From the Institute of Environmental Medicine,
Division of Toxicology and Neurotoxicology, and
Division of Work Environment Toxicology,
Karolinska Institutet, Stockholm, Sweden

Constitutive and TCDD-Induced Expression of Ah Receptor Responsive Genes with Special Focus on the Brain and Pituitary

Ping Huang

黄平

Stockholm 2003

All previously published papers were reproduced with permission from the publisher.
Corver pictures: double staining of NPY and AhRR in the hypothalamic arcuate nucleus (from paper III).
Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden
© Ping Huang, 2003
ISBN 91-7349-434-8

To my mother

In memory of my father

ABSTRACT

The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and related substances are ubiquitous environmental pollutants causing a number of pathological alterations, the most severe being progressive anorexia and body weight loss. The effects on body weight homeostasis suggest a possible involvement of the nervous system and neuroendocrine areas including the pituitary gland. The studies described in this thesis aimed at investigating the expression of aryl hydrocarbon receptor (AHR) responsive genes in different brain regions and pituitaries after treatment with the AHR ligands — TCDD and beta-naphthoflavone (βNF). The diurnal expression of AHR related genes was also studied.

Male Sprague-Dawley rats and 129/SV/C57BL/6 mice, as well as male mice deficient in the cellular retinol-binding protein (CRBP-I) were used as *in vivo* experimental models. The mouse pituitary AtT-20 cell line was selected as an *in vitro* model. A single oral dose of TCDD (10 μg/kg or 15 μg/kg in rats; 50 μg/kg or 250 μg/kg in mice) was administrated and the animals were sacrificed 1, 3, 6, 7 or 28 days after treatment.

The results revealed that basal mRNAs levels of AHR and aryl hydrocarbon receptor translocator (ARNT) were present ubiquitously in the different brain regions and pituitaries, which provided anatomical and biochemical basis for a possible direct action of AHR ligands on these regions. The expression of cytochrome P450 1A1 (CYP1A1) was dramatically increased after TCDD treatment and the pituitary was found the most sensitive among the tissues investigated. In contrast to CYP1A1, the expression changes of AHR and ARNT mRNAs were limited. The expression of AHR repressor (AHRR) was induced by TCDD or βNF *in vivo* and *in vitro*.

To shed some light on central mechanisms involved in the wasting syndrome induced by TCDD, we investigated the expression of several hypothalamic neuropeptides regulating food intake. Both body weight stimulatory (neuropeptide Y, NPY; melanin concentrating hormone, MCH), and inhibitory (cocaine- and amphetamine-regulated transcript, CART; proopiomelanocortin, POMC) neuropeptides were affected by TCDD, which indicated that the central peptidergic circuitries had been dysregulated by TCDD. Results from immunohistochemical double staining analyses showed that AHRR co-localized with NPY-, CART-, MCH- and orexin- immunopositive neurons. Xenobiotic responsive elements (XREs) were found in these neuropeptide genes with the exception of MCH. Furthermore, the proteins encoded by the POMC gene, the adrenocorticotrophic hormone (ACTH) and β-endorphin, were increased in AtT-20 cells after TCDD exposure. These data suggest that body weight loss and wasting syndrome after TCDD treatment may be induced by AHR-mediated regulation of neuropeptides in the brain and in the pituitary.

The study on the mice that lacked the cellular retinol-binding protein (CRBP-I) showed that the CRBP-I -/- mice have a significantly higher basal expression of the AHRR gene in the pituitary compared to the wild type mice. The vitamin A homeostasis seems to play a role on the TCDD-induced activation of AHR-regulated gene transcription.

The results of a 24-h expression study in rats showed a significant diurnal variation of CYP1A1 mRNA level in the anterior and posterior pituitary, as well as in the liver. A daily upregulation of CYP1A1 and period (PER2) mRNAs was observed in anti-phase to the AHR and ARNT mRNAs in the liver.

The high inducibility of CYP1A1 and AHRR, as well as hypothalamic neuropeptides changes that may explain the anorexia and body weight loss in response to TCDD exposure, indicate that the brain and pituitary gland may be direct targets for TCDD.

LIST OF PUBLICATIONS

I. **Huang P.**, Rannug A, Ahlbom E, Håkansson H., Ceccatelli S. (2000) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the expression of cytochrome P450 1A1, the aryl hydrocarbon receptor, and the aryl hydrocarbon receptor nuclear translocator in rat brain and pituitary. *Toxicology and Applied Pharmacology*. **169**, 159-167.

II. **Huang P.**, Ceccatelli S., Håkansson H., Grandison L., Rannug A. (2002) Constitutive and TCDD-induced expression of Ah receptor responsive genes in the pituitary. *NeuroToxicology*, **23**, 783-793.

III. *Fetissov S.O., ***Huang P.**, Zhang Q., Mimura J., Fujii-Kuriyama Y., Rannug A., Hökfelt T., Ceccatelli S. (2002) Hypothalamic neuropeptides in TCDD-induced wasting syndrome with reference to Ah receptor repressor expression. (*Submitted to Toxicology and Applied Pharmacology*)

IV. **Huang P.**, Ceccatelli S., Hoegberg P., Håkansson H., Rannug A. (2003) TCDD-induced expression of AH receptor responsive genes in the pituitary and brain of cellular retinol-binding protein (CRBP-I) knockout mice. (*manuscript*)

V. **Huang P.**, Ceccatelli S., Rannug A. (2002) A study on diurnal mRNA expression of CYP1A1, AHR, ARNT, and PER2 in rat pituitary and liver. *Environmental Toxicology and Pharmacology*, **11**, 119-126.

* Equal contribution to this study

CONTENTS

List of publications

List of Abbreviations

1. Introduction 1

1.1. The bHLH/PAS proteins, AHR and ARNT 1
1.1.1. Transcriptional regulation 1
1.1.2. The structure of AHR and ARNT 1
1.1.3. The functions of AHR-ARNT 3
1.1.3.1. Mediating induction of xenobiotic metabolizing enzymes 3
1.1.3.2. Possible physiological roles of AHR-ARNT 4
1.1.4. The regulation of AHR dependent genes 5
1.1.4.1. Negative feedback regulation 5
1.1.4.2. Cross-talk to other signaling pathways 6
1.1.4.3. Modulation of the AHR level 7
1.1.5. Ligands for AHR 8
1.1.5.1. Exogenous ligands for AHR 8
1.1.5.2. Possible endogenous ligand/s for AHR 8
1.2. The toxic effects of TCDD 8
1.2.1. Carcinogenicity and cell cycle control 9
1.2.2. Effects on the central nervous system (CNS) 10
1.2.2.1. Neurotransmitters and biochemical changes 10
1.2.2.2. Neurobehavioural changes 10
1.2.3. Effects on the hypothalamic-pituitary axis 11
1.2.3.1. Hypothalamus and regulation of energy balance 11
1.2.3.2. Neuropeptides, energy homeostasis and food intake 11
1.2.3.3. The role of pituitary in energy homeostasis and hormone regulation 13
1.2.4. Other effects on endocrine functions 15
1.2.5. Effects on retinoid homeostasis 16
1.2.5.1. CRBP-I and vitamin A metabolism 16
1.2.5.2. Retinoid metabolites and CNS development 16
1.2.5.3. Retinoid metabolism disruption by TCDD 17
1.2.5.4. CYP enzymes and metabolism of retinoids 17
1.2.5.5. CRBP-I knockout mice as a model for studies of TCDD toxicity 18

2. Aims of the study 19

3. Materials and methods 20

3.1. Design of *in vivo* studies (paper I-V) 20
3.1.1. Treatment of SD-rats with TCDD (paper I, III) 20
3.1.2. Treatment of 129/SV/C57BL/6 mice with TCDD (paper II, IV) 20
3.1.3. Treatment of CRBP-I genotype -/-, -/+, +/+ mice with TCDD (paper IV) 21
3.1.4. Biological rhythms in SD-rats (paper V) 21
3.2. Design of *in vitro* studies (paper II) 21
3.2.1. AtT-20 cell culture 21
3.2.2. Primary culture of anterior pituitary cells 21
3.3. mRNA analysis (paper I-V) 22
3.3.1. RT-PCR analysis (paper I, II, IV, V) 22

3.3.2. *In situ* hybridization analysis (paper III) 23
3.4. Protein analysis (paper I-IV) 24
3.4.1. Western Blot (paper I, II, IV) 24
3.4.2. Immunohistochemistry (paper III, IV) 25
3.4.3. Immunocytochemistry (paper II) 26
3.5. Statistical analysis 26

4. Results and Discussion 27

4.1. CYP1A1, AHR and ARNT mRNAs are constitutively expressed in the brain and pituitary of mice and rats (paper I, II, IV, V) 27
4.2. TCDD induced the expression of CYP1A1 with the pituitary being the most responsive organ (paper I, II, IV) 27
4.3. Induced expression of AHRR in response to TCDD or βNF exposure (paper II-IV) 28
4.4. Changes in the expression of hypothalamic neuropeptides in response to TCDD exposure (paper III) 29
4.5. Modulation of AHR-regulated gene expression in CRBP-I genotype mice (paper IV) 31
4.6. Diurnal rhythms of CYP1A1 mRNA expression in the rat pituitary (paper V) 32

5. Conclusions 33

6. Acknowledgements 34

7. References 36

8. Paper I-V 56

LIST OF ABBREVIATIONS

ACTH	adrenocorticotropic hormone
ADPF	AHR degradation promoting factor
AHR	aryl hydrocarbon receptor
AHRR	aryl hydrocarbon receptor repressor
ANOVA	analysis of variance
AIP	Ah receptor-interacting protein
Arc	arcuate nucleus
ARNT	aryl hydrocarbon receptor nuclear translocator
BBB	blood brain barrier
bHLH/PAS	basic helix-loop-helix/Per-Arnt-Sim
BMAL	brain and muscle ARNT-like protein
βNF	β-naphthoflavone
bw	body weight
CART	cocaine- and amphetamine-regulated transcript
cDNA	complementary deoxyribonucleic acid
CNS	central nervous system
CRBP	cellular retinol-binding protein
CYP1A1	cytochrome P450 1A1
CYP1A2	cytochrome P450 1A2
CYP1B1	cytochrome P450 1B1
HIF	hypoxia inducible factor
hsp	heat-shock protein
IS	internal standard
LHA	lateral hypothalamic area
3-MC	3-methylcholanthrene
MCH	melanin concentrating hormone
α-MSH	α-melanocyte stimulating hormone
mRNA	messenger ribonucleic acid
NPY	neuropeptide Y
PAH	polycyclic aromatic hydrocarbons
PCR	polymerase chain reaction
PER	*period*
POMC	proopiomelanocortin
RAR	retinoid acid receptor
RT	reverse transcription
SD	standard deviation
SEM	standard error of the mean
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
XRE	xenobiotic responsive elements

1. INTRODUCTION

1.1. The bHLH/PAS proteins, AHR and ARNT

1.1.1. Transcriptional regulation

The aryl hydrocarbon receptor (AHR) is a cytoplasmic regulatory protein that mediates transcriptional responses to ligands, such as the environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) (Hankinson, 1995; Schmidt and Bradfield, 1996; Rowlands and Gustafasson, 1997; Sogawa and Fujii-Kuriyama 1997). The AHR is a prototypical member of the basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) class of transcription factors. It is normally found in the unliganded state in the cytoplasm forming a complex with heat-shock protein (hsp90) and other AHR interaction factors, such as an immunophilin chaperone referred to as either AIP (Ah receptor-interacting protein, Ma and Whitlock, 1997), ARA9 (Carver et al., 1998), or XAP2 (Meyer et al., 1998) and p23 (a co- chaperone of 23 kDa, Kazlauskas et al., 1999). These proteins are thought to maintain the unliganded AHR in a state responsive to ligand binding. In response to ligand binding within the PAS domain, the cytosolic AHR translocates to the nucleus and forms a heterodimer with another bHLH/PAS regulatory protein — aryl hydrocarbon receptor nuclear translocator (ARNT). The AHR-ARNT complex binds to regulatory sequences (xenobiotic responsive elements, XRE) in the 5′ transcriptional regulating regions and enhances the transcription of biotransformation genes as well as genes involved in cell growth, differentiation and cellular homeostasis. The XRE (Fujisawa-Sehara et al., 1987) is only a small segment of DNA (the pentanucleotide core sequence is 5′-GCGTG-3′), which can be located more than one thousand bases from the initiation site for transcription. Induction of cytochrome P4501A1 (CYP1A1) is one of the most sensitive and well-characterized responses to ligands that bind to the AHR. The enhancer region of the CYP1A1 gene contains multiple copies of XREs, which accounts for a marked increase in CYP1A1 mRNA and protein expression in response to AHR dependent activation (Figure 1).

1.1.2. The structure of AHR and ARNT

The AHR is approximately 100 kDa in size, and is found in most tissues and cells. It was discovered by Poland and co-workers in 1976 (Poland et al., 1976). A bHLH domain is located close to the N terminus (Figure 2). The basic region contributes to DNA binding, and the HLH region is involved in protein-protein dimerization. The N-terminal region also contains the AHR nuclear localization signal and a nuclear export signal. C terminal to the bHLH domain is a region of approximately 300 amino acids designated the “PAS” domain (Ema et al.,1992; Burbach et al., 1992). The name is based on the first three proteins identified containing this motif: the *Drosophila* Period (Per, a protein involved in circadian rhythm), human ARNT (the AHR nuclear translocator), and *Drosophila* Single-minded (Sim, a CNS developmental protein). The PAS domain contains two subdomains termed the PAS A and PAS B repeats, and the C-terminal of bHLH-PAS proteins contain a glutamine-rich domain.

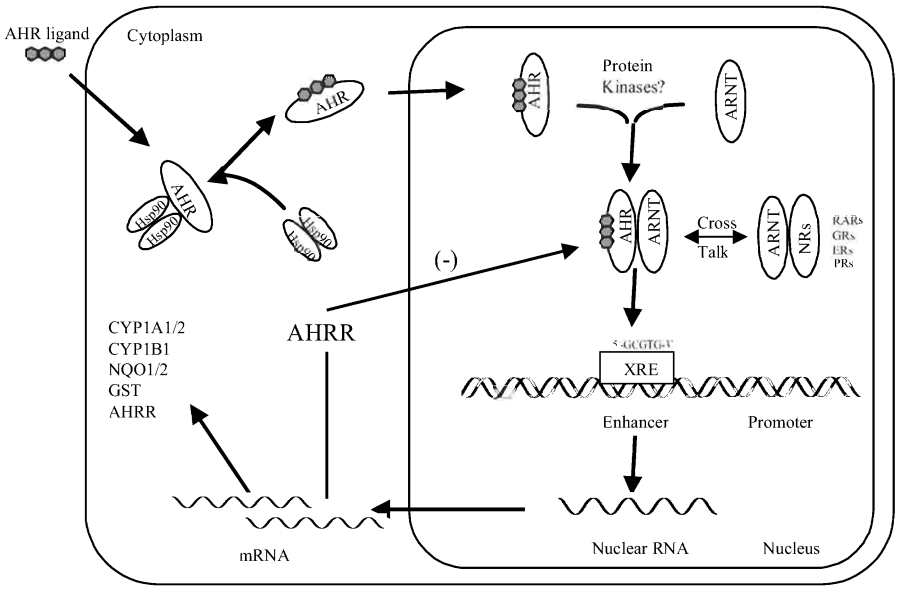

Figure 1. Working model for AHR pathway

The AHR normally resides in the cytoplasm in a complex that also contains a dimer of hsp90. On activation by its ligand, AHR translocates into the nucleus and exchanges its chaperones for ARNT. The AHR-ARNT heterodimer then binds to the xenobiotics responsive element (XRE) with the pentanucleotide core sequence of 5´-GCGTG-3´ and activates transcription of downstream target genes. The AHR repressor (AHRR) may form a feedback inhibition loop by competing for the binding of ARNT with AHR and by actively repressing transcription from XRE-driven promoters. (Modified from Gu et al., 2000).

ARNT cDNA was cloned one year before the AHR (Hoffman et al., 1991). Subsequent studies revealed that ARNT is a nuclear protein which heterodimerizes with liganded AHR, thereby generating a DNA-binding transcription factor (Reyes et al., 1992; Hankinsson, 1995). ARNT functions as a nuclear dimerization partner, rather than as a translocator for AHR (Pollenz et al., 1994). The structural organization of ARNT is similar to that of AHR (Figure 2) (Lindebro et al., 1995; Whitelaw et al., 1994; Jain et al., 1994; Reisz-Porszasz et al., 1994; Li et al., 1994; Numayama-Tsuruta et al., 1997; Eguchi et al., 1997).

The ARNT homologue, ARNT2, has been cloned from mouse and rat (Drutel et al., 1996; Hirose et al., 1996). The ARNT2 protein has been proposed to play a role in PAH/dioxin signal transduction by acting as an alternative partner of AHR (Hirose et al., 1996). ARNT is expressed in many tissues (Carver et al., 1994; Petersen, 2000), but ARNT2, in contrast, is primarily expressed in the brain and kidney (Hirose et al., 1996).

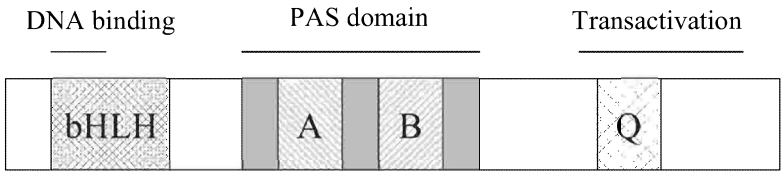

Figure 2. Domains of the bHLH-PAS protein family

The PAS domain contains two PAS subunit repeats, termed PAS-A and PAS-B. The PAS domain contributes to dimerization, ligand and hsp90 binding, respectively. The basic region (b) contributes to DNA binding. Domain Q is a glutamine-rich (Q-rich) region involved in transactivation.

It has been suggested that the DNA binding activity by the AHR-ARNT complex requires phosphorylation of both proteins, whereas dimerization between the AHR and ARNT requires phosphorylation of ARNT (Berghard et al., 1993). It is known that activated protein kinase C is required for AHR-mediated signal transduction (Chen and Tukey 1996; Long et al., 1998), and previous evidence from *in vitro* studies indicates that the ARNT binds to the 3´end of XRE (Swanson et al., 1995; Bacsi et al., 1995), whereas AHR binds to the 5´end of XRE.

1.1.3. The functions of AHR-ARNT

The PAS domain in some proteins has been shown to be involved in developmental processes and in adaptation to environmental changes (Gu et al., 2000). Proteins carrying the PAS domain are involved in the monitoring of changes in light, redox potential, oxygen and small ligands (Gonzalez and Fernandez-Salguero, 1998; Taylor and Zhulin, 1999).

1.1.3.1. Mediating induction of xenobiotic metabolizing enzymes

One important effect of AHR activation is an adaptive response to xenobiotic substances. Vertebrates adapt to adverse chemical environments by up-regulating batteries of xenobiotic metabolizing enzymes, thus decreasing the biological half-life of the harmful chemicals.

The role of cytochrome P450 in xenobiotic metabolism

Cytochrome P450 genes (P450s) consist of a multigene superfamily of mixed-function monooxygenases which are responsible for phase I oxidative metabolism of a wide range of structurally diverse substrates (Smith et al., 1998). They participate in many important endogenous biochemical processes, including biosynthesis of steroid hormones (Spink et al., 1992), retinoids (Tomita et al., 1996) and prostaglandins. Some of the target genes triggered by AHR activation are CYP1A1, CYP1B1, CYP1A2, the glutathione S transferase Ya subunit and quinone oxidoreductase (Whitlock, 1999).

Regulation of cytochrome P450 1A1 expression

A number of factors may affect the regulation of CYP1A1 gene expression, and different mechanisms of regulation may be involved. The major regulation of P450 genes in eukaryotes is at the transcriptional and post transcriptional level (Ramana and Kohli 1998). The induction of CYP1A1 gene transcription mediated by AHR has been extensively studied. CYP1A1 contains numerous XRE sequence motifs located approximately 1kb upstream from the transcriptional start site. Binding of the AHR-ARNT complex to these sequences leads to initiation of transcription of the CYP1A1 gene (Fujisawa-Sehara et al., 1988). DNase I footprinting experiments have revealed that binding of ligand-activated AHR-ARNT complex to the upstream XRE enhancer sequence disrupts the chromatin structure and allows access of general transcription factors to the promoter without disrupting the intervening chromatin (Elferink, et al., 1990; Morgan and Whitlock, 1992; Okino and Whitlock, 1995; Wu and Whitlock, 1993). In addition to the XREs, a GC rich BTE (basic transcription element) has been characterized in the CYP1A1 promoter. This element is localized immediately upstream to the TATA box. The XREs and the BTE element, together with the TATA sequence, are necessary for full induction of the CYP1A1 gene transcription in response to AHR ligands (Imataka et al.,1992).

AHR-ARNT up-regulates CYP1A1 transcription. However, negative regulation of CYP enzymes may also occur in a tissue- or species-specific pattern. The CYP1A1 promoter contains a number of negative regulatory elements (Boucher et al. 1993, 1995) which have been identified by DNA footprint analysis of heterologously expressed promoter constructs (Smith et al., 1998).

1.1.3.2. Possible physiological roles of AHR-ARNT

Up to date, a number of data show that the AHR-ARNT complex has a physiological purpose. AHR is much more than a regulator of drug metabolizing enzymes (review by Puga et al., 2002).

Liver development, vascular development and immune responses

AHR knockout mice commonly show defects in liver development, decreased weight, and poor fecundity (Fernandez-Salguero et al., 1995; Schmidt et al., 1996; Lahvis and Bradfield, 1998). These mice were found to have a number of abnormal phenotypes, including deficiency of the peripheral immune system primarily affecting the total number of B and T cells in the spleen. They were also found to have defects in the development of the vascular structures. The AHR null mice exhibit massive portosystemic shunting, immature sinusoidal architecture in the liver, persistent hyaloid arteries in the eyes, and aberrations in kidney vascular patterns (Lahvis et al., 2000). Such results imply that AHR plays an important developmental role. Among the most consistent phenotypes was an altered liver pathogenesis that was associated with accelerated rates of apoptosis. Published data suggests that this may be related to an abnormal accumulation of hepatic retinoic acid, which activates transforming growth factor β, resulting in stimulation of apoptosis. AHR may directly or indirectly control levels of cytochrome P450 that is responsible for catabolizing retinoic acid (Gonzalez and Fernandez-Salguero, 1998). Indeed, AHR seems to be involved in the

development of liver and the immune system, reproductive function, cell growth and differentiation, and vascular development in multiple organs (see Ma, 2001).

Circadian rhythms

The PAS domains of several proteins appear to serve as regulators in circadian rhythmicity. Diurnal changes in light and temperature are fundamental environmental variations that challenge terrestrial species. In order to adapt to the environmental change, circadian rhythms of various biological activities are maintained through both an internal clock and respond to environmental cues, which keep that clock in tune (Dunlap, 1999; Gu et al., 2000).

The bHLH-PAS protein PER2 is involved in control of circadian rhythms. Cellular oscillators of clock genes are expressed after binding of the bHLH-PAS heterodimer of CLOCK and BMAL (brain and muscle ARNT-like protein) to the recognition sequence (E-box) in DNA. The binding causes transiently increased levels of several mRNAs and proteins. The proteins feedback, after a lag phase, to depress the level of their own transcripts, and sustain a circadian rhythmicity (Dunlap, 1999). PER2, together with another PER protein (PER1) and cryptochrome proteins (CRY1, CRY2), is an essential component of negative regulation of CLOCK and BMAL1 (Chang and Reppert, 2001).

Ligand activation of the AHR-ARNT complex may possibly also affect biological rhythms (see under 1.2.4.).

1.1.4. The regulation of AHR dependent genes

1.1.4.1. Negative feedback regulation

Enzyme mediated ligand depletion

As mentioned above, AHR mediated gene regulation occurs mainly via ligand binding. The ligand-activated AHR-ARNT complex acts as a transcriptional factor to alter expression of a battery of genes, including some drug-metabolism enzymes such as CYP1A1, CYP1A2, and CYP1B1. Induced expression of the metabolizing enzymes of the AHR gene battery is thought to enhance the elimination of the inducing agent and/or its metabolites. Based on this important function, the enzymes activated by AHR-ARNT system would contribute to the ligand depletion and exclusion.

AHR repressor (AHRR)

A recently identified aryl hydrocarbon receptor repressor, termed as AHRR, also belongs to the bHLH/PAS protein family. The AHRR shares a high homology to the AHR, but lacks the ligand binding PAS-B region (Mimura et al., 1999; Baba et al., 2001). AHRR may dimerize with ARNT, and then bind to the XRE sequence motif in the absence of ligand; where the AHRR-ARNT complex is transcriptionally inactive in reporter gene assays. Moreover, the AHRR can inhibit transcriptional activity of the ligand-activated AHR-ARNT complex in a dose-dependent manner, suggesting a role for AHRR as a repressor of AHR function (Mimura et al., 1999; Baba et al., 2001). The 5′-flanking sequence of the AHRR gene contains functional XREs that have been shown to mediate the activation of AHRR expression in the presence of ligands for the AHR (Mimura et al., 1999; Karchner et al., 2002). According to the proposed model (Mimura et al., 1999), AHRR provides negative regulation of AHR function: the

ligand-activated AHR induces the expression of its own inhibitory protein (AHRR), which acts by competing with the receptor for the recruitment of ARNT and binding to target DNA. The AHRR functions as a transcriptional repressor and may directly inhibit gene expression from XRE-liked promoters (Mimura et al., 1999; Baba et al., 2001; Gu et al., 2000; Mimura and Fujii-Kuriyama, 2002; Kazlauskas, 2002).

Ligand-dependent degradation of AHR —ubiquitin-proteasome pathway

Another regulatory factor involved in the AHR negative control is the AHR degradation promoting factor (ADPF). ADPF is a labile factor, promoting ubiquitination of agonist-activated AHR in nuclei, which leads to rapid degradation of the AHR protein through the 26S proteasome pathway, thereby negatively controlling the transcriptional activity of nuclear AHR (Ma et al., 2000). The AHR is ubiquinated following TCDD exposure, which induces a rapid reduction in the level of AHR protein in cells, possibly through the 26S-proteasome mediated degradation (for review see Ma, 2001; Pollenz, 2002). The process of AHR ubiquitination and degradation by the 26S-proteasome is ligand dependent. Such ligand dependent degradation of AHR was seen as a decrease in binding of labeled [^{3}H-]TCDD in cells (Harris et al., 1989; Prokipeak and Okey, 1991; Giannone et al., 1995), decreased binding to XRE in ligand-treated cells (Reick et al., 1994) and depleted AHR protein levels in cells (Pollenz, 1996; Pollenz et al., 1998).

1.1.4.2. Cross-talk to other signaling pathways

Cross-talk to RAR

To date, some evidence indicates that the AHR signaling pathway may cross-talk with other signaling pathways (Delescluse et al., 2000). In keratinocytes, it has been reported that the expression of the AHR is dependent on the state of differentiation and seems to be affected by retinoic acid (Wanner et al., 1995). Therefore, cross talk could exist between AHR and the Retinoid Acid Receptors (RARs) signaling pathway (Figure 1). TCDD has been suggested to exert its toxic action by directly modulating RARs action (Lorick et al., 1998).

Cross-talk to PTK

In addition, protein tyrosine kinase (PTK) activated signal transduction pathways may be involved in the induction of CYP1A1. This is based on some evidence of ligand independent activation of CYP1A1 (Backlund et al., 1997; 1999) and activation caused by some compounds belonging to different chemical families which are not structurally related or not prototypical AHR ligands, but can induce CYP1A1 (Delescluse et al., 2000). More investigations are needed to identify the role of PTK in activation of the AHR.

Cross-talk to ER

There is evidence for cross-talk between AHR and estrogen receptor (ER), therefore data showing that TCDD can produce anti-estrogenic effects by decreasing the estrogen synthesis and increasing estrogen metabolism (Heimler et al., 1998; Spink et al., 1990; 1994). This evidence may be directly related to TCDD effects on the AHR. A reduction in the concentration of ER is seen in various mouse tissues after TCDD exposure, and reduction in ER is AHR-dependent. Such effects may be

transcriptional, because the level of ER mRNA is also decreased (Zacharewski et al., 1991; Tian et al., 1998). Anti-estrogenic effects of TCDD probably reflect decreased responsiveness of target cells to hormone. TCDD antagonizes the induction of cathepsin D gene expression by estradiol in cultured human mammary cells (Krishnan et al., 1995). Anti-estrogen effects by TCDD and anti-dioxin effects by estrogen may imply that there are interactions and cross-regulation between the two signaling systems (Kharat and Saatcioglu, 1996; Ricci et al., 1999; see Okino and Whitlock, 2000).

Cross-talk to NF-κB

AHR interacts with nuclear factor kappa B (NF-κB) in a ligand-dependent manner and that both transcriptionally modulate each other. Many physiological functions adversely affected by TCDD are known to be regulated by NF-κB, such as immune activation, maintenance of skin differentiation, control of cell proliferation and survival, as well as induction of xenobiotic metabolizing enzymes (review by Tian et al., 2002). AHR and NF-κB form a heterodimer that suppresses gene transcription through the XRE or NF-κB binding elements in reporter assays in hepatoma cells (Tian et al., 1999). The AHR and the RelA subunit of NF-κB cooperate to bind to NF-κB binding elements and induce c-myc gene expression in mammary cells (Kim et al., 2000). These findings may provide a new explanation for AHR mediating immune modulation, inflammatory responses and apoptosis.

1.1.4.3. Modulation of the AHR level

Time of day (Richardson et al., 1998), stage of cellular differentiation (Hayashi et al., 1995; Wanner et al., 1995; Shimba et al., 1998), and hormone status (Chaffin et al., 2000) have all been reported to modulate AHR expression.

Effect of TCDD on AHR levels

Exposure to exogenous chemicals, including AHR ligands (notably TCDD), has also been shown to modulate AHR expression levels (Sloop and Lucier, 1987; Bunce et al., 1990; Franc et al., 2001a, b; see review by Pollenz, 2002). The effect of TCDD on AHR levels is dependent on dose, duration of exposure, and the tissue/organism studied. The experimental evidence has revealed that repeated or persistent, low-dose TCDD treatments increase the AHR expression (Slooper and Lucier, 1987; Franc et al., 2001a, b). Cytosolic AHR protein and mRNA were elevated, suggesting a pretranslational mechanism. It has also been previously shown that, following short-term treatment of cultured cells with TCDD, AHR levels are depleted immediately following treatment through the ubiquitin-proteasome pathway (Pollenz, 1996; Giannone et al., 1998) and that this depletion persists up to 72 h — the maximum time-frame tested. *In vivo*, an acute 10 or 50 μg/kg dose of TCDD produces a similarly pronounced depletion in AHR levels at early time points (hours) (Pollenz et al., 1998, Franc et al., 2001a). However, within 10-14 days, hepatic tissue recovers from this initial depletion and AHR levels are restored (Franc et al., 2001b). A similar transient depletion followed by recovery is also evident in other tissues (Pollenz et al., 1998). These above observations indicate that, with persistent TCDD exposure *in vivo*, a balance between degradation and synthesis is established so that AHR levels remain relatively constant in the long term.

1.1.5. Ligands for AHR

1.1.5.1. Exogenous ligands for AHR

AHR binds several different chemicals: including halogenated aromatic hydrocarbons like dibenzo-*p*-dioxins and dibenzofurans as well as polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene, β-naphthoflavone (βNF), and 3-methylcholanthrene (3-MC). Among these compounds, the ligand with the highest binding affinity is TCDD, which is why AHR is also known as the dioxin receptor (Whitlock, 1993). Common structural features among AHR ligands are that they are planar or can become coplanar and that they may be included into a 6.8 X 13.7 Å rectangle (Gillner et al., 1993; Rannug et al., 1991).

1.1.5.2. Possible endogenous ligand/s for AHR

Phylogenetic studies have revealed that AHR is an ancient protein, homologous in both vertebrates and invertebrates (Hahn et al., 1997; Powell-Coffman et al., 1998). The AHR evolved at least 400-500 million years ago (Hahn et al., 1994; Hahn et al., 1997). TCDD is a synthetic chemical that has been introduced into the environment relatively recently. Before such man-made aromatic hydrocarbons existed in the world, other compounds must have represented the natural ligand for the receptor. However, the endogenous ligand for the AHR remains unknown so far. Certain indoles and other plant compounds are potent agonists of AHR and function as enzyme inducers. Dietary indole derivatives, such as indolo[3,2-*b*]carbazole (ICZ), bind to the AHR with high affinity (Bjeldanes et al., 1991; Gillner et al., 1985; 1993) and function as agonists to the AHR (Chen et al., 1995; Kleman et al., 1994). It has also been suggested that endogenous ligands for the AHR may be formed after UV irradiation of tryptophan (Rannug et al., 1987). Two tryptophan photoproducts with high AHR binding affinity, 6-formylindolo[3,2-*b*]carbazole (FICZ) and 6,12-diformylindolo[3,2-*b*]carbazole (dFICZ), have been characterized (Rannug et al., 1995). FICZ has been shown to be the most potent AHR ligand of all compounds tested (Rannug et al., 1987; Wei et al., 1998, 1999). However, it is worth emphasizing here that such suggested/or possible AHR endogenous ligands do not have TCDD-like toxic effects.

Some observations based on AHR knockout mice suggest that endogenous ligands for the AHR may have important physiological roles in development (Fernandez-Salguero et al., 1995; Schmidt et al., 1996; Mimura et al., 1997; Lahvis et al., 1998; Gonzalez et al., 1995). For example, the appearance of hepatic defects and accelerated aging in AHR knockout mice suggest that liver development and maintenance of homeostasis may require an endogenous ligand.

1.2. The toxic effects of TCDD

TCDD is the most toxic of the polyhalogenated aromatic hydrocarbons (Figure 3) and is also the most potent ligand for the AHR in the group of xenobiotics. Toxicity and risk assessment of TCDD and dioxin-like compounds have been extensively studied (Ahlborg et al., 1992; Birnbaum 1994; Pohjanvirta and Tuomisto 1994; van den Berg et al., 1994; Bernes 1998). Dioxins are widespread environmental pollutants

and have never been intentionally manufactured (except for scientific purposes). Due to their lipophilicity, chemical stability, and low efficiency in detoxification, dioxins have become the prototypic toxic contaminants accumulated within the food chain. Human exposure to dioxin-like substances is mainly from fish, meat, and milk products. Moreover, dioxins (particularly the tetra- and more highly chlorinated congeners) are extremely stable in the environment, persisting for decades (Boening 1998). Due to its resistance to metabolism, TCDD tends to persist in the body, and its half-life in humans is estimated to be 10 years (Pirkle et al., 1989).

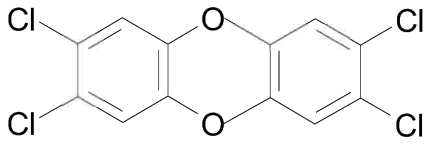

Figure 3. Chemical structure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

Exposure to TCDD can result in a variety of outcomes, including anorexia, carcinogenesis, reproductive toxicity, immune dysfunction, hyperkeratosis, hepatotoxicity, thymic involution and teratogenesis (Pohjanvirta and Tuomisto, 1994; Gasiewicz, 1997; Peterson et al., 1993). The toxic and biochemical response to TCDD and related congeners depends on a number of factors, such as dose, age, sex, animal strain and species, target organs and cells. Embryos and fetuses of all species are the most susceptible to dioxins.

1.2.1. Carcinogenicity and cell cycle control

Some agonists of the AHR are potent tumor promoters in the rodent liver and skin. TCDD is a powerful tumor promoter (Knutson and Poland, 1982; Poland et al., 1982). Tumor promotion by TCDD is likely to be mediated by AHR. Tumor promoters may affect the rate of division, terminal differentiation or death (apoptosis) of tumor precursor cells (Schwarz et al., 2000).

TCDD and certain structurally related compounds increase the incidence of liver neoplasms in rodents upon chronic treatment. Studies on tumor promotion in the liver of female rats suggest a non-genotoxic mechanism for the induction of neoplasms by TCDD. The ability of TCDD to enhance proliferation and inhibit apoptotic processes in focal hepatic lesions further supports an indirect mechanism of carcinogenicity (Dragan and Schrenk, 2000). It seems that TCDD is a multi-site carcinogen in several animal species and possibly in humans as well, whereas natural AHR ligands like dietary indoles tend to protect against cancer.

There are reports showing that dioxin has both blocking and enhancing effects, acting as a promoter blocker to reduce the incidence of certain cancers, and as a tumor promoter in other types of cancers (Kayajanian 1997; Bertazzi et al., 1993; Fingerhut, 1990; Fingerhut et al., 1991). An early study has shown that TCDD can inhibit cell proliferation (Gierthy and Crane, 1984). Exposure to dioxin is associated with decreased incidence of mammary, uterine, and pituitary tumors in female rats (Kociba et al., 1978). Since the growth of such neoplasms is often estrogen-dependent, these

findings suggest that TCDD has an antiestrogen effect. Women exposed to TCDD in an occupational setting do not exhibit a decreased incidence of breast or uterine cancer (Adami et al., 1995); however, women who were exposed to high levels of TCDD during an industrial accident appear to have a lower incidence of both types of neoplasms (Bertazzi et al., 1993). The latter epidemiological evidence is consistent with an antiestrogen effect of TCDD (see Okino and Whitlock, 2000). In this case, TCDD seems to inhibit estrogen-induced breast and endometrial cancer (Safe, 2001).

TCDD toxicity may also induce apoptotic cell death. One of the most pronounced apoptotic processes associated with TCDD is thymic atrophy with characteristic morphological and biochemical changes (see Robertson and Orrenius, 2000). Atrophy of the thymus is a consistent finding in mammals poisoned by TCDD. Suppression of thymus-dependent cellular immunity, particular in young animals, may contribute to death (Boening, 1998).

Overall, TCDD can induce cell cycle arrest in some cell types, while acting as a potent tumor promoter based on some data from animal studies (Puga et al., 2002). Thus, it has been demonstrated that TCDD has a dual effect, inducing proliferation or cell death, dependent on the cell type.

1.2.2. Effects on the central nervous system (CNS)

TCDD-induced effects have been well characterized in hepatic and extrahepatic cells, whereas relatively little is known about the toxic effects of TCDD on the CNS (Pohjanvirta and Tuomisto 1994; Pohjanvirta et al., 1989). TCDD does not penetrate well into the CNS and the concentration found in the brain is far lower than in liver or adipose tissues. However, the behavioural alterations observed in animals exposed to TCDD, such as anorexia, body weight loss, altered sexual and reproductive behaviour (Mably et al., 1992a,b), suggest that the nervous system might be affected by TCDD.

1.2.2.1. Neurotransmitters and biochemical changes

Earlier studies have shown biochemical effects, such as enzyme induction (Elovaara et al., 1977; Unkila, et al. 1995) and changes in the concentration of selected neurotransmitters (Pohjanvirta, et al. 1989; Russell, et al. 1988; Tuomisto, et al. 1990) in the brain after TCDD exposure. TCDD increases the turnover of brain serotonin, along with increased levels of the serotonin precursor, tryptophan, in brain and plasma (Pohjanvirta and Tuomisto 1994; Rozman et al., 1991; Tuomisto et al., 1990; Unkila et al., 1994; 1999). TCDD may also cause injury to rat hippocampal neurons in culture, based on an increase in intracellular calcium (Hanneman et al., 1996). Furthermore, TCDD induces oxidative stress and cell damage, including cytochrome c reduction, lipid peroxidation and DNA-single-strand breakage, in murine brain *in vivo* (Hassoun et al., 1998).

Induction of cytochrome P-450 enzymes has been reported to occur in the brain after exposure to polycyclic aromatic hydrocarbons and TCDD (Anandatheerthavarada et al., 1990; Das et al., 1982; Rouet et al., 1981; Schilter and Omiecinski 1993, Unkila et al., 1993a). An increase in CYP1A1 has been reported in both mitochondria and microsomal brain fractions in rats (Das, et al. 1982; Kapitulnik et al., 1987).

1.2.2.2. Neurobehavioural changes

Alterations in motor activity were observed in laboratory animals treated with TCDD (Seefeld et al., 1984; Potter et al., 1986a; Tuomisto et al., 1993). Prenatal exposure to TCDD (0.3 μg/kg) on GD19 led to hearing deficits (Gordon et al., 1995, 1996). A similar exposure paradigm resulted in changes in locomotor activity and learning behaviour (Thiel et al., 1994). Prenatal exposure through the dam to 1 μg/kg on GD15 in rat or 2 μg/kg on GD12 in hamsters resulted in a permanent depression of the core body temperature (Gordon et al., 1995, 1996). Along these lines, it has been shown that prenatal exposure to TCDD in Rhesus monkeys leads to deficits in object learning (Schantz et al., 1996). The evidence as mentioned above indicate that TCDD can cause various types of brain damage.

Treatment of animals with TCDD induces an altered temporal pattern of food intake, anorexia, depletion of energy stores, loss of body weight, and severe wasting followed ultimately by death (Seefeld et al., 1984; Christian et al., 1986; Russell et al., 1988; Pohjanvirta and Tuomisto, 1990; Tuomisto et al., 1995, 1999, 2000; Unkila et al., 1995; for review see Pohjanvirta and Tuomisto, 1994; Birnbaum and Tuomisto, 2000). In weanlings the TCDD response is manifested by cessation of weight gain, whereas in adults it is characterized by weight loss, which is observed after both acute and chronic exposure to TCDD and related compounds. By careful examination of the metabolic features of the TCDD-induced wasting syndrome, it was revealed that the body weight loss is due to decreased food intake (Seefeld et al., 1984), and a hypothesis that TCDD may lower the body weight set point has been advanced (Keesey and Hirvonen, 1997).

Alterations in body weight, as well as permanent depression of the core body temperature, imply that the hypothalamus is affected by TCDD.

1.2.3. Effects on the hypothalamic-pituitary axis

1.2.3.1. Hypothalamus and regulation of energy balance

Although many regions of the brain are involved in energy homeostasis, food intake and body weight are mainly regulated via neural circuits located in the hypothalamus. The specific neurons in the arcuate nucleus of the hypothalamus act as sensors of whole-body energy status and initiate downstream responses designed to maintain fuel stores at a constant level. Substantial progress in the understanding of how the body weight set point is controlled has been made by characterization of the hypothalamic circuitries, that regulate energy intake and expenditure, and by clarifying the important role of neuropeptides (see Schwartz et al., 2000).

1.2.3.2. Neuropeptides, energy homeostasis and food intake

The characteristic wasting caused by TCDD intoxication could result from either reduced energy intake or induced energy expenditure (Pohjanvirta and Tuomisto, 1994). In this regard, several molecules might be involved in the effect of TCDD on neuroendocrine areas that influence food intake and energy expenditure. These energy balance regulating molecules can be divided into three groups: *i)* leptin and its receptor; *ii)* orexigenic peptides (which stimulate feeding), anorexigenic peptides

(which decrease food intake), and their receptors; *iii)* cytokines and their receptors (see Jeanrenaud and Rohner-Jeanrenaud, 2001). Basically, orexigenic and anorexigenic peptides are essential for normal body weight balance (Broberger et al., 1998a; Hokfelt et al., 2000; Broberger and Hokfelt, 2001).

Neuropeptide Y (NPY) neurons in the arcuate nucleus project to many hypothalamic nuclei and to several extrahypothalamic sites (Broberger et al., 1998b). NPY stimulates food intake (Stanley and Leibowitz, 1985) via Y1 and Y5 receptors (Broberger et al., 1997; Goumain et al., 1998). In addition to being involved in food intake, NPY influences insulin secretion and increases the activity of the hypothalamus-pituitary-adrenal axis.

Orexin has been identified as a small peptide expressed in the lateral hypothalamic area (LHA), which results in the stimulation of feeding (Sakurai et al., 1998). Fasting and hypoglycemia increase orexin expression in the LHA (Griffond et al., 1999; Mondal et al., 1999). Subsequently the orexin was shown to be involved in sleep and narcolepsy (Chemelli et al., 1999; Lin et al., 1999). Consistent with the suspected role of the lateral hypothalamus as an appetite-stimulating center, intraventricular administration of orexin resulted in an over 10-fold increase in food intake 1 h after injection (De Lecea et al., 1998, Meier, 1998).

Melanin-concentrating hormone (MCH) is produced in the LHA, by different cells from those that produce orexin. MCH is generally considered to be an orexigenic peptide (Qu et al., 1996; Rossi et al., 1997) and MCH release stimulates food intake. MCH neurons display an abundant innervation of the cerebral cortex (Bittencourt and Elias, 1998) and therefore may participate in the behavioral component of feeding. However, it could be suggested that physiological activation of feeding behavior via the MCH system might also require an appropriate change of its co-transmitter CART.

Cocaine- and amphetamine-regulated transcript (CART) is expressed in many brain areas and co-expressed with *proopiomelanocortin* (POMC) in the arcuate nucleus of the hypothalamus. CART inhibits food intake and promotes weight loss (Elmquist et al., 1999; Kuhar et al., 1999a, b; 2000). Injection of CART peptide into the brain ventricular system of the rat causes a rapid inhibition of feeding (Lambert et al., 1997; 1998; Kristensen et al., 1998). In addition, CART inhibits the increase in feeding caused by NPY, and CART mRNA is regulated by leptin. In addition to the hypothalamic localization, the CART is also found in the posterior pituitary (very high concentration), anterior pituitary, adrenal medulla, and blood.

Both the hypothalamus and the pituitary can produce *POMC*. POMC is a neuropeptide precursor molecule (Figure 4) which is cleaved by endopeptidases to yield mature peptides like adrenocorticotrophic hormone (ACTH), β-lipotropin (β-LP), β-endorphin, and α-melanocyte stimulating hormone (α-MSH) (Castro and Morrison, 1997). Measurements of POMC mRNA levels are frequently used to identify and to characterize the neurons that produce α-MSH (Barsh and Schwartz, 2002). POMC-derived peptides contribute to the regulation of energy homeostasis (Yeo et al., 2000). The POMC gene seems to play an unclear yet crucial role in body weight regulation. POMC-derived peptides activate MC4-R (one of melanocortin receptors), resulting in

reduced food uptake and increased energy expenditure, which leads to maintenance of body weight (Krude and Gruters, 2000).

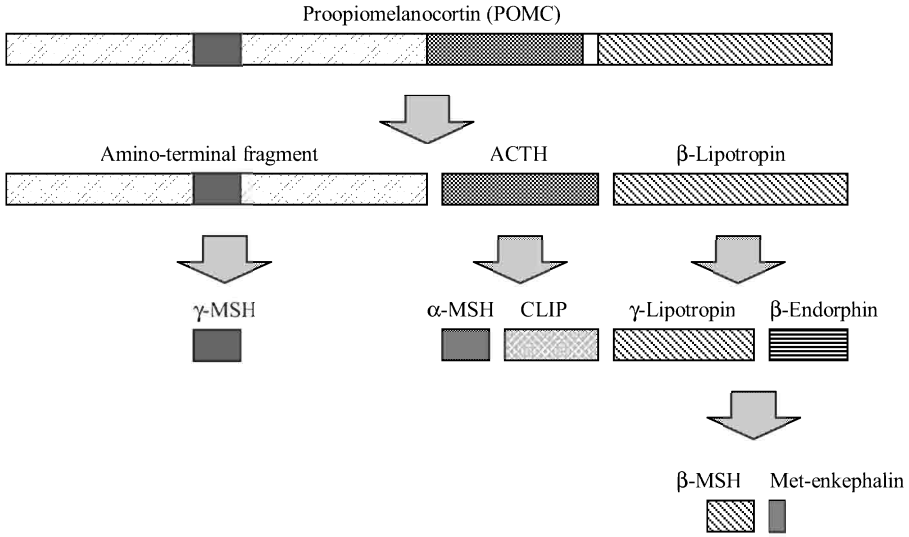

Figure 4. POMC derived peptides

The figure indicates the structure of POMC and the products of posttranslational processing. MSH: melanocyte stimulating hormone. CLIP: corticotropin-like intermediate lobe peptide.

1.2.3.3. The role of pituitary in energy homeostasis and hormone regulation

Localization and anatomy of the pituitary

The pituitary is located in a bony cavity (the sella turcica) at the base of the brain below the hypothalamus with which it has an important anatomic and functional relationship (Ikeda et al., 1988, Collu et al., 1989). It can be divided into two parts, the anterior that develops from ectoderm, and posterior that develops from the hypothalamus (Figure 5). The anterior pituitary, or adenohypophysis, can be subdivided into pars distalis, pars intermedia and pars tuberalis. The cells of the anterior pituitary were originally classified using techniques for staining intracellular granules as acidophils, basophils, and chromophobe cells (Findling and Tyrrell, 1990). The posterior lobe consists of fibers and endings of the hypothalamic neurohypophyseal tract and has a rich blood supply (Wilson et al., 1998).

Pituitary hormones

There are at least six major anterior pituitary hormones — corticotropin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), growth hormone (GH), prolactin (PRL), and thyrotropin — whose biosynthesis, structure, function and secretory control have been well characterized and which can be measured accurately in tissues and body fluids. These pituitary synthesized hormones control growth, development, and function of target organs including the thyroid gland, adrenal cortex, gonads, and breasts. Each pituitary hormone is regulated by hypothalamic

regulatory hormones that are synthesized in the hypothalamus and transported from the median eminence to the anterior pituitary via the hypothalamic-pituitary portal circulation. Hypothalamic hormones bind to specific high-affinity membrane receptors in pituitary cells to regulate hormone secretion. With the exception of prolactin, anterior pituitary hormones are under feedback regulation via the hormones secreted by the target glands on which the pituitary hormones act. Thus, signals from the brain and the periphery interact to regulate pituitary hormone secretion and maintain a normal endocrine state. The feedback control occurs at both the pituitary and hypothalamic levels.

Oxytocin and arginine vasopressin are the main peptides known to be secreted from the posterior lobe of the pituitary in humans and other mammals. These two neurohypophyseal hormones are synthesized in the hypothalamus, transported and stored in the posterior lobe, and released into the circulation.

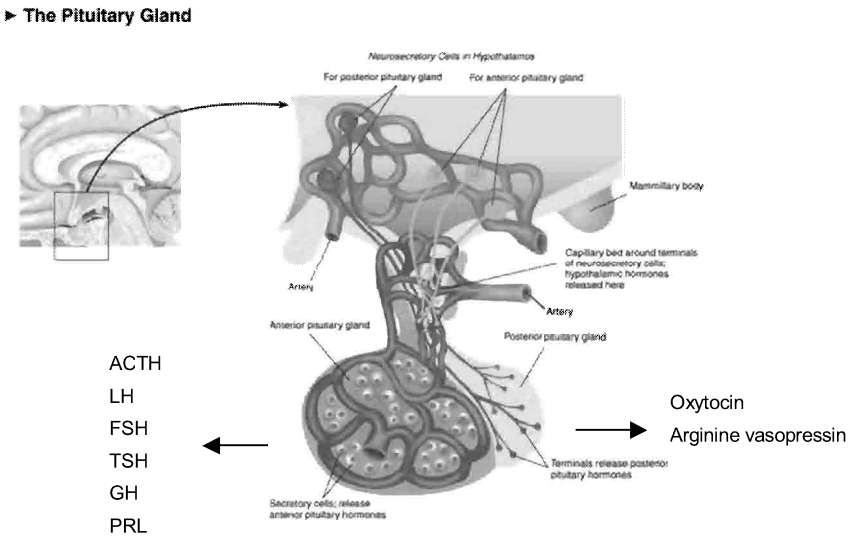

Figure 5. Schematic drawing of the human pituitary gland

(Modified from Driesen, JL. 2000)

Pituitary hormones secretion is altered by TCDD

Several hormones secreted by the anterior pituitary are altered by TCDD. TCDD causes changes in plasma prolactin (Jones et al., 1987; Russell et al., 1988; Moore et al., 1989), adrenocorticotropin (Bestervelt et al., 1991; 1993a; 1993b), β-endorphin (Pohjanvirta et al., 1993), and LH (Ruangwises et al., 1991; Moore et al., 1989). Concentrations of thyroid-stimulating hormone are frequently found to be increased by TCDD, as assessed by radioimmuno-assays (Potter et al., 1986b; Pohjanvirta et al., 1989) or by bioassays (Bastomsky, 1977). Hence, the anterior lobe of the pituitary gland may be a target for TCDD toxicity, and may exhibit both exaggerated and suppressed responses to its regulatory hormones (see Pohjanvirta and Tuomisto, 1994).

The effects of TCDD on the pituitary may induce alterations in neuropeptides such as POMC and its derived peptides. Recent studies have shown that TCDD increases ACTH levels in pituitary cell primary cultures and beta-endorphin-like immunoreactivity in TCDD susceptible rats (Pohjanvirta et al., 1993; Bestervelt et al., 1991, 1998a). These results have led to the speculation that TCDD may affect ACTH concentration by increasing the rate of transcription of the POMC gene by an AHR-mediated process (Bestervelt et al., 1993a,b, 1998a,b). Bestervelt and colleagues (1998b) have reported that the ACTH secretion is AHR-mediated in the rodent pituitary. These authors suggest that the ACTH regulation might depend on the binding of the activated AHR-ARNT regulatory complex to an XRE core sequence that appears in the 5′-flanking region of the proopiomelanocortin gene.

TCDD exposure results in pituitary atrophy

Earlier studies have shown that TCDD induces pituitary atrophy. A non-lethal dose (25 μg/kg) of TCDD at day 32 resulted in a slight uniform atrophy of mainly the chromophobe cells of the pituitary, which was much more pronounced by a lethal dose (125 μg/kg) of TCDD (Gorski et al., 1988). It was also reported that there is less intense staining in acidophilic cells of the anterior lobe in rat pups exposed to 5 μg/kg TCDD postnatally on days 0, 7, and 14 and killed on day 25 (Vos and Moore, 1974). TCDD decreased pituitary weight in adult rats as an early effect, developing by day 4, and resulting in shrinking of the pituitary gland. This appeared to be a primary response (Pohjanvirta et al., 1993). Decreases in pituitary gland weight and epididymal sperm numbers were found in TCDD-exposed male ICR offspring mice (Theobald and Peterson, 1997). How these phenomena are regulated is still unknown.

1.2.4. Other effects on endocrine functions

A wide spectrum of endocrine perturbations including changes in plasma levels of thyroxin, adrenocorticotropin, gonadotropins, corticosterone, insulin, melatonin, testosterone, as well as tissue concentrations of estrogen-, progesterone- and glucocorticoid receptors have been reported after TCDD exposure in various experimental models (Bestervelt et al., 1993a, 1993b; Li et al., 1997; Pohjanvirta et al., 1996; Pohjanvirta and Tuomisto 1994; Russell et al., 1988; Unkila et al., 1995). These alterations point to a direct effect of TCDD on the function of several endocrine organs, including the adrenal gland, pancreas, thyroid gland, testis, ovaries and pineal gland.

Although very few measurements have been performed on the effect of TCDD on diurnal rhythms of endocrine functions, a shift in circadian feeding rhythms (Christian et al., 1986; Pohjanvirta et al, 1988; Pohjanvirta and Tuomisto, 1990), in prolactin secretion and response to prolactin (Jones et al., 1987), and in corticosterone levels (DiBartolomeis et al., 1987) have been observed in rats. These data suggest that TCDD could interfere with biological rhythms. Recently, it was reported that TCDD also has an effect on circadian rhythms in deer mice (Miller et al., 1999).

1.2.5. Effects on retinoid homeostasis

1.2.5.1. CRBP-I and vitamin A metabolism

Vitamin A metabolites, known as retinoids that include retinol, retinal, retinoic acid (RA), retinyl palmitate and retinoyl glucuronide (Zile, 1992), are potent regulators of gene transcription and play important roles in cell proliferation and differentiation (Gudas et al., 1994; Mangelsdorf, et al., 1994).

The multiple biological actions of retinoids are mediated and influenced by two classes of proteins: retinoid receptors (RARs, RXRs) and retinoid-binding proteins (RBPs, CRBPs, CRABPs) (Noy, 2000). Within a cell, all-*trans*-retinol and its oxidation product, all-*trans*-retinal, are associated with one of the two isoforms of cellular retinol-binding proteins (CRBP-I and CRBP-II). Both proteins interact with both the all-*trans*-retinol and the all-*trans*-retinal. CRBP-I shows a much higher binding affinity for its ligands than CRBP-II. (Li et al., 1986; MacDonald and Ong, 1987). CRBP-I is expressed in multiple tissues, both in the fetus and in the adult. In the adult, CRBP-I is abundant in the liver, kidney, lung, eye and reproductive organs (Eriksson et al., 1984; Kato et al., 1984; De Leeuw et al., 1990; Wardlaw et al., 1997), and is also widely distributed in the pituitary, choroid plexus of brain, hypothalamus, hippocampus, caudate/putamen and olfactory bulb (Ruberte et al., 1992; 1993; Zetterström et al., 1994; 1999). In contrast, CRBP-II is restricted to the small intestine in the adult (Ong, 1984; Ong and Page, 1987).

In the adult brain, retinol bound to retinol-binding protein (RBP) in circulation is transported across the blood-brain barrier (BBB) in the endothelial cells of brain microvasculature and in the cuboidal epithelial cells of the choroid plexus. In the cell, CRBP takes up the retinol released by RBP (MacDonald et al., 1990). Retinol is then facilitated to retinyl ester for storage or converts to RA, which then binds to either the RA receptor (RAR) or the retinoid X receptor (RXR) within the nucleus and modulates gene expression (Malik et al., 2000).

CRBP-I has been associated with two main functions, the regulation of vitamin A storage and the synthesis of RA (Noy, 2000). As CRBP-I binds not only to retinol but also to retinal (Noy, 2000), it has been postulated that CRBP-I can direct retinol and retinal to specific enzymes while restricting them from others, thereby influencing the metabolic fate (activation, catabolism, or storage) of the retinol that enters the cell (Napoli et al., 1992; Boerman and Napoli, 1996). It has been shown that CRBP-I is essential for vitamin A homeostasis (Ghyselinck et al., 1999).

1.2.5.2. Retinoid metabolites and CNS development

Vitamin A and its derivatives, the retinoids, have an important role in the prenatal development of the nervous system. During brain development, retinoic acid is involved in events leading to cessation of cell proliferation and terminal differentiation (Takahashi et al., 1999; Summerbell and Maden, 1990; Cheung and Ip, 1998; Wuarin et al., 1990; Durston et al., 1989; for review see Malik et al., 2000). Maden and his colleagues (1998) observed that deficiency in vitamin A and its metabolites, particular RA, alters the CNS development of quail embryos. First, the posterior hindbrain fails to develop because the cells that form this part of the CNS in the very early embryo die by apoptosis. Second, the neural tube fails to extend

neurites into the periphery both *in vivo* and *in vitro*. Third, the neural crest cells throughout the embryo die by apoptosis (Maden et al., 1998). The acute administration of RA to aged mice restores the levels of retinoid receptor to presenescent (adult) levels (Etchamendy et al., 2001). In addition, vitamin A functions in the regulation of dopaminergic system in the brain and pituitary gland, therefore, controls coordination of movement as well as the synthesis and secretion of pituitary hormone (Wolf, 1998).

1.2.5.3. Retinoid metabolism disrupted by TCDD

TCDD and similar environmental contaminants cause a significant decrease in hepatic retinoid storage, accompanied by an increase in the level of vitamin A compound in rat serum and kidney, where it undergoes elimination (Thunberg et al., 1979; see Nilsson and Håkansson, 2002; Birnbaun and Tuomisto, 2000). The hepatic vitamin A reduction occurs both after a single dose and following long-term low-dose exposure to dioxin-like compounds (Nilsson et al., 1996; 2000; Kelley et al., 2000, Fattore et al., 2000). TCDD induces significant reductions in retinol and retinyl ester concentrations in the liver, lung, intestine and adrenals, whereas serum retinol binding protein (RBP) exhibited a 150 % increase in the serum of TCDD-treated rats (Brouwer et al., 1989). Moreover, wildlife and fish exposed to dioxin-like substances also show effects on retinoid levels (see Rolland, 2000). Other reports implicate that TCDD can alter retinoid receptor expression (Weston et al., 1995) or function (Lorick et al., 1998).

On the other hand, laboratory animals fed with a vitamin A-deficient diet show signs of toxicity that are similar to the effects observed in animals treated with TCDD. These include hypophagia, loss of body weight, loss of hepatic vitamin A and accumulation of renal retinoids, impaired growth, defective reproduction, developmental abnormalities, and impaired immune function (Innami et al., 1974; Kimbrough 1974; Jurek, et al., 1990). Other factors such as alcohol can also cause similar effects. Hepatic microsomal cytochrome P450 and CRBP were significantly reduced in old rats after ethanol feeding (Mobarhan et al., 1991).

1.2.5.4. CYP enzymes and metabolism of retinoids

It is possible that the AHR-regulated enzymes may have a function in retinoid homeostasis. All-*trans*-retinol and all-*trans*-retinal metabolism are known to be catalyzed by several enzymes including the cytochrome P450s, alcohol dehydrogenases, short-chain dehydrogenase/reductases, and aldehyde dehydrogenases (Duester, 1996, 2000). CYP1A2 and CYP3A4 have been suggested as the major CYP enzymes catalyzing hepatic oxidative conversion of all-*trans*-retinol to all-*trans*-retinal and to all-*trans*-retinoic acid in humans (Chen et al., 2000). In addition, the human extrahepatic enzymes CYP1A1 and CYP1B1 have been found to exhibit exceptionally high activity in catalyzing the biosynthesis of all-*trans* retinol to all-*trans* retinoic acid (Zhang et al., 2000; Chen et al., 2000). The human CYP1A2 has also been implicated in the process of metabolism of 9-cis retinal to 9-cis retinoic acid (Zhang et al., 2000). Impaired CYP1A1 expression can result in the low synthesis of retinoic acid in mouse xeroderma pigmentosum fibroblasts (Ding et al., 2001). A family of retinoic acid-catabolizing cytochrome P450 proteins (P450-RAIs or CYP26) has also been described

in several species including humans (White et al., 1996; Fujii et al., 1997; MacLean et al., 2001).

In the case of TCDD exposure, retinoids may be oxidized by one or several TCDD-induced enzymes to more water-soluble molecules, and thereafter filtered and excreted. TCDD is well known to induce the high expression of CYP1A1, CYP1A2 and CYP1B1. Induction of these enzymes could increase the oxidation of retinoids and lead to a increased excretion and a net decrease of retinoid levels in tissues (for review, see Nilsson and Håkansson, 2002). An AHR/ARNT-binding site has been predicted in a murine retinal dehydrogenase gene, suggesting that dioxins binding to the AHR may affect the synthesis of retinoic acid (Wang et al., 2001). An early study revealed that cytochrome P450s catalyzed the oxidation of retinol and contributed to the hepatic vitamin A depletion seen in TCDD exposure and alcoholic feeding (Lieber et al., 1987).

The observations described above imply that there is an association between the CYP enzymes and retinoid oxidation, and the AHR-related regulation appears to be involved in retinoid homeostasis.

1.2.5.5. CRBP-I knockout mice as a model for studies of TCDD toxicity

Experimental data suggest that CRBP act as a chaperone that protects retinol from unspecific metabolism and that CRBP interacts with certain retinoid-metabolizing enzymes. Mutant mice with an inactivated CRBP gene show decreased liver retinyl ester storage, faster elimination of liver retinoids, and have been suggested to display an increased vitamin A oxidation (for review see Napoli, 2000). However, CRBP-I null mice show no symptoms of vitamin A deficiency when fed with a normal vitamin A containing diet (Ghyselinck et al., 1999). CRBP-I upholds a central position in the retinoid system in the crossroad between storage and activation, and is expressed in the adult mouse brain. Moreover, it is currently unknown whether any of the AHR-mediated toxicity mechanisms can be associated with the retinoid system. Thus, we believe that the CRBP-I knockout mouse could constitute an interesting tool to study the role of the retinoid system in the induced AHR/ARNT signaling machinery in the brain.

Recently, the retinoic acid signaling pathway is proposed to be involved in toxicity processing and cross-talk with the classic AHR signaling pathway (Delescluse et al., 2000). Since the CRBP-I and -II may be involved in retinoic acid synthesis from retinol (Boerman and Napoli, 1996; White et al., 1996), and CRBP-I plays an important role in vitamin A homeostasis, these phenomena prompted us to investigate further the regulation pattern of the AHR responsive genes in CRBP-I knockout mice.

2. AIMS OF THE STUDY

Most effects of TCDD are considered to be AHR mediated. The AHR-ARNT complex recognizes xenobiotic response elements within target genes, and triggers a series of biological functions and metabolism of xenobiotics. The overall aims of the present investigation were to study the constitutive and TCDD-induced expression of AHR responsive genes in the brain and pituitary, and to identify mechanisms involved in TCDD-induced effects on body weight.

The more specific objectives were:

- To investigate the effects of exposure to low doses of TCDD on the central nervous system and the pituitary gland;

- To investigate the expression of hypothalamic neuropeptides that regulate food intake in TCDD-exposed rodents;

- To investigate the changes of AH receptor-responsive genes expression and the interaction with retinoids homeostasis in CRBP-I genotype mice treated with TCDD;

- To examine the diurnal mRNA levels of AH receptor-responsive genes in the pituitary;

- To identify possible markers which can be used to evaluate toxicity of TCDD and TCDD-like compounds.

3. MATERIALS AND METHODS

In order to elucidate the TCDD effects on the brain and pituitary more accurately, both *in vivo* and *in vitro* models were used in the present study.

3.1. Design of *in vivo* studies (papers I-V)

The animals used were male Sprague-Dawley rats and male 129/SV/C57BL/6 mixed genetic background mice from B&K, Stockholm, Sweden. Breeding pairs of CRBP-I knockout (-/-) and wild type (+/+) mice of the same mixed genetic background (129/SV and C57BL/6) were kindly provided by Dr. N. Ghyselinck (1999) for breeding and experimentation at Karolinska Institutet, Stockholm, Sweden. Animals were housed in polypropylene cages and checked twice daily. Animals were kept in air-conditioned quarters at a temperature of 25°C, under a controlled photoperiod (14 hrs light: 10 hrs darkness), and with free access to food and tap water. Procedures used in animal experimentation were in compliance with the Karolinska Institutet guide lines in the care and use of laboratory animals. All animal experiments were approved by the local ethical committee.

3.1.1. Treatment of SD-rats with TCDD (paper I, III)

Oral administration by gavage was used in this study, to imitate the exposure condition for humans who are exposed to TCDD mainly via food. A single oral dose of 10 μg TCDD/kg body weight (bw) was administered in the male Sprague-Dawley rats. This dose has previously been shown to have only small effects on the growth and general well-being of the rats, but causing liver enlargement, thymus atrophy, and a marked induction of CYP1A1 (Håkansson and Ahlborg 1985; Håkansson et al., 1994). At three different time points (1, 3, 28 days) after treatment, the rats were anesthetized with pentobarbital and killed by decapitation. The brains, including cortex, cerebellum, olfactory bulb, caudate-putamen, hypothalamus, hippocampus and substantia nigra were dissected with the meninges left in situ according to Palkovits and Brownstein (Palkovits and Brownstein 1992). The pituitaries were removed from the skull, and dissected into anterior and posterior lobes.

Another set of male SD-rats was treated with a single oral dose of 15 μg TCDD/kg bw and killed on day 6. The brain was dissected and immersed in ice-cold phosphate-buffered saline and frozen immediately thereafter. The tissues were cut at 14 μm sections using a cryostat for *in situ* analysis.

3.1.2. Treatment of 129/SV/C57BL/6 mice with TCDD (paper II, IV)

A single dose of 50 or 250 μg TCDD/kg bw was administered to 56-day-old male mice by gavage. At two different time points (7, 28 days) after treatment, the mice were anesthetized with pentobarbital and killed by decapitation. The pituitaries were removed from the skull, and subjected to RNA and protein level analysis.

Table 1. TCDD treatment and study designs

Paper	Species	Day	Treatment
I	Male Sprague-Dawley rat	0: 1, 3, 28:	Oral administration of 10 μg TCDD/kg bw Termination
II	Male 129/SV/C57BL/6 mouse (wild type)	0: 7, 28:	Oral administration of 50 or 250 μg TCDD/kg bw Termination
III	Male Sprague-Dawley rat	0: 6:	Oral administration of 15 μg TCDD/kg bw Termination
IV	Male CRBP-I -/-, -/+, +/+ genotype mouse	0: 7, 28:	Oral administration of 50 or 250 μg TCDD/kg bw Termination
V	Male Sprague-Dawley rat		Untreated Samples were collected at circadian time 8:00 am; 12:00 am; 16:00 pm; 20:00 pm; 0:00 am; 4:00 am.

3.1.3. Treatment of CRBP-I genotype -/-, -/+, +/+ mice with TCDD (paper IV)

Mice of the same mixed genetic background (129/SV and C57BL/6) were bred, in which the gene encoding CRBP-I had been inactivated (Ghyselinck et al., 1999). A single dose of 50 or 250 μg TCDD /kg bw was administered to male 56-day-old CRBP-I mice homozygous (-/-), heterozygous (-/+), and wild type (+/+) by gavage. The CRBP-I (-/+) and (+/+) mice were used as genotype control. At two different time points (7, 28 days) after treatment, the mice were anethesized with pentobarbital and killed by decapitation. The brain was dissected and the pituitary was collected.

3.1.4. Biological rhythms in SD-rats (paper V)

In the biological rhythm experiment, male Sprague-Dawley rats were sacrificed at six different time points (08:00 am, 12:00 am, 16:00 pm, 20:00 pm, 00:00 am and 04:00 am). The anterior and posterior pituitaries, as well as liver, were sampled.

3.2. Design of *in vitro* studies (paper II)

3.2.1. AtT-20 cell culture

The AtT-20 cell line, derived from a mouse pituitary tumor and producing ACTH, was used. These cells were grown in Ham´s F-10 with 2 mM glutamine, 2.5 % Fetal Bovine Serum (FBS), 15 % heat inactivated horse serum and 100 μg/ml penicillin-streptomycin. The cells were treated with TCDD or βNF at different doses and time points.

3.2.2. Primary culture of anterior pituitary cells

Primary cultures of anterior pituitary cells were used in the studies. Anterior pituitary cells were derived from male Sprague-Dawley rats and maintained in DMEM

supplemented with 10 % inactivated FCS and 0.5 % (v/v) penicillin-streptomycin. The cells were exposed to 2 μM or 6 μM βNF for 3 h.

3.3. mRNA analysis (paper I-V)

3.3.1. RT-PCR analysis (paper I, II, IV, V)

The mRNA levels of CYP1A1, CYP1A2, AHR, ARNT, AHRR, POMC, and PER2, were determined with a highly sensitive RT-PCR method.

3.3.1.1. Total RNA preparation and cDNA synthesis

Total RNA was isolated following the protocol of QIAGEN's RNeasy Mini Kit. RNase-free DNase I was used to minimize the contamination with genomic DNA. First-strand cDNA was synthesized using random hexamers as a primer according to a protocol of SuperScript ^{TM} II RT. The cDNA product was stored at -20 °C or -70 °C.

3.3.1.2. Primers and competitive internal standard synthesis

The primers were designed using Oligo 5.0 (National Biosciences, Inc.) based on the corresponding sequences from GeneBank. The competitor, named "internal standard" (IS), was designed and synthesized following a previously reported method (Celi et al, 1993). The variation in length between internal standard templates and specific target sequences was set to less than 20 %.

3.3.1.3. Competitive PCR

The competitive PCR reaction system contained a 2 μl product of the reverse transcription reaction and 1 μl IS with a suitable concentration in a total of 20 μl reaction volume. The final concentrations of other reagents in the system were as follows: 1×PCR buffer II (Life Technologies); 1.5 mM $MgCl_2$; 200 μM each dNTP; 200 nM each primer; and 0.5 U *Taq* DNA polymerase.

3.3.1.4. Detection of PCR-amplified products and analysis

PCR products from the competitive quantitative RT-PCR were detected using a Gel-Doc instrument (BIO-RAD) equipped with the Molecular Analyst software. Aliquots of PCR-amplified products (10 μl) were electrophoresed on 1.6 % agarose gel and visualized with ethidium bromide staining. Specificity was confirmed by the expected sizes of the amplified products. The density of each band was quantitated using the Molecular Analyst software. The results were expressed as the ratio of peak areas between the test sample and the IS. A fixed amount of target cDNA was co-amplified with a series of diluted IS by PCR. The log of the ratio of peak area, between target cDNA and IS, was then plotted against the log of amount of the IS (moles). A straight line was obtained. At the point of equivalence, where the ratio of target cDNA/IS was 1, the amount of target cDNA in the test sample equaled the amount of IS moles.

The processing of semi-quantitative RT-PCR was similar to that described above but without the IS template. The procedure of quantifying cDNA/mRNA of a specific gene by RT-PCR is summarized in Figure 6. All PCR reactions were linear at the PCR cycling conditions used in the present study. Within each run, linearity was ensured by varying the input amount of cDNA and varying PCR cycling number.

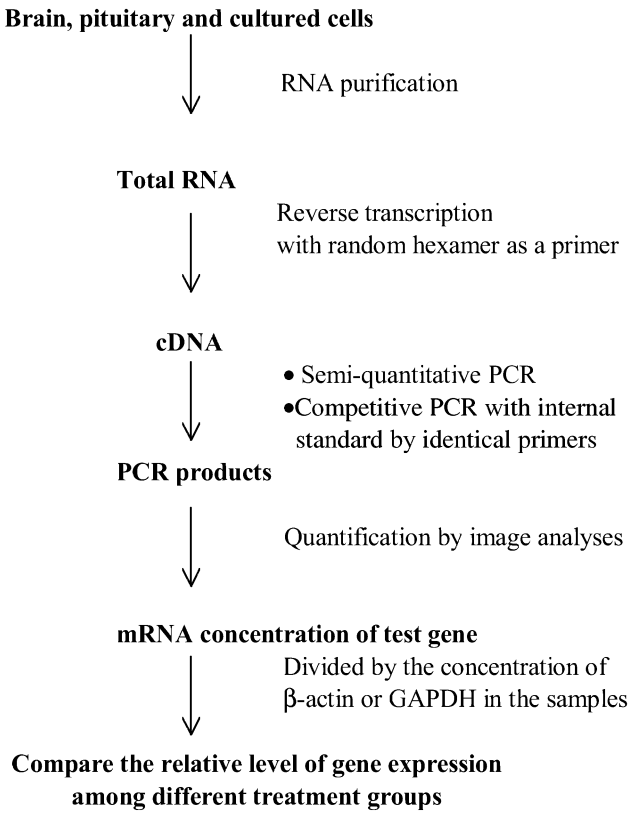

Figure 6. Procedures of quantitative RT-PCR methodology

3.3.2. *In situ* hybridization analysis (Paper III)

3.3.2.1. Probe preparation

Antisense oligoprobes complimentary to mRNAs of NPY, POMC, CART, MCH and orexin/hypocretin were synthesized by CyberGene AB (Huddinge, Sweden) based on the previously reported gene sequence (Larhammar et al., 1987; Drouin and Goodman, 1980; Douglass et al., 1995; Nahon et al., 1989; de Lecea et al., 1998; Sakurai et al., 1998). The oligonucleotide probes were labeled at the 3'end using terminal deoxynucleotidyltransferase with [α-^{35}S]dATP to the specific activities from 1-4 x 10^6 cpm/ng oligonucleotide. The oligoprobes were purified through QIA quick spin columns. Dithiothreitol was added to a final concentration of 10 mM.

3.3.2.2. *In situ* hybridization

Sections were hybridized as described previously (Schalling et al., 1988; Dagerlind et al., 1992). Briefly, air-dried sections were incubated in the hybridization buffer with labeled specific probes in a humidified chamber for 16-18 h at 42°C. After hybridization, the sections were washed in 1xSSC at 55°C, brought to room temperature over 30 min, then washed in distilled water, dehydrated and air-dried. The slides were dipped into Ilford K5 nuclear emulsion. After exposure at 4°C for 2-6 days, the slides were developed in Kodak D19, fixed in Kodak 3000 A&B and rinsed in water. Developed slides were mounted with glycerol and coverslipped for analysis in a Nikon Microphot-FX microscope equiped with a dark field condenser. For specific control of *in situ* hybridization, adjacent sections were incubated with an excess (x100) of unlabeled probe.

3.3.2.3. Quantification

Every 6th section from serial sectioning through the rostro-caudal dimension of the hypothalamus giving in total 10 sections for each mRNA was analyzed using a microscope equipped with a dark-field condenser (Nikon Microphot-FX) and a digital camera DC 300 (Leica Microsystems, Heerbrugg, Switzerland). An area containing labeled neurons was randomly selected (using a x20 objective) within the arcuate nucleus for NPY, POMC and CART or within the perifornical part of the lateral hypothalamic area for CART, MCH and orexin, and was saved as a 2088x1552 pixel grayscale TIFF image. The intensity of mRNA labeling was measured using Scion Image 4.0 (Maryland 21701, USA). Each captured image was calibrated to 256 pixel grey values (0 = white and 256 = black), and every labeled neuronal profile was individually selected, and its mean pixel density was measured. The background level was measured in a separate image from outside the section area. We assumed that silver grain density overlying individual neurons correlated directly with their level of mRNA expression. To estimate silver grain density, the mean pixel density was converted into relative optic density (ROD) using formula -log[(256-greyvalue)/256]. The background ROD level was calculated in the same way and was ultimately subtracted from the neuronal ROD. We used unpaired t-test to compare means of ROD between TCDD-treated and control rats. Data are expressed as Mean±SD.

3.4. Protein analysis (paper I-IV)

3.4.1. Western Blotting (paper I, II, IV)

The variation of protein expression after exposure to TCDD or βNF was determined by Western Blotting. Extracts from the pituitary, brain tissues or cultured cells were separated by SDS-PAGE, and transferred to nitrocellulose membranes. The membranes were probed with primary antibodies against CYP1A1, ACTH, or the house keeping protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH), then detected with an appropriate horseradish peroxidase (HRP) conjugated secondary antibody. The chemiluminescence from the bound HRP antibodies was detected on X-ray autoradiography films.

3.4.2. Immunohistochemistry (paper III, IV)

Pituitary sections: Immunohistochemistry for CYP1A1 and AHRR proteins was performed on the mouse frozen pituitary sections. Fourteen µm-thick sections were cut from tissue blocks, air-dried and fixed in Zamboni´s fixative (for CYP1A1) or 4 % paraformaldehyd (AHRR). The avidin-biotin-peroxidase complex (ABC) method was applied for CYP1A1 detection. The endogenous peroxidase activity was blocked by 0.3 % solution of H_2O_2 in methanol, followed by a thorough rinsing with PBS. After blocking unspecific binding by incubating the sections with 2 % bovine serum albumin (BSA), the sections were incubated with primary antibody overnight at 4°C, rinsed with PBS, incubated with secondary antibody in 2 % BSA at room temperature for 30 minutes. Then the sections were rinsed in PBS, followed by incubation with ABC for 45 min at room temperature. Color visualization was accomplished by using diamino-benzidine (DAB) substrate kit for 10 minutes. All incubations were carried out in a humidified chamber. Diluted haematoxylin was used for gentle nuclear counterstaining.

Immunohistochemistry for the AHRR protein was performed by incubating the fixed sections in 4 % normal goat serum plus 0.3 % Triton-X100 to block the unspecific binding. The sections were then incubated with a primary antibody against AHRR supplemented with 0.3 % Triton-X100 and 2 % BSA in PBS overnight at 4°C. After rinsing with PBS, the sections were incubated with FITC-conjugated secondary antibody with 0.3 % Triton-X100 in PBS for 1 h at room temperature, then rinsed with PBS. The sections were mounted in glycerol-PBS containing 0.1 % phenylenediamine. The pituitary sections were then examined with an Olympus BX60 fluorescence microscope. Images were collected with a Hamamatsu digital camera ORCA II.

Brain sections: Male Balb/cX129SV adult mice and Sprague-Dawley rats were used for immunohistochemical analysis of hypothalamic sections. In order to detect peptides/proteins more readily in neuronal cell bodies, we blocked centrifugal axonal transport in some mice by injecting colchicine (30 µg in 5 µl of 0.9 % NaCl) into the lateral ventricle under anesthesia with a mixture of 1ml Midasolam (5 mg/ml) and 1 ml Hypnorm (2.7 ml/kg), both given i.p. After colchicine injection 24-h, intact mice and rats were anaesthetized with sodium pentobarbital and perfused via the ascending aorta with Tyrode's Ca^{2+} free solution at 37°C, followed by a mixture of 4 % paraformaldehyde and 0.4 % picric acid in 0.16 M phosphate buffer (pH 6.9, 37°C) (Zamboni and De Martino, 1967). The brains were rapidly dissected out.

The tyramide signal amplification (TSA) Plus TMR System (Adams, 1992) was used to detect AHRR. For double-labeling, the TSA technique was followed by conventional immunohistochemistry (Coons, 1958) with primary antisera against NPY, CART, MCH and orexin. Secondary antibodies conjugated with fluorescein were used. Sections were mounted in a mixture of glycerol, 0.1 M phosphate buffered saline (3:1), pH 7.4, and containing 0.1 % para-phenylenediamine as anti-fading agent (Platt and Michael, 1983). After processing, the sections were examined in a Bio-Rad Radiance Plus confocal laser scanning system, installed on a Nikon Eclipse E600 fluorescence microscope. Digital images resulting from the microscopy were optimized for image resolution and images with double labeling were merged in Adobe PhotoShop 6.0.

3.4.3. Immunocytochemistry (paper II)

Immunocytochemistry was performed to detect the AHRR, ACTH and β-endorphin expressions in the AtT-20 cell line *in vitro*. Glass coverslips were pretreated with 0.05 mg/ml poly-L-lysine. After exposure to TCDD (1 pM and 1 nM) for 30 h, AtT-20 cells grown on coverslip were fixed with ice-cold 80 % methanol (v/v) for 30 minutes. The coverslips were incubated in a humid chamber at 4°C overnight with antibodies against the AHRR, ACTH and β-endorphin respectively diluted at 1:1000 in PBS, supplemented with 0.3 % Triton-X100 and 0.5 % BSA. The cells were then rinsed with PBS, and incubated with appropriate FITC-conjugated secondary antibodies diluted at 1:80 in PBS containing 0.3 % Triton-X100 for 30 minutes at room temperature. After three rinses with PBS, coverslips were mounted in glycerol-PBS containing 0.1 % phenylenediamine. The AtT-20 cells were then examined with an Olympus BX60 fluorescence microscope. Images were collected with a Hamamatsu digital camera ORCA II.

3.5. Statistical analysis

Values are given as mean ± SD in the tables and mean ± SEM in the figures. To analyze data from *in vivo* studies, which were non-normally distributed, the nonparametric test Kruskall-Wallis one-way ANOVA was used to compare mRNA levels from the different time points or doses. Student´s *t* test and the pair-wise Mann-Whiney U-tests were then used in comparison among different groups. Reported p-values were two-tailed and $p<0.05$ was considered statistically significant.

To analyze the changes in the body and liver weight of CRBP-I genotype mice, all data were passed through the test for normal distribution (Kolmogorov-Smirnov) without transformation by using the SPSS 11.0 software.

4. RESULTS AND DISCUSSION

4.1. CYP1A1, AHR and ARNT mRNAs are constitutively expressed in the brain and pituitary of mice and rats (paper I, II, IV, V)

Our study shows that CYP1A1, AHR and ARNT mRNAs are constitutively expressed in the brain and pituitary of rodents. The constitutive CYP1A1 expression is only slightly higher than the background. The basal levels of CYP1A1 mRNA in both anterior and posterior pituitary are similar to the levels in liver, but significantly higher compared to the basal expression in most rodent brain regions examined. Overall, constitutive levels of AHR and ARNT in all areas investigated are higher compared to CYP1A1. The results demonstrate that CYP1A1, AHR and ARNT mRNAs are ubiquitously present in the central nervous system and in the pituitary, and that TCDD may exert a direct action on these regions.

Although the presence of CYP1A1, AHR, ARNT has been repeatedly reported in brain tissues, their distributions in the brain are often contradictory (Anandatheerthavarada, et al. 1990; Das, et al. 1982; Dey et al., 1999; Kainu, et al. 1995; Kapitulnik, et al. 1987; Schilter and Omiecinski 1993; Warner, et al. 1988, Morse, et al. 1998; Petersen et al., 2000). The expression of the AHR and ARNT genes in the rat and developing mouse brain is proven by *in situ* hybridization and immunochemical analysis (Abbott et al., 1995; Kainu et al., 1995). With these methods, the highest expression of both AHR and ARNT is reported in the olfactory bulb, hippocampus, and cerebral and cerebellar cortices. Surprisingly, these mRNAs are undetectable in the areas, which are thought to be possible targets for TCDD toxic effects, such as the hypothalamic nuclei (Kainu, et al. 1995). The RT-PCR technique applied in the present study is more sensitive than *in situ* hybridization. Our data provide evidence that CYP1A1 is constitutively transcribed in the central nervous system and pituitary. Moreover, our study confirms previous knowledge on the distribution of AHR and ARNT mRNAs, providing new information on their presence in neuroendocrine relevant areas, such as the hypothalamus and the pituitary.

The localization of AHR, ARNT and CYP1A1 in CNS and in neuroendocrine regions provides an anatomical and biochemical basis for the impairment of neuronal and endocrine functions observed in dioxin intoxication.

4.2. TCDD induced the expression of CYP1A1 with the pituitary being the most responsive organ (paper I, II, IV)

After exposure to TCDD, an increase in the expression of CYP1A1 mRNA is observed in all regions examined, with the highest levels found in the anterior and posterior pituitary. The results of our studies show a high sensitivity of pituitary cells to TCDD. Only minor changes in the expression of AHR and ARNT mRNAs are caused by TCDD treatment in present studies. The balance between synthesis and degradation of AHR seems to be maintained at an equilibrium in the case of TCDD exposure, such that the animal is not likely to be sensitized to this environmental toxicant. The changes in the CYP1A1 expression appear early, whereas the changes in expression of AHR and ARNT are mainly of a delayed type and seen at the latest time point in the TCDD-dosed rats. In CRBP-I -/-, -/+, and +/+ mice, a two-fold increase in AHR mRNA levels is observed in the pituitary and the liver after TCDD

treatment. The protein expression of CYP1A1, determined with specific antibodies by Western blot analysis, shows a significant increase in the pituitary from the TCDD-treated rats and mice, as well as in the TCDD or βNF-treated AtT-20 cells. Immunohistochemical results reveal that CYP1A1 positive stained cells are present in the neurohypophysis, the pars intermedia and the pars distalis in the pituitaries of both CRBP-I +/+ and -/- mice after TCDD treatment. The localization of CYP1A1 protein is overlapped with the AHRR protein positive area.

Although it is believed that the blood-brain barrier may serve as a protective diffusion barrier and that dioxin does not readily cross the blood brain barrier (Pohjanvirta et al., 1990; Unkila et al., 1993b), the present results indicate that both the central nervous system and the pituitary may be direct targets for TCDD, as indicated by the time-dependent induction of CYP1A1 mRNA observed in all areas examined after exposure to TCDD. The potent induction of CYP1A1 mRNA in the pituitary resulted in a clear increase in CYP1A1 protein level after TCDD or βNF treatment as well.

The present study found that the most prominent induction of CYP1A1 did occur in the tissues with the highest basal mRNA levels of AHR and ARNT, i.e. the pituitary, as well as the liver. The results suggest that the pituitary is very sensitive to TCDD. This could be partially explained by the higher TCDD levels in the pituitary, which is highly exposed due to its dense vascularization.

4.3. Induced expression of AHRR in response to TCDD or βNF exposure (paper II-IV)

The present studies, in addition to showing high CYP1A1 inducibility in the brain and pituitary, also demonstrate an induced expression of the AHRR gene in the brain, pituitary, and in AtT-20 cells in response to TCDD or βNF. The increased expression of AHRR is concomitant to that of CYP1A1.

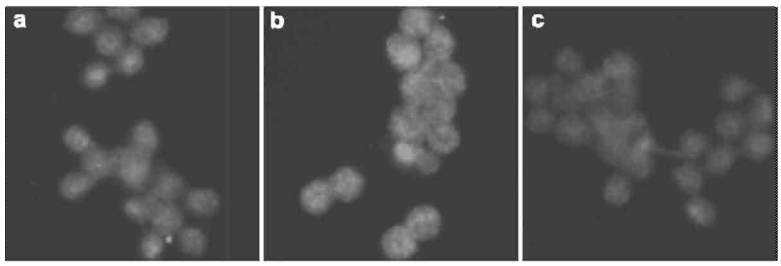

Figure 7. AHRR expression in the AtT-20 cells exposed to TCDD for 30 h

a. TCDD 1nM; b. TCDD 1pM; c. Control.

The basal mRNA level of AHRR in the pituitary is constitutively higher than that found in different brain regions or in the AtT-20 cells. The level of AHRR protein is also found to be increased by TCDD in the pituitaries of CRBP-I +/+ and -/- mice *in vivo* (**Paper IV**) and in the AtT-20 cells *in vitro* (Figure 7). These observations confirm the previous finding that AHRR might be involved in the AHR regulation pathway by competing with AHR (Mimura et al., 1999; Baba et al., 2001; Karchner et

al., 2002). A rapid and pronounced induction of AHRR in the brain and pituitary by AHR ligands supports the notion that the AHRR exerts a tissues-specific regulatory role (Mimura et al., 1999). The CYP1A1 protein has also been suggested to have an important function in the metabolism of endogenous ligands, and therefore a role in the regulation of the AHR-response (Chang and Puga, 1998; Wei et al., 2000). Thus, the AHR functions seem to have a feedback regulation in the brain and pituitary, by both the CYP1A1 protein and the AHRR protein.

Subcellular localization of AHRR immunoreactivity (-ir) as revealed in the mouse brain is confined to the nuclei of neurons, in agreement with previously shown constitutive expression of AHRR-GFP fusion protein in the nuclei of COS7 cells in culture (Mimura et al., 1999). AHRR-ir is found in all main hypothalamic subdivisions, with different intensities. AHRR-ir is also present in extrahypothalamic areas, including hippocampus and cortex. Absorption test with AHRR completely abolishes AHRR-ir in the mouse brain confirming the specificity of the staining. More interesting, by double-staining mouse brain with this specific AHRR antibody and with individual neuropeptide antibodies, AHRR-ir is found to be co-localized with NPY and CART in the arcuate nucleus, and with MCH and orexin in the lateral hypothalamus and zona incerta. Such anatomical and immunohistochemical data support the hypothesis that the AHRR may have a role in the regulation of neuropeptides in the central nervous system after TCDD exposure.

In summary, AHRR can be induced in the brain and pituitary by xenobiotic compounds both *in vivo* and *in vitro*.

4.4. Changes in the expression of hypothalamic neuropeptides in response to TCDD exposure (paper III)

After TCDD treatment, body weight loss is a consistent clinical sign in most animal species. It is also by far the prototypic feature for the characteristic toxicity of TCDD. Decreased body weight in the SD-rat treated with TCDD has been observed in our experiments. Body weight gain observed during 6 days after a single dose of TCDD is lower in TCDD-treated rats vs. control, accompanied by changes in gene expression of hypothalamic neuropeptides.

A characteristic pattern of cellular distribution of NPY, POMC and CART mRNAs is observed throughout the rostro-caudal extension of the arcuate nucleus (Arc) in both control and TCDD treated rats. Quantifications of the *in situ* hybridization signal in the individual arcuate nucleus neurons show that mRNA levels of all three neuropeptides are altered significantly in TCDD-treated rats vs. controls. In the LHA, TCDD-treated rats show an increased MCH mRNA level compared with control animals, in contrast the change of CART as well as orexin mRNA levels does not reach significance. Both body weight stimulatory and inhibitory neuropeptides are activated, indicating that the central peptidergic circuitries have been deregulated by TCDD. The mechanism underlying this deregulation may involve both direct and indirect actions of TCDD. For a direct action of TCDD, the target neurons should possess both AHR and ARNT and the neuropeptide genes should have XRE. We have shown that the expression of AHR and ARNT mRNAs is ubiquitously present in all rat brain regions, including the caudate-putamen, cerebellum, cortex, hypothalamus, substantia nigra, hippocampus and olfactory bulb (**Paper I**), as well as in mouse brain regions (**Paper IV**). Moreover, our results show that the AHRR protein is found to co-localize with neuropeptides in

neurons. Both the arcuate nucleus neurons immunopositive for NPY and CART, and the lateral hypothalamic area neurons immunopositive for orexin and MCH, have AHRR-positive nuclei. A relatively higher intensity of AHRR immunostaining in the arcuate neurons suggests that they have a more pronounced AHR-mediated signal transduction mechanism than those neurons located in deeper hypothalamic structures.

Table 2. Relative mRNA levels of POMC in the pituitary gland and pituitary cell cultures treated with TCDD or βNF

Samples	Treatment	Time	n	POMC Mean±SD‡
CRBP-I knockout mouse				
CRBP-I genotype				
-/-	Control	7 days	5	3.49 ± 0.61
-/-	TCDD 50 μg/kg	7 days	9	8.58 ± 5.10*
-/-	TCDD 250 μg/kg	7 days	5	5.19 ± 1.47*
-/-	Control	28 days	5	3.35 ± 0.87
-/-	TCDD 50 μg/kg	28 days	3	5.61 ± 1.76*
-/-	TCDD 250 μg/kg	28 days	4	7.10 ± 1.96**
-/+	Control		4	7.30 ± 3.13
-/+	TCDD 250 μg/kg	7 days	3	12.99 ± 1.70
-/+	TCDD 50 μg/kg	28 days	3	7.88 ± 2.37
-/+	TCDD 250 μg/kg	28 days	2	7.08 ± 1.64
+/+	Control	7 days	5	4.65 ± 1.77
+/+	TCDD 50 μg/kg	7 days	4	13.32 ± 4.32**
+/+	TCDD 50 μg/kg	28 days	4	8.78 ± 0.79**
Rat primary anterior pituitary cell culture				
	Control	3 h	4	1.30 ± 0.10
	βNF 2μM	3 h	4	1.60 ± 0.18*
	βNF 6μM	3 h	4	1.70 ± 0.30*
Mouse pituitary AtT-20 cell				
	Control	3 h	4	9.65 ± 0.55
	TCDD 1pM	3 h	4	10.19 ± 1.06
	TCDD 1nM	3 h	4	11.55 ± 0.27***
	Control	3 h	5	10.90 ± 1.86
	βNF 2μM	3 h	4	12.82 ± 1.06
	βNF 6μM	3 h	5	12.36 ± 0.87

‡The unit of POMC mRNA relative levels is X 10^{-18} moles / 100 ng total RNA as analyzed by competitive quantitive RT-PCR.
*p<0.05; **p<0.01; ***p<0.01 compared to the controls, Student *t*test.

Screening the neuropeptide genes for the presence of the XRE revealed that, with the exception of MCH, all contain xenobiotic responsive elements. Therefore, TCDD may directly regulate gene expression of NPY, POMC, CART and orexin in the hypothalamic neurons.

In addition, significantly increased levels of POMC mRNA are observed after TCDD or βNF treatment in the pituitaries of mice with different CRBP-I genotype, SD-rat primary anterior pituitary cells in culture and AtT-20 cells (Table 2). The changes in mRNA are reflected by corresponding changes in ACTH and β-endorphin proteins encoded by POMC transcripts after TCDD exposure in AtT-20 cells in this study (**Paper II**). These results are in agreement with previous reports that TCDD increases ACTH levels in rat pituitary cell primary cultures and β-endorphin-like immunoreactivity in the TCDD susceptible rat (Pohjanvirta et al., 1993; Bestervelt et al., 1991, 1998a). In the promoter region of the human POMC gene three XREs, which act as binding sites for the AHR, are found. Thus, the mRNA level of POMC may be induced by xenobiotics in the pituitary. These findings support and extend the previous observation that TCDD acts at the ACTH level in the pituitary. Our data suggest that TCDD-induced body weight loss may be related to the regulation of POMC gene expression in the pituitary.

The presence of several XRE sequences in the promoter region and in the intron of neuropeptide genes suggests that the up-regulation of hypothalamic neuropeptides expression in the brain may play a role in the wasting syndrome induced by TCDD through an AHR mediated process. The present data suggest, for the first time, that the AHR pathway may be involved in the regulation of these neuropeptide genes in the brain after TCDD treatment.

4.5. Modulation of AHR-regulated gene expression in CRBP-I genotype mice (paper IV)

The AHR-ARNT regulated genes, CYP1A1, CYP1A2, AHR and AHRR are up-regulated by TCDD in CRBP-I wild type as well as in the mutant (-/-, -/+) mice.

The present study shows some modulation of the TCDD-induced gene expression by the deficiency of the retinol binding protein. A major finding is the high basal mRNA and protein levels of AHRR in the pituitary. The high basal expression of AHRR mRNA, which is approximately 8-fold higher in the pituitary compared to the other tissues investigated, suggests that there is a tight control system that is activated via the AHR/ARNT pathway in the pituitary. The results also indicate that AHRR may have a physiological role in the pituitary gland.

Another interesting aspect is that mice which lacked the CRBP-I protein (-/-) are found to have a significantly higher basal expression of AHRR gene in the pituitary compared to the wild type (CRBP-I +/+) mice. The high basal expression of AHRR in the pituitary of CRBP-I -/- mice may permit the AHRR protein to accumulate in the cell, influence other regions through the hormones secretion and cause prolonged effects. Indeed, in the cerebellum, cortex and hypothalamus there is a prolonged expression of AHRR mRNA observed in the -/- mice when compared with the -/+ and +/+ mice. We also found slightly lower induction of CYP1A1, AHR and AHRR mRNA expression in the CRBP-I null mice when compared to the +/+ mice. These phenomena may be related to the high basal mRNA level of AHRR in the pituitaries of CRBP-I null mice.

It has been documented before that the AHR-ARNT pathway is involved in TCDD-induced retinoid homeostasis (see Nilsson and Hakansson, 2002), and the CYP system has been reported to play a role in retinoid metabolism (Duester, 1996; White et al., 1996; Chen et al., 2000; Zhang et al., 2000; Ding et al., 2001). Based on the observation of gene modulation in CRBP-I genotype mice, we conclude that the vitamin A homeostasis seems to have some influence to the TCDD-induced activation of AHR-regulated gene transcription. A crossregulation between the AHR and the retinoid system seems to exist and deserves further studies.

4.6. Diurnal rhythms of CYP1A1 mRNA expression in the rat pituitary (paper V)

To further characterize the expression of CYP1A1, AHR, and ARNT in the pituitary, we have also investigated the diurnal rhythms of their mRNA levels. The results of this study show temporal patterns in the mRNA expression of CYP1A1, AHR and ARNT in the pituitary from Sprague-Dawley rats. In addition, the diurnal expression of the PER2 gene, encoding for a PAS protein involved the regulation of the mammalian clock genes CLOCK/BMAL1, is also demonstrated. The abundance of PER2 mRNA is found to vary 2.6-fold in both the anterior and the posterior pituitary between the highest and lowest levels in the daily cycle. A pronounced antiphase expression of some genes was observed between the anterior and the posterior pituitary. For example, a very tightly regulated diurnal variation in CYP1A1 mRNA was observed which exhibited an opposite expression in the anterior and the posterior pituitary. Our present data are in agreement with the previous findings of temporal peaks in CYP1A1 protein or enzyme activity (Tredger and Chhabra, 1977; Plewka et al., 1992), as well as temporal changes in AHR and ARNT protein levels in the liver (Richardson et al., 1998).

Molecular circadian pacemakers consist of genes encoding proteins that can activate or repress gene transcription. The AHR-ARNT heterodimeric protein shares a great similarity with the known components of the mammalian circadian response pathway, the CLOCK/BMAL1 complex. They are the bHLH-PAS proteins that utilize promoter sequences, the XRE-sequence and the E-box sequence, which share high sequence homology. Additionally, their transcriptional products, the CYP1A1 protein and the PER proteins (by interaction with CRY proteins), act as repressors which turn off transcription of their own genes. The repressor proteins, CYP1A1 and PER, have a limited lifespan and eventually decay. When being activated again, a new wave of repressor gene transcription commences. This study found that the expression of CYP1A1 and PER2 was highly coordinated in the liver of the rat. This observation suggests a possible interaction between the AHR-ARNT and the CLOCK/BMAL systems, or a similar response to the daily environmental changes.

Overall, this study illustrates that the CYP1A1 gene, as well as the bHLH-PAS genes, AHR, ARNT and PER2, display daily variations in mRNA levels. The results on diurnal rhythms also reveal the importance of the time factor when comparing gene expression in experimental studies.

CONCLUSIONS

- The results provide evidence for the presence of the AHR and AHR responsive genes in the central nervous system and in the pituitary, as well as the ability of TCDD to alter their expressions. The AHR-ARNT system is considered as a defense system against toxic chemicals. The high inducibility of CYP1A1, increased expression of AHRR in the brain and pituitary after TCDD treatment, as well as the tissue specific expression patterns observed in the present studies, suggest that AHR-ARNT, CYP1A1 and AHRR may also play a physiological role in controlling neuroendocrine functions.

- The observed up-regulation of neuropeptide expression involved in both orexigenic and anorexigenic alteration in the brain suggests that neuropeptides might be involved in TCDD-induced wasting syndrome.

- Vitamin A homeostasis seems to have some influence on the TCDD-induced activation of AHR-regulated gene transcription, based on the observations from CRBP-I genotype mice.

- The pronounced rhythmicity in the expression of CYP1A1 indicates a tightly regulated function of the CYP1A1 protein in the pituitary. The results on diurnal rhythms also point to the importance of the time factor in comparisons of gene expression in experimental studies.

- Taken together, the alterations of gene expression observed in animals exposed to TCDD indicate that the central nervous system and the pituitary may be direct targets for dioxin and dioxin like compounds with high AHR binding affinity.

ACKNOWLEDGEMENTS

This work was performed at the Division of Toxicology and Neurotoxicology, and the Division of Work Environment Toxicology, National Institute of Environmental Medicine, Karolinska Institutet. I would like to express my sincere gratitude to my colleagues and friends who have contributed to this thesis, especially:

Professor **Sandra Ceccatelli** and Associate Professor **Agneta Rannug**, my supervisors, for giving me this great opportunity to work with the intriguing TCDD project, and for super scientific guidance, solid support and valuable advice. Also, for your constant help and your constructive discussions. I am deeply grateful for everything I have learned and for many unforgettable and enjoyable times in the Lab.

Professor **Sten Orrenius**, Head of the Division, for creating a pleasant stimulating research environment and for the excellent scientific guidance.

Professor **Göran Pershagen**, the head of IMM, for giving me the opportunity to work at the Institute.

Professor **Tomas Hökfelt** and Dr. **Serguei O. Fetissov**, my co-authors, for sharing your great knowledge in Neurosciences and neuropeptides, for scientific and technical guidance, for nice co-work and discussions in the dioxin project.
Professor **Helen Håkansson**, and **Pi Hoegberg**, my co-authors, for sharing your great knowledge in TCDD toxicity, for well-designed animal experiments, for contributions, discussions and comments to my papers and manuscript.
Drs. **Lindsey Grandison**, **Eva Ahlbom**, **Qing Zhang**, my co-authors, for your invaluable collaborations and essential contribution.

All past and present colleagues in the Neurotoxicology group for providing a warm, helpful and fun working environment. Dr. **Elisabetta Dare** and **Roshan Tofighi**, for endless chats about life and technical problems; **Eric Sleeper** for proof reading my Licentiate thesis; **Christoffer Tamm** for help with the computer; Dr. **Adrienne Gorman** for fun and good discussions, and for the kind help with the application for travel funding; **Nesar Uddin Akanda**, **Michele Benelli**, **Ming Chen**, **Marta Dabrio**, Dr. **Mia Emgard**, **Elena Fonfria**, Dr. **Mario Götz**, Dr. **Tiejun Shi,** and **Maria Trieb,** for nice discussions and good times.
All of my colleagues in the Work Environment Toxicology group: Associate Professor **Margareta Warholm**, for giving me useful comments; Dr. **Yudan Wei** for being my Chinese friend at work; Dr. **Anna-Karin Alexandrie** for help and support during the preparation of the thesis; **Rainer Tuominen**, **Margareta Ståhlberg** and **Anna-Karin Nilsson** for the good times together.
All the past and present members of the "Blue Group": Professor **Boris Zhivotovsky** for sharing your knowledge on apoptosis; Dr. **Bengt Fadeel** for your generosity; Dr. **Bertrand Joseph** for technical advice on RPA method, Dr. **Leta Nutt** for proof reading my thesis; **Pavel Grudinkin** for help with the computer; Dr. **John Robertson** for nice discussions about TCDD-induced apoptosis in the pituitary; Drs. **Babett Bartling**, **Joya Chandra**, **Jessica Ekedahl**, **Mari Enoksson, Bettina Gleiss**,

Vladimir Gogvadze, **Therese Hemström**, **Suzanne Iverson**, **Maria Karpova**, **Andrej Kropotov**, **Camilla Köhler**, **Afshin Samali**, **Margareta Sandström**, **Jan Schmidt-Mende**, **Agnieszka Sobczak-Pluta**, **Helene Stridh** and **Wanlaya Uthaisang**, for being helpful in the Lab and the good times together.

Birgitta Blacker and **Eva Undsen**, the secretaries in our Institute, for always being cheerful and helpful; for invaluable help in many situations; for many on-time announcements on the funding application and important information.

Professor **Jan Hillert,** Professor **Lennart Hammarström**, for generously accepting me to work in your laboratories for a period of time, for your scientific guidence and nice discussons.

Associate Professor **Anders Bergendorff,** Associate Professor **Monica Nordberg**, for sharing your knowledge in toxicology and nice discussions in the courses.
Christina Trossvik for skillful assistance in working with the animals.
Dr. **Maria Backlund,** for kindly supplying the CYP1A1 antibody and helping with the Western Blotting. **Simi Anastasia**, **Edyta Bajak**, **Brita Palm**, **Miyuki Shimoji**, **Atchasai Siritontikorn**, **Jue Wang**, and **Mattias Öberg**, for your encouragement and nice times together.
Everyone else in the IMM who helped me throughout this work.

Professor **Shangda Cai**, for leading me to the field of medical biology.

To all my dear friends outside the lab and previous colleagues, especially Drs. **Lei Pei**, **Tao Wang**, **Huihong Cheng**, **Xuxia Wu**, **Qing Liu**, **Wenjie Bao**, **Li Liu, Jinjing Pei**, **Huixin Wang**, **Zhong He**, **Yu Shi**, **Fengqing Xiang**, **Liping Luo**, **Shuang Liu**, **Fengming Lu**, **Yanzhong Hu** and **Qiang Pan**, for your warm friendship, encouragement, wonderful times together, and Chinese speaking.

My parents, for your endless love and encouragement. My younger sister **Yi Huang** and her hunsband **Shaowen Qiu**, my younger brother **Fan Huang** and her wife **Yanyan Jia**, for your love, encouragement, and helpfulness. My nieces **Zhuyun Huang** and **Leran Qiu**, for nice time together in Paris.

Finally, my dear husband **Wenxin Huang**, for sharing the sweetness and bitterness of life together, for teaching me new techniques, giving me experimental advice and helping me make figures on the computer. Your love, encouragement and support, have made my study in Sweden possible.

This work was supported by the grant 12X-10815-04B from the Swedish Medical Research Council; the Swedish Foundation for Strategic Environmental Research; the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning; the Commission of the European Communition; and by the Swedish Environmental Protection Board.

1 REFERENCES

Abbott, B. D., Birnbaum, L. S., and **Perdew, G. H.** (1995). Developmental expression of two members of a new class of transcription factors: I. Expression of aryl hydrocarbon receptor in the C57BL/6N mouse embryo. *Dev Dyn* **204**, 133-43.

Adami, H. O., Lipworth, L., Titus-Ernstoff, L., Hsieh, C. C., Hanberg, A., Ahlborg, U., Baron, J., and **Trichopoulos, D.** (1995). Organochlorine compounds and estrogen-related cancers in women. *Cancer Causes Control* **6**, 551-66.

Adams, J. C. (1992). Biotin amplification of biotin and horseradish peroxidase signals in histochemical stains. *J Histochem Cytochem* **40**, 1457-63.

Ahlborg, U. G., Brouwer, A., Fingerhut, M. A., Jacobson, J. L., Jacobson, S. W., Kennedy, S. W., Kettrup, A. A., Koeman, J. H., Poiger, H., and **Rappe, C.,** (1992). Impact of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls on human and environmental health, with special emphasis on application of the toxic equivalency factor concept. *Eur J Pharmacol* **228**, 179-99.

Anandatheerthavarada, H. K., Shankar, S. K., and **Ravindranath, V.** (1990). Rat brain cytochromes P-450: catalytic, immunochemical properties and inducibility of multiple forms. *Brain Res* **536**, 339-43.

Andersson, T., Forlin, L., Olsen, S., Fostier, A., and **Breton, B.** (1993). Pituitary as a target organ for toxic effects of P4501A1 inducing chemicals. *Mol Cell Endocrinol* **91**, 99-105.

Antonsson, C. (1999). *Inducible and constitutive modes of gene regulation by the dioxin receptor and Arnt.* (Thesis). In The Department of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institutet. Stockholm, Sweden.

Baba, T., Mimura, J., Gradin, K., Kuroiwa, A., Watanabe, T., Matsuda, Y., Inazawa, J., Sogawa, K., and **Fujii-Kuriyama, Y.** (2001). Structure and expression of the Ah receptor repressor gene. *J Biol Chem* **276**, 33101-10.

Backlund, M., Johansson, I., Mkrtchian, S., and **Ingelman-Sundberg, M.** (1997). Signal transduction-mediated activation of the aryl hydrocarbon receptor in rat hepatoma H4IIE cells. *J Biol Chem* **272**, 31755-63.

Backlund, M., Weidolf, L., and **Ingelman-Sundberg, M.** (1999). Structural and mechanistic aspects of transcriptional induction of cytochrome P450 1A1 by benzimidazole derivatives in rat hepatoma H4IIE cells. *Eur J Biochem* **261**, 66-71.

Bacsi, S. G., Reisz-Porszasz, S., and **Hankinson, O.** (1995). Orientation of the heterodimeric aryl hydrocarbon (dioxin) receptor complex on its asymmetric DNA recognition sequence. *Mol Pharmacol* **47**, 432-8.

Barsh, G. S., and **Schwartz, M. W.** (2002). Genetic approaches to studying energy balance: perception and integration. *Nat Rev Genet* **3**, 589-600.

Bastomsky, C. H. (1977). Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Endocrinology* **101**, 292-6.

Berghard, A., Gradin, K., Pongratz, I., Whitelaw, M., and **Poellinger, L.** (1993). Cross-coupling of signal transduction pathways: the dioxin receptor mediates induction of cytochrome P-450IA1 expression via a protein kinase C-dependent mechanism. *Mol Cell Biol* **13**, 677-89.

Bernes, C. (1998). *Persistent organic pollutants: A Swedish view of an international problem.* Swedish Environmental Protection Agency, Stockholm.

Bertazzi, A., Pesatori, A. C., Consonni, D., Tironi, A., Landi, M. T., and **Zocchetti, C.** (1993). Cancer incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-para-dioxin. *Epidemiology* **4**, 398-406.

Bestervelt, L. L., Cai, Y., Piper, D. W., Nolan, C. J., Pitt, J. A., and **Piper, W. N.** (1993a). TCDD alters pituitary-adrenal function. I: Adrenal responsiveness to exogenous ACTH. *Neurotoxicol Teratol* **15**, 365-7.

Bestervelt, L. L., Nolan, C. J., Cai, Y., Maimansomsuk, P., Mousigian, C. A., and **Piper, W. N.** (1991). Tetrachlorodibenzo-p-dioxin alters rat hypothalamic endorphin and mu opioid receptors. *Neurotoxicol Teratol* **13**, 495-7.

Bestervelt, L. L., Pitt, J. A., Nolan, C. J., Cai, Y., Piper, D. W., Dybowski, J. A., Dayharsh, G. A., and **Piper, W. N.** (1998a). In vitro 2,3,7,8-tetrachlorodibenzo-p-dioxin interference with the anterior pituitary hormone adrenocorticortropin. *Toxicol Sci* **44**, 107-15.

Bestervelt, L. L., Pitt, J. A., Nolan, C. J., and **Piper, W. N.** (1993b). TCDD alters pituitary-adrenal function. II: Evidence for decreased bioactivity of ACTH. *Neurotoxicol Teratol* **15**, 371-6.

Bestervelt, L. L., Pitt, J. A., and **Piper, W. N.** (1998b). Evidence for Ah receptor mediation of increased ACTH concentrations in primary cultures of rat anterior pituitary cells exposed to TCDD. *Toxicol Sci* **46**, 294-9.

Birnbaum, L. S. (1994). The mechanism of dioxin toxicity: relationship to risk assessment. *Environ Health Perspect* **102 Suppl 9**, 157-67.

Birnbaum, L. S., and **Tuomisto, J.** (2000). Non-carcinogenic effects of TCDD in animals. *Food Addit Contam* **17**, 275-88.

Bittencourt, J. C., and **Elias, C. F.** (1998). Melanin-concentrating hormone and neuropeptide EI projections from the lateral hypothalamic area and zona incerta to the medial septal nucleus and spinal cord: a study using multiple neuronal tracers. *Brain Res* **805**, 1-19.

Bjeldanes, L. F., Kim, J. Y., Grose, K. R., Bartholomew, J. C., and **Bradfield, C. A.** (1991). Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8- tetrachlorodibenzo-p-dioxin. *Proc Natl Acad Sci U S A* **88**, 9543-7.

Boening, D. W. (1998). Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin to several ecological receptor groups: a short review. *Ecotoxicol Environ Saf* **39**, 155-63.

Boerman, M. H., and **Napoli, J. L.** (1996). Cellular retinol-binding protein-supported retinoic acid synthesis. Relative roles of microsomes and cytosol. *J Biol Chem* **271**, 5610-6.

Boucher, P. D., Piechocki, M. P., and **Hines, R. N.** (1995). Partial characterization of the human CYP1A1 negatively acting transcription factor and mutational analysis of its cognate DNA recognition sequence. *Mol Cell Biol* **15**, 5144-51.

Boucher, P. D., Ruch, R. J., and **Hines, R. N.** (1993). Specific nuclear protein binding to a negative regulatory element on the human CYP1A1 gene. *J Biol Chem* **268**, 17384-91.

Broberger, C., De Lecea, L., Sutcliffe, J. G., and **Hokfelt, T.** (1998a). Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. *J Comp Neurol* **402**, 460-74.

Broberger, C., and **Hokfelt, T.** (2001). Hypothalamic and vagal neuropeptide circuitries regulating food intake. *Physiol Behav* **74**, 669-82.

Broberger, C., Johansen, J., Johansson, C., Schalling, M., and **Hokfelt, T.** (1998b). The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proc Natl Acad Sci U S A* **95**, 15043-8.

Broberger, C., Landry, M., Wong, H., Walsh, J. N., and **Hokfelt, T.** (1997). Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. *Neuroendocrinology* **66**, 393-408.

Brouwer, A., Hakansson, H., Kukler, A., Van den Berg, K. J., and **Ahlborg, U. G.** (1989). Marked alterations in retinoid homeostasis of Sprague-Dawley rats induced by a single i.p. dose of 10 micrograms/kg of 2,3,7,8- tetrachlorodibenzo-p-dioxin. *Toxicology* **58**, 267-83.

Bunce, N. J., Landers, J. P., Schneider, U. A., Safe, S. H., and **Zacharewski, T. R.** (1990). Chlorinated trans stilbenes: Competitive binding to the AH receptor, induction of cytochrome P-450 monooxygenase activity and partial 2,3,7,8-TCDD antagonism. *Toxicol. Environ. Chem.* **28**, 217-229.

Burbach, K. M., Poland, A., and **Bradfield, C. A.** (1992). Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor. *Proc Natl Acad Sci U S A* **89**, 8185-9.

Carver, L. A., Hogenesch, J. B., and **Bradfield, C. A.** (1994). Tissue specific expression of the rat Ah-receptor and ARNT mRNAs. *Nucleic Acids Res* **22**, 3038-44.

Carver, L. A., LaPres, J. J., Jain, S., Dunham, E. E., and **Bradfield, C. A.** (1998). Characterization of the Ah receptor-associated protein, ARA9. *J Biol Chem* **273**, 33580-7.

Castro, M. G., and **Morrison, E.** (1997). Post-translational processing of proopiomelanocortin in the pituitary and in the brain. *Crit Rev Neurobiol* **11**, 35-57.

Celi, F. S., Zenilman, M. E., and **Shuldiner, A. R.** (1993). A rapid and versatile method to synthesize internal standards for competitive PCR. *Nucleic Acids Res* **21**, 1047.

Chaffin, C. L., Trewin, A. L., and **Hutz, R. J.** (2000). Estrous cycle-dependent changes in the expression of aromatic hydrocarbon receptor (AHR) and AHR-nuclear translocator (ARNT) mRNAs in the rat ovary and liver. *Chem Biol Interact* **124**, 205-16.

Chang, C. Y., and **Puga, A.** (1998). Constitutive activation of the aromatic hydrocarbon receptor. *Mol Cell Biol* **18**, 525-35.

Chang, D. C., and **Reppert, S. M.** (2001). The circadian clocks of mice and men. *Neuron* **29**, 555-8.

Chemelli, R. M., Willie, J. T., Sinton, C. M., Elmquist, J. K., Scammell, T., Lee, C., Richardson, J. A., Williams, S. C., Xiong, Y., Kisanuki, Y., Fitch, T. E., Nakazato, M., Hammer, R. E., Saper, C. B., and **Yanagisawa, M.** (1999). Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* **98**, 437-51.

Chen, H., Howald, W. N., and **Juchau, M. R.** (2000). Biosynthesis of all-trans-retinoic acid from all-trans-retinol: catalysis of all-trans-retinol oxidation by human P-450 cytochromes. *Drug Metab Dispos* **28**, 315-22.

Chen, Y. H., Riby, J., Srivastava, P., Bartholomew, J., Denison, M., and **Bjeldanes, L.** (1995). Regulation of CYP1A1 by indolo[3,2-b]carbazole in murine hepatoma cells. *J Biol Chem* **270**, 22548-55.

Chen, Y. H., and **Tukey, R. H.** (1996). Protein kinase C modulates regulation of the CYP1A1 gene by the aryl hydrocarbon receptor. *J Biol Chem* **271**, 26261-6.

Cheung, W. M., and **Ip, N. Y.** (1998). Interaction of neurotrophins and retinoic acid in neuronal differentiation. *Mol Cells* **8**, 496-502.

Christian, B. J., Inhorn, S. L., and **Peterson, R. E.** (1986). Relationship of the wasting syndrome to lethality in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Appl Pharmacol* **82**, 239-55.

Collu, R., Ducharme, J. R., and **Guyda, H. J.** (1989). *Pediatric Endocrinology*. Raven press, New York.

Coons, A. H. (1958). Fluorescent antibody methods. In: *General Cytochemical Methods* (J.F. Danielli, Ed.), pp.399-422. Academic Press, New York.

Dagerlind, A., Friberg, K., Bean, A. J., and **Hokfelt, T.** (1992). Sensitive mRNA detection using unfixed tissue: combined radioactive and non-radioactive in situ hybridization histochemistry. *Histochemistry* **98**, 39-49.

Das, M., Seth, P. K., Dixit, R., and **Mukhtar, H.** (1982). Aryl hydrocarbon hydroxylase of rat brain mitochondria: properties of, and effect of inhibitors and inducers on, enzyme activity. *Arch Biochem Biophys* **217**, 205-15.

de Lecea, L., Kilduff, T. S., Peyron, C., Gao, X., Foye, P. E., Danielson, P. E., Fukuhara, C., Battenberg, E. L., Gautvik, V. T., Bartlett, F. S., 2nd, Frankel, W. N., van den Pol, A. N., Bloom, F. E., Gautvik, K. M., and **Sutcliffe, J. G.** (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A* **95**, 322-7.

De Leeuw, A. M., Gaur, V. P., Saari, J. C., and **Milam, A. H.** (1990). Immunolocalization of cellular retinol-, retinaldehyde- and retinoic acid-binding proteins in rat retina during pre- and postnatal development. *J Neurocytol* **19**, 253-64.

Delescluse, C., Lemaire, G., de Sousa, G., and **Rahmani, R.** (2000). Is CYP1A1 induction always related to AHR signaling pathway? *Toxicology* **153**, 73-82.

Dey, A., Jones, J. E., and **Nebert, D. W.** (1999). Tissue- and cell type-specific expression of cytochrome P450 1A1 and cytochrome P450 1A2 mRNA in the mouse localized in situ hybridization. *Biochem Pharmacol* **58**, 525-37.

DiBartolomeis, M. J., Moore, R. W., Peterson, R. E., Christian, B. J., and **Jefcoate, C. R.** (1987). Altered regulation of adrenal steroidogenesis in 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated rats. *Biochem Pharmacol* **36**, 59-67.

Ding, J., Ichikawa, M., Furukawa, A., Tomita, S., Tanaka, K., and **Ichikawa, Y.** (2001). Low synthesis of retinoic acid due to impaired cytochrome P450 1a1 expression in mouse xeroderma pigmentosum fibroblasts. *Int J Biochem Cell Biol* **33**, 603-12.

Douglass, J., McKinzie, A. A., and **Couceyro, P.** (1995). PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. *J Neurosci* **15**, 2471-81.

Dragan, Y. P., and **Schrenk, D.** (2000). Animal studies addressing the carcinogenicity of TCDD (or related compounds) with an emphasis on tumour promotion. *Food Addit Contam* **17**, 289-302.

Drouin, J., and **Goodman, H. M.** (1980). Most of the coding region of rat ACTH beta--LPH precursor gene lacks intervening sequences. *Nature* **288**, 610-3.

Drutel, G., Kathmann, M., Heron, A., Schwartz, J. C., and **Arrang, J. M.** (1996). Cloning and selective expression in brain and kidney of ARNT2 homologous to the Ah receptor nuclear translocator (ARNT). *Biochem Biophys Res Commun* **225**, 333-9.

Duester, G. (1996). Involvement of alcohol dehydrogenase, short-chain dehydrogenase/reductase, aldehyde dehydrogenase, and cytochrome P450 in the control of retinoid signaling by activation of retinoic acid synthesis. *Biochemistry* **35**, 12221-7.

Duester, G. (2000). Families of retinoid dehydrogenases regulating vitamin A function: production of visual pigment and retinoic acid. *Eur J Biochem* **267**, 4315-24.

Dunlap, J. C. (1999). Molecular bases for circadian clocks. *Cell* **96**, 271-90.

Durston, A. J., Timmermans, J. P., Hage, W. J., Hendriks, H. F., de Vries, N. J., Heideveld, M., and **Nieuwkoop, P. D.** (1989). Retinoic acid causes an anteroposterior transformation in the developing central nervous system. *Nature* **340**, 140-4.

Eguchi, H., Ikuta, T., Tachibana, T., Yoneda, Y., and **Kawajiri, K.** (1997). A nuclear localization signal of human aryl hydrocarbon receptor nuclear translocator/hypoxia-inducible factor 1beta is a novel bipartite type recognized by the two components of nuclear pore- targeting complex. *J Biol Chem* **272**, 17640-7.

Elferink, C. J., Gasiewicz, T. A., and **Whitlock, J. P., Jr.** (1990). Protein-DNA interactions at a dioxin-responsive enhancer. Evidence that the transformed Ah receptor is heteromeric. *J Biol Chem* **265**, 20708-12.

Elmquist, J. K., Elias, C. F., and **Saper, C. B.** (1999). From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron* **22**, 221-32.

Elovaara, E., Savolainen, H., Parkki, M. G., Aitio, A., and **Vainio, H.** (1977). Neurochemical effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Wistar and Gunn rats. *Res Commun Chem Pathol Pharmacol* **18**, 487-94.

Ema, M., Sogawa, K., Watanabe, N., Chujoh, Y., Matsushita, N., Gotoh, O., Funae, Y., and **Fujii-Kuriyama, Y.** (1992). cDNA cloning and structure of mouse putative Ah receptor. *Biochem Biophys Res Commun* **184**, 246-53.

Eriksson, U., Das, K., Busch, C., Nordlinder, H., Rask, L., Sundelin, J., Sallstrom, J., and **Peterson, P. A.** (1984). Cellular retinol-binding protein. Quantitation and distribution. *J Biol Chem* **259**, 13464-70.

Etchamendy, N., Enderlin, V., Marighetto, A., Vouimba, R. M., Pallet, V., Jaffard, R., and **Higueret, P.** (2001). Alleviation of a selective age-related relational memory deficit in mice by pharmacologically induced normalization of brain retinoid signaling. *J Neurosci* **21**, 6423-9.

Fattore, E., Trossvik, C., and **Hakansson, H.** (2000). Relative potency values derived from hepatic vitamin A reduction in male and female Sprague-Dawley rats following subchronic dietary exposure to individual polychlorinated dibenzo-p-dioxin and dibenzofuran congeners and a mixture thereof. *Toxicol Appl Pharmacol* **165**, 184-94.

Fernandez-Salguero, P., Pineau, T., Hilbert, D. M., McPhail, T., Lee, S. S., Kimura, S., Nebert, D. W., Rudikoff, S., Ward, J. M., and **Gonzalez, F. J.**

(1995). Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science* **268**, 722-6.

Findling, J. W., and **Tyrrell, J.** (1990). Anterior pituitary gland. In Basic and Clinical Endocrinology (F. S. Greenspan, ed.), p80. APPLETON & LANGE, Norwalk, CT/San Mateo, CA.

Fingerhut, M. (1990). *Mortality among U.S. Workers Employed in the Production of Chemicals Contaminated with 2,3,7,8-TCDD.* NIOSH, Cincinnati, OH.

Fingerhut, M. A., Halperin, W. E., Marlow, D. A., Piacitelli, L. A., Honchar, P. A., Sweeney, M. H., Greife, A. L., Dill, P. A., Steenland, K., and **Suruda, A. J.** (1991). Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p- dioxin. *N Engl J Med* **324**, 212-8.

Franc, M. A., Pohjanvirta, R., Tuomisto, J., and **Okey, A. B.** (2001a). In vivo up-regulation of aryl hydrocarbon receptor expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in a dioxin-resistant rat model. *Biochem Pharmacol* **62**, 1565-78.

Franc, M. A., Pohjanvirta, R., Tuomisto, J., and **Okey, A. B.** (2001b). Persistent, low-dose 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure: effect on aryl hydrocarbon receptor expression in a dioxin-resistance model. *Toxicol Appl Pharmacol* **175**, 43-53.

Fujii, H., Sato, T., Kaneko, S., Gotoh, O., Fujii-Kuriyama, Y., Osawa, K., Kato, S., and **Hamada, H.** (1997). Metabolic inactivation of retinoic acid by a novel P450 differentially expressed in developing mouse embryos. *Embo J* **16**, 4163-73.

Fujisawa-Sehara, A., Sogawa, K., Yamane, M., and **Fujii-Kuriyama, Y.** (1987). Characterization of xenobiotic responsive elements upstream from the drug-metabolizing cytochrome P-450c gene: a similarity to glucocorticoid regulatory elements. *Nucleic Acids Res* **15**, 4179-91.

Fujisawa-Sehara, A., Yamane, M., and **Fujii-Kuriyama, Y.** (1988). A DNA-binding factor specific for xenobiotic responsive elements of P- 450c gene exists as a cryptic form in cytoplasm: its possible translocation to nucleus. *Proc Natl Acad Sci U S A* **85**, 5859-63.

Gasiewicz, T. A. (1997). Exposure to dioxin and dioxin-like compounds as a potential factor in developmental disabilities. *Mental Retardation & Developmental Disabilities Research Reviews.* **3**, 230-238.

Ghyselinck, N. B., Bavik, C., Sapin, V., Mark, M., Bonnier, D., Hindelang, C., Dierich, A., Nilsson, C. B., Hakansson, H., Sauvant, P., Azais-Braesco, V., Frasson, M., Picaud, S., and **Chambon, P.** (1999). Cellular retinol-binding protein I is essential for vitamin A homeostasis. *Embo J* **18**, 4903-14.

Giannone, J. V., Li, W., Probst, M., and **Okey, A. B.** (1998). Prolonged depletion of AH receptor without alteration of receptor mRNA levels after treatment of cells in culture with 2,3,7,8- tetrachlorodibenzo-p-dioxin. *Biochem Pharmacol* **55**, 489-97.

Giannone, J. V., Okey, A. B., and **Harper, P. A.** (1995). Characterization of polyclonal antibodies to the aromatic hydrocarbon receptor. *Can J Physiol Pharmacol* **73**, 7-17.

Gierthy, J. F., and **Crane, D.** (1984). Reversible inhibition of in vitro epithelial cell proliferation by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Appl Pharmacol* **74**, 91-8.

Gillner, M., Bergman, J., Cambillau, C., Alexandersson, M., Fernstrom, B., and **Gustafsson, J. A.** (1993). Interactions of indolo[3,2-b]carbazoles and related

polycyclic aromatic hydrocarbons with specific binding sites for 2,3,7,8-tetrachlorodibenzo- p-dioxin in rat liver. *Mol Pharmacol* **44**, 336-45.

Gillner, M., Bergman, J., Cambillau, C., Fernstrom, B., and **Gustafsson, J. A.** (1985). Interactions of indoles with specific binding sites for 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver. *Mol Pharmacol* **28**, 357-63.

Gonzalez, F. J., and **Fernandez-Salguero, P.** (1998). The aryl hydrocarbon receptor: studies using the AHR-null mice. *Drug Metab Dispos* **26**, 1194-8.

Gonzalez, F. J., Fernandez-Salguero, P., Lee, S. S., Pineau, T., and **Ward, J. M.** (1995). Xenobiotic receptor knockout mice. *Toxicol Lett* **82-83**, 117-21.

Gordon, C. J., Gray, L. E., Jr., Monteiro-Riviere, N. A., and **Miller, D. B.** (1995). Temperature regulation and metabolism in rats exposed perinatally to dioxin: permanent change in regulated body temperature? *Toxicol Appl Pharmacol* **133**, 172-6.

Gordon, C. J., Yang, Y., and **Gray, L. E., Jr.** (1996). Autonomic and behavioral thermoregulation in golden hamsters exposed perinatally to dioxin. *Toxicol Appl Pharmacol* **137**, 120-5.

Gorski, J. R., Muzi, G., Weber, L. W., Pereira, D. W., Arceo, R. J., Iatropoulos, M. J., and **Rozman, K.** (1988). Some endocrine and morphological aspects of the acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Toxicol Pathol* **16**, 313-20.

Goumain, M., Voisin, T., Lorinet, A. M., and **Laburthe, M.** (1998). Identification and distribution of mRNA encoding the Y1, Y2, Y4, and Y5 receptors for peptides of the PP-fold family in the rat intestine and colon. *Biochem Biophys Res Commun* **247**, 52-6.

Griffond, B., Risold, P. Y., Jacquemard, C., Colard, C., and **Fellmann, D.** (1999). Insulin-induced hypoglycemia increases preprohypocretin (orexin) mRNA in the rat lateral hypothalamic area. *Neurosci Lett* **262**, 77-80.

Gu, Y. Z., Hogenesch, J. B., and **Bradfield, C. A.** (2000). The PAS superfamily: sensors of environmental and developmental signals. *Annu Rev Pharmacol Toxicol* **40**, 519-61.

Gudas, L. J., Sporn, M. B., and **Roberts, A. B.** (1994). Cellular Biology and Biochemistry of the Retinoids. In *THE RETINOIDS: Biology, Chemistry, and Medicine* (M. B. Sporn, A. B. Roberts and D. S. Goodman, eds.), p. 443. Raven Press, Ltd., New York.

Hahn, M. E., Karchner, S. I., Shapiro, M. A., and **Perera, S. A.** (1997). Molecular evolution of two vertebrate aryl hydrocarbon (dioxin) receptors (AHR1 and AHR2) and the PAS family. *Proc Natl Acad Sci U S A* **94**, 13743-8.

Hahn, M. E., Poland, A., Glover, E., and **Stegeman, J. J.** (1994). Photoaffinity labeling of the Ah receptor: phylogenetic survey of diverse vertebrate and invertebrate species. *Arch Biochem Biophys* **310**, 218-28.

Hakansson, H., and **Ahlborg, U. G.** (1985). The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the uptake, distribution and excretion of a single oral dose of [11,12-3H]retinyl acetate and on the vitamin A status in the rat. *J Nutr* **115**, 759-71.

Hakansson, H., Johansson, L., Manzoor, E., and **Ahlborg, U. G.** (1994). Effect of 2,3,7,8-tetrachlorodibenzoy-p-dioxin on the hepatic 7- ethoxyresorufin O-deethylase activity in four rodent species. *Eur J Pharmacol* **270**, 279-84.

Hankinson, O. (1995). The aryl hydrocarbon receptor complex. *Annu Rev Pharmacol Toxicol* **35**, 307-40.

Hanneman, W. H., Legare, M. E., Barhoumi, R., Burghardt, R. C., Safe, S., and Tiffany-Castiglioni, E. (1996). Stimulation of calcium uptake in cultured rat hippocampal neurons by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicology* **112**, 19-28.

Harris, M., Zacharewski, T., Astroff, B., and **Safe, S.** (1989). Partial antagonism of 2,3,7,8-tetrachlorodibenzo-p-dioxin-mediated induction of aryl hydrocarbon hydroxylase by 6-methyl-1,3,8- trichlorodibenzofuran: mechanistic studies. *Mol Pharmacol* **35**, 729-35.

Hassoun, E. A., Wilt, S. C., Devito, M. J., Van Birgelen, A., Alsharif, N. Z., Birnbaum, L. S., and **Stohs, S. J.** (1998). Induction of oxidative stress in brain tissues of mice after subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Sci* **42**, 23-7.

Hayashi, S., Okabe-Kado, J., Honma, Y., and **Kawajiri, K.** (1995). Expression of Ah receptor (TCDD receptor) during human monocytic differentiation. *Carcinogenesis* **16**, 1403-9.

Heimler, I., Rawlins, R. G., Owen, H., and **Hutz, R. J.** (1998). Dioxin perturbs, in a dose- and time-dependent fashion, steroid secretion, and induces apoptosis of human luteinized granulosa cells. *Endocrinology* **139**, 4373-9.

Hirose, K., Morita, M., Ema, M., Mimura, J., Hamada, H., Fujii, H., Saijo, Y., Gotoh, O., Sogawa, K., and **Fujii-Kuriyama, Y.** (1996). cDNA cloning and tissue-specific expression of a novel basic helix-loop- helix/PAS factor (Arnt2) with close sequence similarity to the aryl hydrocarbon receptor nuclear translocator (Arnt). *Mol Cell Biol* **16**, 1706-13.

Hoffman, E. C., Reyes, H., Chu, F. F., Sander, F., Conley, L. H., Brooks, B. A., and **Hankinson, O.** (1991). Cloning of a factor required for activity of the Ah (dioxin) receptor. *Science* **252**, 954-8.

Hokfelt, T., Broberger, C., Xu, Z. Q., Sergeyev, V., Ubink, R., and **Diez, M.** (2000). Neuropeptides--an overview. *Neuropharmacology* **39**, 1337-56.

Huang, P., Ceccatelli, S., Håkansson, H,, Grandison, L., and **Rannug, A.** (2002). Constitutive and TCDD-induced expression of Ah responsive genes in the pituitary. *Neurotoxicology* **23**, 783-793.

Huang, P., Ceccatelli, S., and **Rannug, A.** (2002). A study on diurnal mRNA expression of CYP1A1, AHR, ARNT, and PER2 in rat pituitary and liver. *Environmental Toxicology and Pharmacology* **11**, 119-126.

Huang, P., Rannug, A., Ahlbom, E., Hakansson, H., and **Ceccatelli, S.** (2000). Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the expression of cytochrome P450 1A1, the aryl hydrocarbon receptor, and the aryl hydrocarbon receptor nuclear translocator in rat brain and pituitary. *Toxicol Appl Pharmacol* **169**, 159-67.

Ikeda, H., Suzuki, J., Sasano, N., and **Niizuma, H.** (1988). The development and morphogenesis of the human pituitary gland. *Anat Embryol* **178**, 327-36.

Imataka, H., Sogawa, K., Yasumoto, K., Kikuchi, Y., Sasano, K., Kobayashi, A., Hayami, M., and **Fujii-Kuriyama, Y.** (1992). Two regulatory proteins that bind to the basic transcription element (BTE), a GC box sequence in the promoter region of the rat P-4501A1 gene. *Embo J* **11**, 3663-71.

Innami, S., Nakamura, A., and **Nagayama, S.** (1974). Polychlorobiphenyl toxicity and nutrition. II. PCB toxicity and vitamin A (2). *J Nutr Sci Vitaminol* **20**, 363-70.

Jain, S., Dolwick, K. M., Schmidt, J. V., and Bradfield, C. A. (1994). Potent transactivation domains of the Ah receptor and the Ah receptor nuclear translocator map to their carboxyl termini. *J Biol Chem* **269**, 31518-24.

Jeanrenaud, B., and **Rohner-Jeanrenaud, F.** (2001). Effects of neuropeptides and leptin on nutrient partitioning: dysregulations in obesity. *Annu Rev Med* **52**, 339-51.

Jones, M. K., Weisenburger, W. P., Sipes, I. G., and **Russell, D. H.** (1987). Circadian alterations in prolactin, corticosterone, and thyroid hormone levels and down-regulation of prolactin receptor activity by 2,3,7,8- tetrachlorodibenzo-p-dioxin. *Toxicol Appl Pharmacol* **87**, 337-50.

Jurek, M. A., Powers, R. H., Gilbert, L. G., and **Aust, S. D.** (1990). The effect of TCDD on acyl CoA:retinol acyltransferase activity and vitamin A accumulation in the kidney of male Sprague-Dawley rats. *J Biochem Toxicol* **5**, 155-60.

Kainu, T., Gustafsson, J. A., and **Pelto-Huikko, M.** (1995). The dioxin receptor and its nuclear translocator (Arnt) in the rat brain. *Neuroreport* **6**, 2557-60.

Kapitulnik, J., Gelboin, H. V., Guengerich, F. P., and **Jacobowitz, D. M.** (1987). Immunohistochemical localization of cytochrome P-450 in rat brain. *Neuroscience* **20**, 829-33.

Karchner, S. I., Franks, D. G., Powell, W. H., and **Hahn, M. E.** (2002). Regulatory interactions among three members of the vertebrate aryl hydrocarbon receptor family: AHR repressor, AHR1, and AHR2. *J Biol Chem* **277**, 6949-59.

Kato, M., Kato, K., and **Goodman, D. S.** (1984). Immunocytochemical studies on the localization of plasma and of cellular retinol-binding proteins and of transthyretin (prealbumin) in rat liver and kidney. *J Cell Biol* **98**, 1696-704.

Kayajanian, G. (1997). Dioxin is a promoter blocker, a promoter, and a net anticarcinogen. *Regul Toxicol Pharmacol* **26**, 134-7.

Kazlauskas, A. (2002). *Regulation of dioxin receptor function by the Hsp90 chaperone complex.* (Thesis). In the Department of cell and molecular biology, Medical Nobel Institute, p49. Karolinska Institute, Stockholm, Sweden.

Kazlauskas, A., Poellinger, L., and **Pongratz, I.** (1999). Evidence that the co-chaperone p23 regulates ligand responsiveness of the dioxin (Aryl hydrocarbon) receptor. *J Biol Chem* **274**, 13519-24.

Keesey, R. E., and **Hirvonen, M. D.** (1997). Body weight set-points: determination and adjustment. *J Nutr* **127**, 1875S-1883S.

Kelley, S. K., Nilsson, C. B., Green, M. H., Green, J. B., and **Hakansson, H.** (2000). Mobilization of vitamin A stores in rats after administration of 2,3, 7,8-tetrachlorodibenzo-p-dioxin: a kinetic analysis. *Toxicol Sci* **55**, 478-84.

Kharat, I., and **Saatcioglu, F.** (1996). Antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin are mediated by direct transcriptional interference with the liganded estrogen receptor. Cross-talk between aryl hydrocarbon- and estrogen-mediated signaling. *J Biol Chem* **271**, 10533-7.

Kim, D. W., Gazourian, L., Quadri, S. A., Romieu-Mourez, R., Sherr, D. H., and **Sonenshein, G. E.** (2000). The RelA NF-kappaB subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells. *Oncogene* **19**, 5498-506.

Kimbrough, R. D. (1974). The toxicity of polychlorinated polycyclic compounds and related chemicals. *CRC Crit Rev Toxicol* **2**, 445-98.

Kleman, M. I., Poellinger, L., and **Gustafsson, J. A.** (1994). Regulation of human dioxin receptor function by indolocarbazoles, receptor ligands of dietary origin. *J Biol Chem* **269**, 5137-44.

Knutson, J. C., and Poland, A. (1982). Response of murine epidermis to 2,3,7,8-tetrachlorodibenzo-p-dioxin: interaction of the ah and hr loci. *Cell* **30**, 225-34.

Kociba, R. J., Keyes, D. G., Beyer, J. E., Carreon, R. M., Wade, C. E., Dittenber, D. A., Kalnins, R. P., Frauson, L. E., Park, C. N., Barnard, S. D., Hummel, R. A., and **Humiston, C. G.** (1978). Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. *Toxicol Appl Pharmacol* **46**, 279-303.

Krishnan, V., Porter, W., Santostefano, M., Wang, X., and **Safe, S.** (1995). Molecular mechanism of inhibition of estrogen-induced cathepsin D gene expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in MCF-7 cells. *Mol Cell Biol* **15**, 6710-9.

Kristensen, P., Judge, M. E., Thim, L., Ribel, U., Christjansen, K. N., Wulff, B. S., Clausen, J. T., Jensen, P. B., Madsen, O. D., Vrang, N., Larsen, P. J., and **Hastrup, S.** (1998). Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* **393**, 72-6.

Krude, H., and **Gruters, A.** (2000). Implications of proopiomelanocortin (POMC) mutations in humans: the POMC deficiency syndrome. *Trends Endocrinol Metab* **11**, 15-22.

Kuhar, M. J., Adams, L. D., Hunter, R. G., Vechia, S. D., and **Smith, Y.** (2000). CART peptides. *Regul Pept* **89**, 1-6.

Kuhar, M. J., and **Dall Vechia, S. E.** (1999a). CART peptides: novel addiction- and feeding-related neuropeptides. *Trends Neurosci* **22**, 316-20.

Kuhar, M. J., and **Yoho, L. L.** (1999b). CART peptide analysis by Western blotting. *Synapse* **33**, 163-71.

Lahvis, G. P., and **Bradfield, C. A.** (1998). Ahr null alleles: distinctive or different? *Biochem Pharmacol* **56**, 781-7.

Lahvis, G. P., Lindell, S. L., Thomas, R. S., McCuskey, R. S., Murphy, C., Glover, E., Bentz, M., Southard, J., and **Bradfield, C. A.** (2000). Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. *Proc Natl Acad Sci U S A* **97**, 10442-7.

Lambert, P. D., Couceyro, P. R., Koylu, E. O., Ling, N. C., DeSouza, E. B., and **Kuhar, M. J.** (1997). A role for novel CART peptide fragments in the central control of food intake. *Neuropeptides* **31**, 620-1.

Lambert, P. D., Couceyro, P. R., McGirr, K. M., Dall Vechia, S. E., Smith, Y., and **Kuhar, M. J.** (1998). CART peptides in the central control of feeding and interactions with neuropeptide Y. *Synapse* **29**, 293-8.

Larhammar, D., Ericsson, A., and **Persson, H.** (1987). Structure and expression of the rat neuropeptide Y gene. *Proc Natl Acad Sci U S A* **84**, 2068-72.

Li, E., Demmer, L. A., Sweetser, D. A., Ong, D. E., and **Gordon, J. I.** (1986). Rat cellular retinol-binding protein II: use of a cloned cDNA to define its primary structure, tissue-specific expression, and developmental regulation. *Proc Natl Acad Sci U S A* **83**, 5779-83.

Li, H., Dong, L., and **Whitlock, J. P., Jr.** (1994). Transcriptional activation function of the mouse Ah receptor nuclear translocator. *J Biol Chem* **269**, 28098-105.

Li, X., Johnson, D. C., and **Rozman, K. K.** (1997). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) increases release of luteinizing hormone and follicle-stimulating hormone from the pituitary of immature female rats in vivo and in vitro. *Toxicol Appl Pharmacol* **142**, 264-9.

Lieber, C. S., Lasker, J. M., Alderman, J., and **Leo, M. A.** (1987). The microsomal ethanol oxidizing system and its interaction with other drugs, carcinogens, and vitamins. *Ann N Y Acad Sci* **492**, 11-24.

Lin, L., Faraco, J., Li, R., Kadotani, H., Rogers, W., Lin, X., Qiu, X., de Jong, P. J., Nishino, S., and **Mignot, E.** (1999). The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* **98**, 365-76.

Lindebro, M. C., Poellinger, L., and **Whitelaw, M. L.** (1995). Protein-protein interaction via PAS domains: role of the PAS domain in positive and negative regulation of the bHLH/PAS dioxin receptor-Arnt transcription factor complex. *Embo J* **14**, 3528-39.

Long, W. P., Pray-Grant, M., Tsai, J. C., and **Perdew, G. H.** (1998). Protein kinase C activity is required for aryl hydrocarbon receptor pathway-mediated signal transduction. *Mol Pharmacol* **53**, 691-700.

Lorick, K. L., Toscano, D. L., and **Toscano, W. A., Jr.** (1998). 2,3,7,8-Tetrachlorodibenzo-p-dioxin alters retinoic acid receptor function in human keratinocytes. *Biochem Biophys Res Commun* **243**, 749-52.

Ma, Q. (2001). Induction of CYP1A1. The AhR/DRE paradigm: transcription, receptor regulation, and expanding biological roles. *Curr Drug Metab* **2**, 149-64.

Ma, Q., Renzelli, A. J., Baldwin, K. T., and **Antonini, J. M.** (2000). Superinduction of CYP1A1 gene expression. Regulation of 2,3,7, 8- tetrachlorodibenzo-p-dioxin-induced degradation of Ah receptor by cycloheximide. *J Biol Chem* **275**, 12676-83.

Ma, Q., and **Whitlock, J. P., Jr.** (1997). A novel cytoplasmic protein that interacts with the Ah receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Biol Chem* **272**, 8878-84.

Mably, T. A., Bjerke, D. L., Moore, R. W., Gendron-Fitzpatrick, A., and **Peterson, R. E.** (1992a). In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 3. Effects on spermatogenesis and reproductive capability. *Toxicol Appl Pharmacol* **114**, 118-26.

Mably, T. A., Moore, R. W., Goy, R. W., and **Peterson, R. E.** (1992b). In utero and lactational exposure of male rats to 2,3,7,8- tetrachlorodibenzo-p-dioxin. 2. Effects on sexual behavior and the regulation of luteinizing hormone secretion in adulthood. *Toxicol Appl Pharmacol* **114**, 108-17.

MacDonald, P. N., Bok, D., and **Ong, D. E.** (1990). Localization of cellular retinol-binding protein and retinol-binding protein in cells comprising the blood-brain barrier of rat and human. *Proc Natl Acad Sci U S A* **87**, 4265-9.

MacDonald, P. N., and **Ong, D. E.** (1987). Binding specificities of cellular retinol-binding protein and cellular retinol-binding protein, type II. *J Biol Chem* **262**, 10550-6.

MacLean, G., Abu-Abed, S., Dolle, P., Tahayato, A., Chambon, P., and **Petkovich, M.** (2001). Cloning of a novel retinoic-acid metabolizing cytochrome P450, Cyp26B1, and comparative expression analysis with Cyp26A1 during early murine development. *Mech Dev* **107**, 195-201.

Maden, M., Gale, E., and **Zile, M.** (1998). The role of vitamin A in the development of the central nervous system. *J Nutr* **128**, 471S-475S.

Malik, M. A., Blusztajn, J. K., and **Greenwood, C. E.** (2000). Nutrients as trophic factors in neurons and the central nervous system: Role of retinoic acid. *J. Nutr. Biochem.* **11**, 2-13.

Mangelsdorf, D., Umesono, K., and **Evans, RM.** (1994). *The retinoid receptors. In the Retinoids, Biology, Chemistry and Medicine.* Raven Press, New York.

Meier, C. A. (1998). Orexins and anorexins: thoughts for food. *Eur J Endocrinol* **139**, 148-9.

Meyer, B. K., Pray-Grant, M. G., Vanden Heuvel, J. P., and **Perdew, G. H.** (1998). Hepatitis B virus X-associated protein 2 is a subunit of the unliganded aryl hydrocarbon receptor core complex and exhibits transcriptional enhancer activity. *Mol Cell Biol* **18**, 978-88.

Miller, J. D., Settachan, D., Frame, L. T., and **Dickerson, R. L.** (1999). 2,3,7,8-tetrachlorodibenzo-p-dioxin phase advances the deer mouse (Peromyscus maniculaturs) circadian rhythm by altering expression of clock proteins. *Organohalogen Compounds* **42**, 23-28.

Mimura, J., Ema, M., Sogawa, K., and **Fujii-Kuriyama, Y.** (1999). Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. *Genes Dev* **13**, 20-5.

Mimura, J., and **Fujii-Kuriyama, Y.** (2002). Functional role of AhR in the expression of toxic effects by TCDD. *Biochimica et Biophysica Acta* Ms#25458, 1-6. *(in press)*

Mimura, J., Yamashita, K., Nakamura, K., Morita, M., Takagi, T. N., Nakao, K., Ema, M., Sogawa, K., Yasuda, M., Katsuki, M., and **Fujii-Kuriyama, Y.** (1997). Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. *Genes Cells* **2**, 645-54.

Mobarhan, S., Seitz, H. K., Russell, R. M., Mehta, R., Hupert, J., Friedman, H., Layden, T. J., Meydani, M., and **Langenberg, P.** (1991). Age-related effects of chronic ethanol intake on vitamin A status in Fisher 344 rats. *J Nutr* **121**, 510-7.

Mondal, M. S., Nakazato, M., Date, Y., Murakami, N., Yanagisawa, M., and **Matsukura, S.** (1999). Widespread distribution of orexin in rat brain and its regulation upon fasting. *Biochem Biophys Res Commun* **256**, 495-9.

Moore, R. W., Parsons, J. A., Bookstaff, R. C., and **Peterson, R. E.** (1989). Plasma concentrations of pituitary hormones in 2,3,7,8- tetrachlorodibenzo-p-dioxin-treated male rats. *J Biochem Toxicol* **4**, 165-72.

Morgan, J. E., and **Whitlock, J. P., Jr.** (1992). Transcription-dependent and transcription-independent nucleosome disruption induced by dioxin. *Proc Natl Acad Sci U S A* **89**, 11622-6.

Morse, D. C., Stein, A. P., Thomas, P. E., and **Lowndes, H. E.** (1998). Distribution and induction of cytochrome P450 1A1 and 1A2 in rat brain. *Toxicol Appl Pharmacol* **152**, 232-9.

Nahon, J. L., Presse, F., Bittencourt, J. C., Sawchenko, P. E., and **Vale, W.** (1989). The rat melanin-concentrating hormone messenger ribonucleic acid encodes multiple putative neuropeptides coexpressed in the dorsolateral hypothalamus. *Endocrinology* **125**, 2056-65.

Napoli, J. L. (2000). A gene knockout corroborates the integral function of cellular retinol- binding protein in retinoid metabolism. *Nutr Rev* **58**, 230-6.

Napoli, J. L., Posch, K. C., and **Burns, R. D**. (1992). Microsomal retinal synthesis: retinol vs. holo-CRBP as substrate and evaluation of NADP, NAD and NADPH as cofactors. *Biochim Biophys Acta* **1120**, 183-6.

Nilsson, C. B., and **Hakansson, H.** (2002). The retinoid signaling system--a target in dioxin toxicity. *Crit Rev Toxicol* **32**, 211-32.

Nilsson, C. B., Hanberg, A., Trossvik, C., and **Hakansson, H.** (1996). 2,3,7,8-Tetrachlorodibenzo-p-dioxin affects retinol esterification in rat hepatic stellate cells and kidney. *Environ Toxicol Pharmacol* **2**, 17-23.

Nilsson, C. B., Hoegberg, P., Trossvik, C., Azais-Braesco, V., Blaner, W. S., Fex, G., Harrison, E. H., Nau, H., Schmidt, C. K., van Bennekum, A. M., and **Hakansson, H.** (2000). 2,3,7,8-tetrachlorodibenzo-p-dioxin increases serum and kidney retinoic acid levels and kidney retinol esterification in the rat. *Toxicol Appl Pharmacol* **169**, 121-31.

Noy, N. (2000). Retinoid-binding proteins: mediators of retinoid action. *Biochem J* **348 Pt 3**, 481-95.

Numayama-Tsuruta, K., Kobayashi, A., Sogawa, K., and **Fujii-Kuriyama, Y.** (1997). A point mutation responsible for defective function of the aryl- hydrocarbon-receptor nuclear translocator in mutant Hepa-1c1c7 cells. *Eur J Biochem* **246**, 486-95.

Okino, S. T., and **Whitlock, J. P., Jr.** (1995). Dioxin induces localized, graded changes in chromatin structure: implications for Cyp1A1 gene transcription. *Mol Cell Biol* **15**, 3714-21.

Okino, S. T., and **Whitlock, J. P., Jr.** (2000). The aromatic hydrocarbon receptor, transcription, and endocrine aspects of dioxin action. *Vitam Horm* **59**, 241-64.

Ong, D. E. (1984). A novel retinol-binding protein from rat. Purification and partial characterization. *J Biol Chem* **259**, 1476-82.

Ong, D. E., and **Page, D. L.** (1987). Cellular retinol-binding protein (type two) is abundant in human small intestine. *J Lipid Res* **28**, 739-45.

Palkovits, M., and **Brownstein, M. J.** (1992). *Maps and guide to microdissection of the rat brain.* Elsevier, New York.

Petersen, S. L., Curran, M. A., Marconi, S. A., Carpenter, C. D., Lubbers, L. S., and **McAbee, M. D.** (2000). Distribution of mRNAs encoding the arylhydrocarbon receptor, arylhydrocarbon receptor nuclear translocator, and arylhydrocarbon receptor nuclear translocator-2 in the rat brain and brainstem. *J Comp Neurol* **427**, 428-39.

Peterson, R. E., Theobald, H. M., and **Kimmel, G. L.** (1993). Developmental and reproductive toxicity of dioxins and related compounds: cross-species comparisons. *Crit Rev Toxicol* **23**, 283-335.

Pirkle, J. L., Wolfe, W. H., Patterson, D. G., Needham, L. L., Michalek, J. E., Miner, J. C., Peterson, M. R., and **Phillips, D. L.** (1989). Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Vietnam Veterans of Operation Ranch Hand. *J Toxicol Environ Health* **27**, 165-71.

Platt, J. L., and **Michael, A. F.** (1983). Retardation of fading and enhancement of intensity of immunofluorescence by p-phenylenediamine. *J Histochem Cytochem* **31**, 840-2.

Plewka, A., Czekaj, P., Kaminski, M., and **Plewka, D.** (1992). Circadian changes of cytochrome P-450-dependent monooxygenase system in the rat liver. *Pol J Pharmacol Pharm* **44**, 655-61.

Pohjanvirta, R., Laitinen, J., Vakkuri, O., Linden, J., Kokkola, T., Unkila, M., and **Tuomisto, J.** (1996). Mechanism by which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) reduces circulating melatonin levels in the rat. *Toxicology* **107**, 85-97.

Pohjanvirta, R., and **Tuomisto, J.** (1990). 2,3,7,8-Tetrachlorodibenzo-p-dioxin enhances responsiveness to post- ingestive satiety signals. *Toxicology* **63**, 285-99.

Pohjanvirta, R., and **Tuomisto, J.** (1994). Short-term toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals: effects, mechanisms, and animal models. *Pharmacol Rev* **46**, 483-549.

Pohjanvirta, R., Tuomisto, J., and **Vikkula, K.** (1988). Screening of pharmacological agents given peripherally with respect to TCDD-induced wasting syndrome in Long-Evans rats. *Pharmacol Toxicol* **63**, 240-7.

Pohjanvirta, R., Tuomisto, L., and **Tuomisto, J.** (1989). The central nervous system may be involved in TCDD toxicity. *Toxicology* **58**, 167-74.

Pohjanvirta, R., Unkila, M., Tuomisto, J. T., Vuolteenaho, O., Leppaluoto, J., and **Tuomisto, J.** (1993). Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on plasma and tissue beta-endorphin-like immunoreactivity in the most TCDD-susceptible and the most TCDD-resistant rat strain. *Life Sci* **53**, 1479-87.

Pohjanvirta, R., Vartiainen, T., Uusi-Rauva, A., Monkkonen, J., and **Tuomisto, J.** (1990). Tissue distribution, metabolism, and excretion of 14C-TCDD in a TCDD-susceptible and a TCDD-resistant rat strain. *Pharmacol Toxicol* **66**, 93-100.

Poland, A., Glover, E., and **Kende, A. S.** (1976). Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p- dioxin by hepatic cytosol. Evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase. *J Biol Chem* **251**, 4936-46.

Poland, A., Palen, D., and **Glover, E.** (1982). Tumour promotion by TCDD in skin of HRS/J hairless mice. *Nature* **300**, 271-3.

Pollenz, R. S. (1996). The aryl-hydrocarbon receptor, but not the aryl-hydrocarbon receptor nuclear translocator protein, is rapidly depleted in hepatic and nonhepatic culture cells exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Mol Pharmacol* **49**, 391-8.

Pollenz, R. S. (2002). The mechanism of AH receptor protein down-regulation (degradation) and its impact on AH receptor-mediated gene regulation. *Chem Biol Interact* **141**, 41-61.

Pollenz, R. S., Santostefano, M. J., Klett, E., Richardson, V. M., Necela, B., and **Birnbaum, L. S.** (1998). Female Sprague-Dawley rats exposed to a single oral dose of 2,3,7,8- tetrachlorodibenzo-p-dioxin exhibit sustained depletion of aryl hydrocarbon receptor protein in liver, spleen, thymus, and lung. *Toxicol Sci* **42**, 117-28.

Pollenz, R. S., Sattler, C. A., and **Poland, A.** (1994). The aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator protein show distinct subcellular localizations in Hepa 1c1c7 cells by immunofluorescence microscopy. *Mol Pharmacol* **45**, 428-38.

Potter, C. L., Menahan, L. A., and **Peterson, R. E.** (1986a). Relationship of alterations in energy metabolism to hypophagia in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Fundam Appl Toxicol* **6**, 89-97.

Potter, C. L., Moore, R. W., Inhorn, S. L., Hagen, T. C., and **Peterson, R. E.** (1986b). Thyroid status and thermogenesis in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Appl Pharmacol* **84**, 45-55.

Powell-Coffman, J. A., Bradfield, C. A., and **Wood, W. B.** (1998). Caenorhabditis elegans orthologs of the aryl hydrocarbon receptor and its heterodimerization partner the aryl hydrocarbon receptor nuclear translocator. *Proc Natl Acad Sci U S A* **95**, 2844-9.

Prokipcak, R. D., and **Okey, A. B.** (1991). Downregulation of the Ah receptor in mouse hepatoma cells treated in culture with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Can J Physiol Pharmacol* **69**, 1204-10.

Puga, A., Xia, Y., and **Elferink, C.** (2002). Role of the aryl hydrocarbon receptor in cell cycle regulation. *Chem Biol Interact* **141**, 117-30.

Qu, D., Ludwig, D. S., Gammeltoft, S., Piper, M., Pelleymounter, M. A., Cullen, M. J., Mathes, W. F., Przypek, R., Kanarek, R., and Maratos-Flier, E. (1996). A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* **380**, 243-7.

Ramana, K. V., and **Kohli, K. K.** (1998). Gene regulation of cytochrome P450--an overview. *Indian J Exp Biol* **36**, 437-46.

Rannug, A., Rannug, U., Rosenkranz, H. S., Winqvist, L., Westerholm, R., Agurell, E., and **Grafstrom, A. K.** (1987). Certain photooxidized derivatives of tryptophan bind with very high affinity to the Ah receptor and are likely to be endogenous signal substances. *J Biol Chem* **262**, 15422-7.

Rannug, U., Rannug, A., Sjoberg, U., Li, H., Westerholm, R., and **Bergman, J.** (1995). Structure elucidation of two tryptophan-derived, high affinity Ah receptor ligands. *Chem Biol* **2**, 841-5.

Rannug, U., Sjogren, M., Rannug, A., Gillner, M., Toftgard, R., Gustafsson, J. A., Rosenkranz, H., and **Klopman, G.** (1991). Use of artificial intelligence in structure-affinity correlations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) receptor ligands. *Carcinogenesis* **12**, 2007-15.

Reick, M., Robertson, R. W., Pasco, D. S., and **Fagan, J. B.** (1994). Down-regulation of nuclear aryl hydrocarbon receptor DNA-binding and transactivation functions: requirement for a labile or inducible factor. *Mol Cell Biol* **14**, 5653-60.

Reisz-Porszasz, S., Probst, M. R., Fukunaga, B. N., and **Hankinson, O.** (1994). Identification of functional domains of the aryl hydrocarbon receptor nuclear translocator protein (ARNT). *Mol Cell Biol* **14**, 6075-86.

Reyes, H., Reisz-Porszasz, S., and **Hankinson, O.** (1992). Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science* **256**, 1193-5.

Ricci, M. S., Toscano, D. G., Mattingly, C. J., and **Toscano, W. A., Jr.** (1999). Estrogen receptor reduces CYP1A1 induction in cultured human endometrial cells. *J Biol Chem* **274**, 3430-8.

Richardson, V. M., Santostefano, M. J., and **Birnbaum, L. S.** (1998). Daily cycle of bHLH-PAS proteins, Ah receptor and Arnt, in multiple tissues of female Sprague-Dawley rats. *Biochem Biophys Res Commun* **252**, 225-31.

Robertson, J. D., and **Orrenius, S.** (2000). Molecular mechanisms of apoptosis induced by cytotoxic chemicals. *Crit Rev Toxicol* **30**, 609-27.

Rolland, R. M. (2000). A review of chemically-induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. *J Wildl Dis* **36**, 615-35.

Rossi, M., Choi, S. J., O'Shea, D., Miyoshi, T., Ghatei, M. A., and **Bloom, S. R.** (1997). Melanin-concentrating hormone acutely stimulates feeding, but chronic administration has no effect on body weight. *Endocrinology* **138**, 351-5.

Rouet, P., Alexandrov, K., Markovits, P., Frayssinet, C., and **Dansette, P. M.** (1981). Metabolism of benzo[a]pyrene by brain microsomes of fetal and adult rats and mice. Induction by 5,6 benzoflavone, comparison with liver and lung microsomal activities. *Carcinogenesis* **2**, 919-26.

Rowlands, J. C., and **Gustafsson, J. A.** (1997). Aryl hydrocarbon receptor-mediated signal transduction. *Crit Rev Toxicol* **27**, 109-34.

Rozman, K., Pfeifer, B., Kerecsen, L., and **Alper, R. H.** (1991). Is a serotonergic mechanism involved in 2,3,7,8-tetrachlorodibenzo-p- dioxin (TCDD)-induced appetite suppression in the Sprague-Dawley rat? *Arch Toxicol* **65**, 124-8.

Ruangwises, S., Bestervelt, L. L., Piper, D. W., Nolan, C. J., and **Piper, W. N.** (1991). Human chorionic gonadotropin treatment prevents depressed 17 alpha-hydroxylase/C17-20 lyase activities and serum testosterone concentrations in 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated rats. *Biol Reprod* **45**, 143-50.

Ruberte, E., Friederich, V., Chambon, P., and **Morriss-Kay, G.** (1993). Retinoic acid receptors and cellular retinoid binding proteins. III. Their differential transcript distribution during mouse nervous system development. *Development* **118**, 267-82.

Ruberte, E., Friederich, V., Morriss-Kay, G., and **Chambon, P.** (1992). Differential distribution patterns of CRABP I and CRABP II transcripts during mouse embryogenesis. *Development* **115**, 973-87.

Russell, D. H., Buckley, A. R., Shah, G. N., Sipes, I. G., Blask, D. E., and **Benson, B.** (1988). Hypothalamic site of action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Toxicol Appl Pharmacol* **94**, 496-502.

Safe, S. (2001). Molecular biology of the Ah receptor and its role in carcinogenesis. *Toxicol Lett* **120**, 1-7.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., Williams, S. C., Richardson, J. A., Kozlowski, G. P., Wilson, S., Arch, J. R., Buckingham, R. E., Haynes, A. C., Carr, S. A., Annan, R. S., McNulty, D. E., Liu, W. S., Terrett, J. A., Elshourbagy, N. A., Bergsma, D. J., and **Yanagisawa, M.** (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* **92**, 573-85.

Schalling, M., Seroogy, K., Hokfelt, T., Chai, S. Y., Hallman, H., Persson, H., Larhammar, D., Ericsson, A., Terenius, L., and **Graffi, J.** (1988). Neuropeptide tyrosine in the rat adrenal gland--immunohistochemical and in situ hybridization studies. *Neuroscience* **24**, 337-49.

Schantz, S. L., Seo, B. W., Moshtaghian, J., Peterson, R. E., and **Moore, R. W.** (1996). Effects of gestational and lactational exposure to TCDD or coplanar PCBs on spatial learning. *Neurotoxicol Teratol* **18**, 305-13.

Schilter, B., and **Omiecinski, C. J.** (1993). Regional distribution and expression modulation of cytochrome P-450 and epoxide hydrolase mRNAs in the rat brain. *Mol Pharmacol* **44**, 990-6.

Schmidt, J. V., and **Bradfield, C. A.** (1996). Ah receptor signaling pathways. *Annu Rev Cell Dev Biol* **12**, 55-89.

Schmidt, J. V., Su, G. H., Reddy, J. K., Simon, M. C., and **Bradfield, C. A.** (1996). Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. *Proc Natl Acad Sci U S A* **93**, 6731-6.

Schwartz, M. W., Woods, S. C., Porte, D., Jr., Seeley, R. J., and **Baskin, D. G.** (2000). Central nervous system control of food intake. *Nature* **404**, 661-71.

Schwarz, M., Buchmann, A., Stinchcombe, S., Kalkuhl, A., and **Bock, K.** (2000). Ah receptor ligands and tumor promotion: survival of neoplastic cells. *Toxicol Lett* **112-113**, 69-77.

Seefeld, M. D., Corbett, S. W., Keesey, R. E., and **Peterson, R. E.** (1984). Characterization of the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Appl Pharmacol* **73**, 311-22.

Shimba, S., Todoroki, K., Aoyagi, T., and **Tezuka, M.** (1998). Depletion of arylhydrocarbon receptor during adipose differentiation in 3T3-L1 cells. *Biochem Biophys Res Commun* **249**, 131-7.

Sloop, T. C., and **Lucier, G. W.** (1987). Dose-dependent elevation of Ah receptor binding by TCDD in rat liver. *Toxicol Appl Pharmacol* **88**, 329-37.

Smith, G., Stubbins, M. J., Harries, L. W., and **Wolf, C. R.** (1998). Molecular genetics of the human cytochrome P450 monooxygenase superfamily. *Xenobiotica* **28**, 1129-65.

Sogawa, K., and **Fujii-Kuriyama, Y.** (1997). Ah receptor, a novel ligand-activated transcription factor. *J Biochem (Tokyo)* **122**, 1075-9.

Spink, D. C., Eugster, H. P., Lincoln, D. W., 2nd, Schuetz, J. D., Schuetz, E. G., Johnson, J. A., Kaminsky, L. S., and **Gierthy, J. F.** (1992). 17 beta-estradiol hydroxylation catalyzed by human cytochrome P450 1A1: a comparison of the activities induced by 2,3,7,8-tetrachlorodibenzo-p- dioxin in MCF-7 cells with those from heterologous expression of the cDNA. *Arch Biochem Biophys* **293**, 342-8.

Spink, D. C., Hayes, C. L., Young, N. R., Christou, M., Sutter, T. R., Jefcoate, C. R., and **Gierthy, J. F.** (1994). The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on estrogen metabolism in MCF-7 breast cancer cells: evidence for induction of a novel 17 beta-estradiol 4-hydroxylase. *J Steroid Biochem Mol Biol* **51**, 251-8.

Spink, D. C., Lincoln, D. W., 2nd, Dickerman, H. W., and **Gierthy, J. F.** (1990). 2,3,7,8-Tetrachlorodibenzo-p-dioxin causes an extensive alteration of 17 beta-estradiol metabolism in MCF-7 breast tumor cells. *Proc Natl Acad Sci U S A* **87**, 6917-21.

Stanley, B. G., and **Leibowitz, S. F.** (1985). Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. *Proc Natl Acad Sci U S A* **82**, 3940-3.

Summerbell, D., and **Maden, M.** (1990). Retinoic acid, a developmental signalling molecule. *Trends Neurosci* **13**, 142-7.

Swanson, H. I., Chan, W. K., and **Bradfield, C. A.** (1995). DNA binding specificities and pairing rules of the Ah receptor, ARNT, and SIM proteins. *J Biol Chem* **270**, 26292-302.

Takahashi, J., Palmer, T. D., and **Gage, F. H.** (1999). Retinoic acid and neurotrophins collaborate to regulate neurogenesis in adult-derived neural stem cell cultures. *J Neurobiol* **38**, 65-81.

Taylor, B. L., and **Zhulin, I. B.** (1999). PAS domains: internal sensors of oxygen, redox potential, and light. *Microbiol Mol Biol Rev* **63**, 479-506.

Theobald, H. M., and **Peterson, R. E.** (1997). In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-rho- dioxin: effects on development of the male and female reproductive system of the mouse. *Toxicol Appl Pharmacol* **145**, 124-35.

Thiel, R., Koch, E., Ulbrich, B., and **Chahoud, I.** (1994). Peri- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin: effects on physiological development,

reflexes, locomotor activity and learning behaviour in Wistar rats. *Arch Toxicol* **69**, 79-86.

Thunberg, T., Ahlborg, U. G., and **Johnsson, H.** (1979). Vitamin A (retinol) status in the rat after a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Arch Toxicol* **42**, 265-74.

Tian, Y., Ke, S., Denison, M. S., Rabson, A. B., and **Gallo, M. A.** (1999). Ah receptor and NF-kappaB interactions, a potential mechanism for dioxin toxicity. *J Biol Chem* **274**, 510-5.

Tian, Y., Ke, S., Thomas, T., Meeker, R. J., and **Gallo, M. A.** (1998). Transcriptional suppression of estrogen receptor gene expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *J Steroid Biochem Mol Biol* **67**, 17-24.

Tian, Y., Rabson, A. B., and **Gallo, M. A.** (2002). Ah receptor and NF-kappaB interactions: mechanisms and physiological implications. *Chem Biol Interact* **141**, 97-115.

Tomita, S., Okuyama, E., Ohnishi, T., and **Ichikawa, Y.** (1996). Characteristic properties of a retinoic acid synthetic cytochrome P-450 purified from liver microsomes of 3-methylcholanthrene-induced rats. *Biochim Biophys Acta* **1290**, 273-81.

Tredger, J. M., and **Chhabra, R. S.** (1977). Circadian variations in microsomal drug-metabolizing enzyme activities in rat and rabbit tissues. *Xenobiotica* **7**, 481-9.

Tuomisto, J., Pohjanvirta, R., MacDonald, E., and **Tuomisto, L.** (1990). Changes in rat brain monoamines, monoamine metabolites and histamine after a single administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Pharmacol Toxicol* **67**, 260-5.

Tuomisto, J. T., Pohjanvirta, R., Unkila, M., Räisänen, L., and **Tuomisto, J.** (1993). Effects of TCDD on motor activity of TCDD-susceptible and TCDD-resistant rat strains. *In* Abstracts of the 13th International Symposium on Dioxins and Related Compounds. *Organohalogen Compounds* **13**, 313-315.

Tuomisto, J. T., Pohjanvirta, R., Unkila, M., and **Tuomisto, J.** (1995). 2,3,7,8-Tetrachlorodibenzo-p-dioxin-induced anorexia and wasting syndrome in rats: aggravation after ventromedial hypothalamic lesion. *Eur J Pharmacol* **293**, 309-17.

Tuomisto, J. T., Pohjanvirta, R., Unkila, M., and **Tuomisto, J.** (1999). TCDD-induced anorexia and wasting syndrome in rats: effects of diet- induced obesity and nutrition. *Pharmacol Biochem Behav* **62**, 735-42.

Tuomisto, J. T., Viluksela, M., Pohjanvirta, R., and **Tuomisto, J.** (2000). Changes in food intake and food selection in rats after 2,3,7, 8- tetrachlorodibenzo-p-dioxin (TCDD) exposure. *Pharmacol Biochem Behav* **65**, 381-7.

Unkila, M., Pohjanvirta, R., Honkakoski, P., Torronen, R., and **Tuomisto, J.** (1993a). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induced ethoxyresorufin-O-deethylase (EROD) and aldehyde dehydrogenase (ALDH3) activities in the brain and liver. A comparison between the most TCDD-susceptible and the most TCDD-resistant rat strain. *Biochem Pharmacol* **46**, 651-9.

Unkila, M., Pohjanvirta, R., MacDonald, E., and **Tuomisto, J.** (1994). Characterization of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced brain serotonin metabolism in the rat. *Eur J Pharmacol* **270**, 157-66.

Unkila, M., Pohjanvirta, R., and **Tuomisto, J.** (1995). Biochemical effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds on the central nervous system. *Int J Biochem Cell Biol* **27**, 443-55.

Unkila, M., Pohjanvirta, R., and **Tuomisto, J.** (1999). Dioxin-induced perturbations in tryptophan homeostasis in laboratory animals. *Adv Exp Med Biol* **467**, 433-42.

Unkila, M., Tuomisto, J. T., Pohjanvirta, R., MacDonald, E., Tuomisto, L., Koulu, M., and **Tuomisto, J.** (1993b). Effect of a single lethal dose of TCDD on the levels of monoamines, their metabolites and tryptophan in discrete brain nuclei and peripheral tissues of Long-Evans rats. *Pharmacol Toxicol* **72**, 279-85.

Van den Berg, M., De Jongh, J., Poiger, H., and **Olson, J. R.** (1994). The toxicokinetics and metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *Crit Rev Toxicol* **24**, 1-74.

Vos, J. G., and Moore, J. A. (1974). Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Int Arch Allergy Appl Immunol* **47**, 777-94.

Wang, X., Sperkova, Z., and **Napoli, J. L.** (2001). Analysis of mouse retinal dehydrogenase type 2 promoter and expression. *Genomics* **74**, 245-50.

Wanner, R., Brommer, S., Czarnetzki, B. M., and **Rosenbach, T.** (1995). The differentiation-related upregulation of aryl hydrocarbon receptor transcript levels is suppressed by retinoic acid. *Biochem Biophys Res Commun* **209**, 706-11.

Wardlaw, S. A., Bucco, R. A., Zheng, W. L., and **Ong, D. E.** (1997). Variable expression of cellular retinol- and cellular retinoic acid- binding proteins in the rat uterus and ovary during the estrous cycle. *Biol Reprod* **56**, 125-32.

Warner, M., Kohler, C., Hansson, T., and **Gustafsson, J. A.** (1988). Regional distribution of cytochrome P-450 in the rat brain: spectral quantitation and contribution of P-450b,e, and P-450c,d. *J Neurochem* **50**, 1057-65.

Wei, Y. D., Bergander, L., Rannug, U., and **Rannug, A.** (2000). Regulation of CYP1A1 transcription via the metabolism of the tryptophan- derived 6-formylindolo[3,2-b]carbazole. *Arch Biochem Biophys* **383**, 99-107.

Wei, Y. D., Helleberg, H., Rannug, U., and **Rannug, A.** (1998). Rapid and transient induction of CYP1A1 gene expression in human cells by the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole. *Chem Biol Interact* **110**, 39-55.

Wei, Y. D., Rannug, U., and **Rannug, A.** (1999). UV-induced CYP1A1 gene expression in human cells is mediated by tryptophan. *Chem Biol Interact* **118**, 127-40.

Weston, W. M., Nugent, P., and **Greene, R. M.** (1995). Inhibition of retinoic-acid-induced gene expression by 2,3,7,8- tetrachlorodibenzo-p-dioxin. *Biochem Biophys Res Commun* **207**, 690-4.

White, J. A., Guo, Y. D., Baetz, K., Beckett-Jones, B., Bonasoro, J., Hsu, K. E., Dilworth, F. J., Jones, G., and **Petkovich, M.** (1996). Identification of the retinoic acid-inducible all-trans-retinoic acid 4- hydroxylase. *J Biol Chem* **271**, 29922-7.

Whitelaw, M. L., Gustafsson, J. A., and **Poellinger, L.** (1994). Identification of transactivation and repression functions of the dioxin receptor and its basic helix-loop-helix/PAS partner factor Arnt: inducible versus constitutive modes of regulation. *Mol Cell Biol* **14**, 8343-55.

Whitlock, J. P., Jr. (1993). Mechanistic aspects of dioxin action. *Chem Res Toxicol* **6**, 754-63.

Whitlock, J. P., Jr. (1999). Induction of cytochrome P4501A1. *Annu Rev Pharmacol Toxicol* **39**, 103-25.

Wilson, J. D., Foster, D. W., Kronenberg, H. M., and **Larsen, P. R.** (1998). *Williams Textbook of Endocrinology*. W.B. Saunders Company press, USA, Philadelphia.

Wolf, G. (1998). Vitamin A functions in the regulation of the dopaminergic system in the brain and pituitary gland. *Nutr Rev* **56**, 354-5.

Wu, L., and **Whitlock, J. P., Jr.** (1993). Mechanism of dioxin action: receptor-enhancer interactions in intact cells. *Nucleic Acids Res* **21**, 119-25.

Wuarin, L., Sidell, N., and **de Vellis, J.** (1990). Retinoids increase perinatal spinal cord neuronal survival and astroglial differentiation. *Int J Dev Neurosci* **8**, 317-26.

Yeo, G. S., Farooqi, I. S., Challis, B. G., Jackson, R. S., and **O'Rahilly, S.** (2000). The role of melanocortin signalling in the control of body weight: evidence from human and murine genetic models. *Qjm* **93**, 7-14.

Zacharewski, T., Harris, M., and **Safe, S.** (1991). Evidence for the mechanism of action of the 2,3,7,8-tetrachlorodibenzo- p-dioxin-mediated decrease of nuclear estrogen receptor levels in wild- type and mutant mouse Hepa 1c1c7 cells. *Biochem Pharmacol* **41**, 1931-9.

Zamboni, I., and **De Martino, C.** (1967). Buffered picric acid formaldehyde: a new rapid fixative for electron microscopy. *J Cell Biol* **35**, 148A.

Zetterstrom, R. H., Lindqvist, E., Mata de Urquiza, A., Tomac, A., Eriksson, U., Perlmann, T., and **Olson, L.** (1999). Role of retinoids in the CNS: differential expression of retinoid binding proteins and receptors and evidence for presence of retinoic acid. *Eur J Neurosci* **11**, 407-16.

Zetterstrom, R. H., Simon, A., Giacobini, M. M., Eriksson, U., and **Olson, L.** (1994). Localization of cellular retinoid-binding proteins suggests specific roles for retinoids in the adult central nervous system. *Neuroscience* **62**, 899-918.

Zhang, Q. Y., Dunbar, D., and **Kaminsky, L.** (2000). Human cytochrome P-450 metabolism of retinals to retinoic acids. *Drug Metab Dispos* **28**, 292-7.

Zile, M. H. (1992). Vitamin A homeostasis endangered by environmental pollutants. *Proc Soc Exp Biol Med* **201**, 141-53.