25%) solutions [31,67]. Even a change in the texture of the standard feed (pelleted vs. powdered) is sufficient to induce avoidance after TCDD exposure [31]. The avoidance is strikingly persistent: when chocolate was offered immediately after TCDD exposure, practically total avoidance of it evolved and persisted for 6 weeks, after which it started to gradually fade, although by the end of the observation period (76 days), the consumption was still below control level [31]. Interestingly, the well-documented rat strain differences in sensitivity to TCDD [49] are not reflected in susceptibility to the novel food avoidance induced by TCDD: all tested strains/lines exhibited comparable aversive behaviour [32,67]. For example, the ED_{50} values for TCDD-caused avoidance for three differently TCDD-responsive rat lines proved to be 0.36, 1.07 and 0.34 μg/kg [32]. The corresponding LD_{50} values of TCDD for these rat lines (males) are >10,000, 830, and 40 μg/kg [68]. Total abstinence from chocolate in all three lines was seen at 3 μg/kg. Hence, this induced avoidance is one of the most sensitive behavioural effects TCDD has been shown to exert in adult laboratory animals.

In the present study, we were interested in further examining the involvement of the AHR in the avoidance response, and to find out whether in addition to TCDD, also shorter-acting, less potent AHR agonists are able to induce it in rats. This cannot be taken for granted: the wasting syndrome is primarily due to hypophagia and thus represents another conspicuous alteration in feeding behaviour caused by TCDD; however, other types of AHR activators do not elicit it even at high and repeated doses [42]. In the case of the avoidance response, we hypothesized that transient AHR activation could suffice, and thus also other AHR-activators might be able to mount it. To test this, we used three well-established AHR agonists: the endogenously generated potent agonist, tryptophan metabolite FICZ (6-formylindolo(3,2-b)carbazole) [41,72,75,76], β-naphthoflavone (BNF) [23,64] and benzo[*a*]pyrene (BaP) [23,24]. We also tested a novel selective AHR modulator, C2 (*N*-acetyl-*N*-phenyl-4-acetoxy-5-chloro-1,2-dihydro-1-methyl-2-oxo-quinoline-3-carboxamide), which represents an N-hydrogen metabolite of the autoimmune disease drug compound laquinimod. The doses were selected so as to reliably bring about activation of hepatic AHR signalling as assessed by *Cyp1a1* mRNA or protein expression, based on the studies cited above and (for the high dose of C2) our own unpublished data.

While the available pharmacokinetic information for these compounds is insufficient to verify exact half-lives, they are all likely to be shorter-acting than TCDD based on the duration of their CYP1A1 induction potential seen in these studies. In addition, they also have either comparable or lower AHR binding affinities than TCDD. *In vitro*, FICZ binds to the AHR with even somewhat greater affinity than TCDD (Kd values of 0.07 and 0.48 nM, respectively) [53,54], but it is metabolized extensively and rapidly *in vivo*, foremost by CYP1A1 [3,4]. The binding avidity of BaP to the AHR is 4–9 times weaker than that of TCDD but 1.4-fold as high as that of BNF [44,55]. For C2, preliminary studies have suggested that its *in vitro* potency to induce *Cyp1a1* is at least as high as that of TCDD (Mahiout et al., manuscript in preparation).

We also hypothesized that crucial to the avoidance response was specifically the induction of AHR instead of activation of one or more other nuclear receptors that would also result in induction of xenobiotic metabolizing enzymes. Therefore, the avoidance response should not be brought about by a phenobarbital-like metabolic enzyme inducer that activates the constitutive androstane receptor (CAR) instead of AHR: 2,4,6-tryphenyldioxane-1,3 (TPD) [51]. Induction of *Cyp1a1* and *Cyp2b1* were determined as sensitive indices of activation of AHR and CAR, respectively, as they are the drug-metabolizing enzymes specifically regulated by them. We also attempted to clarify the roles of enhanced neophobia and CTA in the avoidance of unfamiliar food behaviour. Chocolate was chosen as the novel food item, as it has been used in the previous studies with TCDD, rats normally find it highly palatable, its consumption is easy to measure, and it keeps well at room temperature.