

Forskningsprogram				
SNAP <input checked="" type="checkbox"/>		REPROSAFE <input type="checkbox"/>		
FLIPP		Inriktning: Ekonomiska styrmedel <input type="checkbox"/>		
		Inriktning: Informationssystem och indikatorer IPP <input type="checkbox"/>		
Projekttitel (svensk): Aromatiska kolväten i luft och aktivering av stress				
Projekttitel (engelsk): Activation of stress response by aromatic hydrocarbons in polluted air				
Huvudsökande	Efternamn: Rannug		Förnamn: Agneta	
	Födelseår: 1949		Kvinna <input checked="" type="checkbox"/> Man <input type="checkbox"/>	
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Medsökande	Efternamn, förnamn, tjänst, organisation, institution:			
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Sammanfattning:				
<p>Bedömning av hälsorisker förknippade med utomhusluften är i mycket hög grad beroende av en detaljerad kännedom om mekanismerna bakom de hälsoeffekter, som tillskrivs de olika komponenterna i förorenad luft. Projektet innebär en ny aspekt på främst kardiovaskulära effekter av föroreningar, som bildas vid förbränning av fossila bränslen och som finns i bilavgaser. Projektet kan snabbt leda till kunskap, som kan få långtgående konsekvenser på riskbedömningen och regleringen av utsläpp till luft. Flera pågående projekt inom SNAP kan vinna på en ökad satsning på att utvärdera de aktuella verkningsmekanismerna, vilka till stor del ännu är okända. Särskilt gäller det de projekt vars målsättning är en identifiering av komponenter i luftföroreningar, som medför de största riskerna, eller som gäller identifiering av särskilt känsliga individer. Nedan beskrivna projekt skulle enkelt kunna integreras med pågående studier inom SNAP genom införandet av upprepade mätningar av kortisolnivåer i saliv i samband med humana exponeringstudier.</p> <p>Påverkas hjärtats autonoma reglering genom exponering för kemiska ämnen, som utlöser centrala stressreaktioner, så är detta en möjlig bidragande orsak till hjärtinfarkt. Projektet går ut på att testa en mekanism, som innebär att kemiska ämnen genom att binda till den s.k. Ah-receptorn aktiverar/förstärker en stressreaktion genom att öka uttrycket av corticotropin-utsöndrande hormon (CRH). Denna förklaring har inte framförts tidigare och bygger på nya fynd från studier av hur ämnet dioxin, som binder starkt till Ah-receptorn, aktiverar hormoner i hjärnan och hypofysen. Den föreslagna mekanismen är möjlig att testa experimentellt med hjälp av tillgänglig molekylärbiologisk metodik. cAMP signalering har en central roll i regleringen av ett stort antal endokrina hormoner i hjärnan och hypofysen. Vi kommer därför att undersöka den effekt som aktivering av Ah-receptorn har på uttrycket av CRH, ensamt och i samverkan med cAMP-beroende stimulering, i hypofysceller som odlas in vitro. En transfektionsassay kommer att tillämpas i vilken reporter-gen-konstruktioner, som kopplats till den 5' regulatoriska regionen i CRH används för att demonstrera CRH mRNA expression efter behandling med polycykliska aromatiska substanser. Försöken baseras på tillgänglig teknik och kan därför hinna generera resultat inom en tvåårsperiod.</p> <p>Resultaten kommer att presenteras vid svenska och internationella möten och konferenser och publiceras i internationella tidskrifter.</p>				
		År 2004	År 2005	
Summa sökta medel per år i kr:		722 800	738 400	

Miljöforskningsnämnden
Ansökan om projektbidrag inom Naturvårdsverkets forskningsprogram

Sökta projektmedel fördelade på kostnadsslag	År 2004 (kr)	År 2005 (kr)
Personalkostnad inkl. soc. avgifter *		
Maria Backlund, post doc (22.500:- x 12 mån x lönerrevision 3% x LBK 53%)	426 000	438 000
Övriga omkostn exkl moms (förbrukningsmtrl, analyser, resor etc)**		
Kemikalier och engångsartiklar	40 000	40 000
Enzymer, fluorescenta prober och kit	70 000	70 000
Datorkostnader	10 000	10 000
Övriga omkostnader (sekreterarhjälp, kopiering, post, tele mm)	10 000	10 000
Delsumma av ovanstående poster:	556 000	568 000
Förvaltningspåslag: ..30.... %	166 800	170 400
Totalsumma per år: (införs sid. 1):	722 800	738 400

*) Specificera namn, tjänst **) Specificera

Samtliga övriga miljörelaterade projekt för vilka de sökande har beviljats anslag eller söker anslag för 2004-2006. OBS Även EU-finansiering.

Projekttitel	Finansiär	Tidsperiod	Sökt kr	Beviljat kr
Tryptophan photoproducts as sensitive indicators of UV-induced oxidative stress	SSI	2003-2004		400 000
A new neuroendocrine signalling pathway	VR	2004-2006	1 775 000	
Dioxin och ljus. Beror dioxinets toxicitet på en felaktig aktivering av melanocortinsystemet	FORMAS	2004-2007	2 393 000	
Genetiska studier av en evolutionärt konserverad ligand/substratbindande domän i Ah-receptorn	ErikPhilip-Sörensen Stiftelse	2004	250 000	

Miljörelaterade projekt för vilka sökande har beviljats anslag för 2000-2003 OBS Även EU-finansiering

Projekttitel	Finansiär	Tidsperiod	Beviljat Kr
Improved risk assessment for acrylamide	RALF	2000-2001	550 000
Studier av individuella skillnader i känslighet för isocyanater	FAS	2002-2003	1 161 000
Genetiska riskfaktorer för lungcancer	Sw Match	2000-2002	600 000

Datum och sökandes underskrift, vilken samtidigt ger Naturvårdsverket tillåtelse att publicera sökandes namn på sin webbplats:	Datum och underskrift av prefekt eller motsvarande med namnförtydligande:
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Ansökan skall bestå av detta formulär jämte högst sex sidor lång projektbeskrivning på **engelska** (strukturerad som den svenska sammanfattningen samt en redovisning av kunskapsläget). Referenser till egna publikationer ges med sifferhänvisning till CV. Andra referenser ges i löpande text. Sökandes och eventuell medsökandes CV får omfatta högst två sidor. Inga bilagor kommer att beaktas vid bedömningen. Ansökan (max 10 A4-sidor, 12 punkters teckenstorlek) skall inlämnas i **original + 15 kopior samt elektroniskt** till ansok@naturvardsverket.se. Häfta ihop ansökan och använd hålat papper. Ansökan skall ha inkommit senast den 15 oktober 2003 till Naturvårdsverket, Forskningssekretariatet, 106 48 STOCKHOLM.

ACTIVATION OF STRESS RESPONSE BY AROMATIC HYDROCARBONS IN POLLUTED AIR

Proposed mechanism: Polycyclic aromatic compounds activate expression of the corticotropin releasing hormone (CRH) gene and mediate an autonomic cardiovascular response to stress.

ENVIRONMENTAL ROLE AND SIGNIFICANCE FOR THE SNAP PROGRAM

Experimental data documenting effects of xenobiotic aromatic compounds on stress-mediated expression of CRH, the central mediator of the hypothalamic pituitary adrenal (HPA) axis-regulated stress response, would represent a credible mechanistic explanation of the cardiovascular effects observed from air pollutants.

In recent years, new scientific evidence has emerged that strengthens the link between ambient exposure to fine particles (commonly measured as PM_{2.5}) and health effects (especially cardiovascular effects). Changes in the autonomic nervous system controlling heart rate variability (HRV) have been documented in several studies (Liao et al., *Env Health Perspect*, 159,187,256; Creason et al., *J Expo Anal Env Epid* 11,2001,116; Pope, et al., *Am Heart J*, 138,199,890; Devlin et al., *Eur Respir J*, 21,2003,76s; Holguin et al., *Epidemiology*, 14,2003,521). Relationship between motor vehicle emissions, in particular diesel exhaust, and lung cancer as well as inflammatory effects in the respiratory tract have been documented. Soot-attached polycyclic aromatic hydrocarbons have been found to contribute to toxicity in epidemiological and controlled exposure studies (WHO Working Group, 2003). Also chlorinated biphenyls, dioxins and dibenzofuranes, which are found on particles are susceptible to long-range transboundary air transport and have been considered by a WHO expert group to contribute significantly to exposure and health risks (Joint WHO/Convention Task Force, 2003).

A suggestion, based on the project described below, would be to introduce repeated measurements of cortisol levels (in saliva), to reflect activation of the HPA axis, in ongoing human studies within the SNAP program. Saliva tests can be added in studies on experimentally exposed subjects as well as in studies on environmental exposures to wood-smoke, diesel exhaust and ozone.

AIM AND KEY QUESTIONS

The project aims at an increased understanding of the effects of air pollution on the cardiovascular function. A mechanistic study is suggested, which is based on the recent research developments on the fundamental physiological role of the aryl hydrocarbon receptor (AHR). The AHR is of biological significance, not only in the induction of xenobiotica metabolizing enzymes as was earlier believed, but also as an ubiquitous transcription factor affecting numerous neuroendocrine functions including the hormones in the hypothalamic pituitary adrenal axis. The proposed study is also based on knowledge about the effects of CRH-related peptides and receptors and their roles as regulators of cardiovascular responses to stress.

- ***Can activation of the aryl hydrocarbon receptor cause corticotropin mediated stress response?*** Studies that we have performed at Karolinska Institutet and Stockholm University over a period of years have pointed to an earlier not recognized role of the AHR in activation of the HPA axis. We have found increased expression in the hypothalamus and the pituitary of several neuroendocrine hormones in response to treatments of rodents and rodent cells with planar lipophilic chemical compounds such as β -naphthoflavone and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Huang P, doctoral thesis, KI 2003).
- ***Can activation of corticotropin hormones explain stress-related cardiovascular effects?*** The corticotropin-releasing hormone, the CRH, is central for the body's ability to respond to stress. Activation of the HPA axis by physical stress, pain, cold, UV-light, emotional stress or proinflammatory cytokines proceeds through the hypothalamic production and release of CRH to integrate endocrine and autonomic responses. The CRH acts within the central nervous system to control immune, reproductive, gastrointestinal, and cardiovascular functions as well as behavior and mood and anxiety.

Based on the scientific data that is reviewed in the following section, that links the AHR with cardiovascular stress, a research project has been designed to study the activation of the human CRH gene and the downstream activation of melanocortin hormones in response to substances that bind to the AHR. This application describes an experimental approach that use mouse pituitary tumor-derived cells (AtT-20) transfected with a human CRH 5' promoter-luciferase fusion gene to examine CRH gene expression after treatment with polycyclic aromatic compounds.

STATE OF THE ART

The AHR. Poland and colleagues identified (Poland et al., JBC, 262,1976,4936) the AHR as a cytosolic receptor, which mediates the induction of aryl hydrocarbon hydroxylase. Since that time, thousands of reports in the scientific literature have described the mechanisms of AHR-regulated induction of xenobiotica metabolizing enzymes. The AHR has been found to be a ligand-dependent transcription factor that regulates the expression of a battery of genes in a wide range of species and tissues (reviewed by Mimura and Fujii-Kuriyama, Biochim Biophys Acta, 1619,2003,263; Denison and Nagy, Ann Rev Pharmacol Toxicol, 43,2003,309). It is now well established that the AHR belongs to the PAS (PerArntSim) super family of proteins. PAS proteins act as sensors of environmental and developmental signals (Taylor and Zhulin, Microbiol Mol Biol Rev, 63,1999,479). Signal transduction pathways via PAS proteins allow organisms to adapt to changes in atmospheric and cellular oxygen [the hypoxia inducible factor (HIF) system] and to entrain an animal's activity to changes in the light cycle (the circadian response pathway). PAS domains are found in a number of light, oxygen and red ox sensors of prokaryotes and plants.

In the cytosol, the AHR exists as a multiprotein complex. Following ligand binding the AHR is presumed to undergo a conformation change that exposes a nuclear localization sequence(s), resulting in translocation of the complex into the nucleus. The ligand-bound AHR dimerizes with the related PAS protein, Arnt, which converts the AHR into its high affinity DNA-binding form. Binding occurs at specific binding sites (xenobiotica responsive elements, XREs) with the core sequence GCGTG. Several important regulatory mechanisms have been identified. They involve efficient metabolic degradation of the ligands through the induced metabolizing enzymes, synthesis of a repressor protein, as well as ubiquitination and degradation of the receptor. In the recent literature more than one hundred genes have been

described to be activated by AHR ligand/s in a cell- and time dependent way. In addition to biotransformation genes and genes coding for AHR, its repressor, several early immediate genes, genes involved in cell division, DNA synthesis, apoptosis, differentiation, morphogenesis, T-cell maturation, oogenesis, spermatogenesis, endocrine signaling, and other essential functions are transcriptionally up- or down-regulated by AHR ligands.

In humans, high inter- and intraindividual (seasonal) variability in the activation of AHR-regulated metabolizing enzymes was documented already in the seventies (Kellerman et al., Cancer Res, 33,1973,1654; Paigen et al., Cancer Res, 41,1981,2757).

A proposed physiological role of the AHR. We have in several publications suggested that the AHR is a sensor for the illuminated environment through the binding of oxidized tryptophan derivatives and that the AHR has a function in adaptation to light. This hypothesis is based on our original findings of i) very high AHR binding affinity of certain tryptophan photoproducts (1) ii) tryptophan dependent induction of AHR-regulated gene expression by UV irradiation of cells in vitro (8) iii) diurnal and seasonal rhythms in AHR-regulated gene expression (12,15) and iv) activation of POMC gene expression via AHR (13).

Thus, tryptophan photoproducts may act as chemical messengers of light carried by the blood from the site of formation to a specific receptor protein - the AHR - to signal changes in the environment and to trigger cell-specific responses. Like many lipid-soluble hormones these ligands are able to cross the plasma membrane and interact with a cytosolic receptor protein. The resulting hormone-receptor complex binds to transcription-control regions in DNA and affects expression of specific genes. In accordance with what is known about hormone signaling, the presence of the photoproducts seems to be strictly regulated and the target cells degrade them via the produced biotransformation enzymes (phase I and phase II enzymes) to terminate the response. This hypothesis was presented at the Dioxin-03 meeting that was held in Boston in August 2003 and was met with great interest.

Numerous aromatic hydrocarbons binds to the AHR and exert toxic effects because compounds such as TCDD, in contrast to the photoproducts, inhibit the normal feed back regulation of the AHR signaling. In addition, certain xenobiotic compounds of the planar polycyclic aromatic hydrocarbon type are substrates for metabolic enzymes that are induced via binding to the AHR and are metabolized into powerful mutagens and carcinogens.

Urban Air Particulates and Activation of AHR-regulated gene expression. Substances present in extracts of urban air particulates were shown by Rune Toftgård and colleagues in 1988 to interact with the AHR in intact cells and to induce cytochrome P-4501A1 (CYP1A1) (Franzén et al., Carcinogenesis, 9,1988,111). Organic compounds from diesel exhaust particles also induce CYP1A1 and other AHR-regulated biotransformation enzymes (Rengasamy et al., J Toxicol Env Health, 66,2003,153).

Corticotropin-Releasing Hormone-Related Peptides and Receptors. The primary hormonal pathway by which the CRH activates stress is via binding to a G-protein-coupled seven-helix cell membrane receptor that causes a cAMP dependent activation of responsive elements in the 5' promoter region of the proopiomelanocortin (POMC) gene. The POMC, in turn, is a precursor protein of adenocorticotropin (ACTH) and alpha melanocyte-stimulating hormone (α MSH) and several other biologically active melanocortins. In 1992-3 five melanocortin receptors (MC1-5) were cloned. The proposed function of POMC derived MC receptors are as follows:

RECEPTOR	LIGANDS	PROPOSED FUNCTION
MC1-R	α -MSH, ACTH	Pigmentation
MC2-R	ACTH	Adrenal function
MC3-R	γ -MSH, α -MSH, ACTH	Cardiovascular regulation
MC4-R	α -MSH, ACTH	Energy homeostasis
MC5-R	α -MSH, ACTH	Exocrine secretion
Opioid receptors	β -Endorphin	Modulation of pain sensation

The MC3-R receptors have been reported to be expressed in various brain areas. In particular, MC3-R receptor mRNA is expressed in the anteroventral periventricular nucleus and posterior hypothalamic area, which, among other things, have been argued to be involved in the neural control of cardiovascular functions. More recently, MC3-R receptors have also been found in human heart (for review see Wikberg et al., *Pharm Res*, 42, 2000, 393).

CRH and Central Regulation of Cardiovascular Adaptations to Stress. The HPA axis is activated through various stimulators that regulate CRH expression in the hypothalamus. Such activators include, protein kinase A and protein kinase C stimulators, norepinephrine, serotonin, acetylcholine, retinoids, Il-1, Il-2, Il-6, NO and CO. When injected intracerebroventricularly, CRH causes cardiovascular responses similar to the effects of stress, increasing heart rate, cardiac output, and mean arterial pressure (Fisher et al., *Regul Pept*, 5,1983,153). The human CRH belongs to a small family of structurally related peptides that includes in addition to CRH the recently identified three proteins urocortin and stresscopin and the stresscopin-related peptide. CRH-related peptides activate two known G-protein-coupled receptors, CRH-R1 and CRH-R2. CRH-R2 receptors are found predominantly in the periphery. High levels of CRH-R2 mRNA exist in cardiac tissue (aorta and myocardium, epicardium and arterioles of the atrium and ventricles). For a review see Vale et al., (*Endocrinologist*, 7, 1997, 3S). The importance of the CRH-related peptide urocortin in the peripheral signaling in the heart by activation of CRH-R2 receptors was recently described (Coste et al., *TCM*, 12,2002,176).

OWN PUBLISHED AND PRELIMINARY FINDINGS

AHR/Arnt induced neuropeptides expression. We have found an important role of the AHR in the pituitary and the hypothalamus (10;13; Huang P, PhD thesis, KI, 2003). A three-fold increase in POMC mRNA was observed in the pituitary of TCDD treated mice. POMC mRNA levels were also increased in the pituitary cell line AtT-20 after exposure to TCDD as well as to the model polycyclic aromatic hydrocarbon β -naphthoflavone. The proteins encoded by POMC translational products, ACTH and β -endorphin, were found with immunocytochemistry staining to be increased in AtT-20 cells after TCDD treatment. Several neuropeptides including POMC, NPY, orexin, MCH and CART were activated by TCDD-treatment and/or were found to be co-expressed with the AHR repressor gene in the rodent hypothalamus (16; Huang P, PhD thesis, KI, 2003). An illustration of a proposed co-regulatory mechanism that take into account both cAMP response elements (CREs) and AHR/Arnt response elements (XREs) is shown in Figure 1. This schematic illustration is based on the findings of increased POMC expression but is suggested to be more general. Support, in the literature, for an AHR/Arnt –regulated activation of CRH expression comes from an observation in monkeys treated with TCDD. Shridhar and coworkers reported that female cynomolgus monkeys expressed significantly increased brain CRH mRNA levels after long term administration of TCDD (Shidhar et al., *Toxicological Sciences*, 63,2001,181).

AHR/Arnt regulatory elements (Figure 2). Preliminary searches for AHR/Arnt regulatory elements using the Signal Scan program (<http://biosci.cbs.umn.edu/software/sigscan.html>) have indicated the presence of 2 AHR/Arnt response elements, XREs, in the first noncoding exon and 7 XREs in the first intron of the human CRH gene (A. Rannug unpublished observations).

Figure 2. Cluster of XREs observed in the 1st intron of the CRH gene. Consensus GCGTG sequences underlined.

NT008183.14

3360-TTAAGGAATA GTCCGCGAAC ACGCGCGCAC ACACACACGC ACGCACGCGC
 3410-GCATAACACAC ACACACATAC GCATAACACAC ATCCATAACAC ACACACACAC
 3460-GCACGCACAC ACATGCATAC

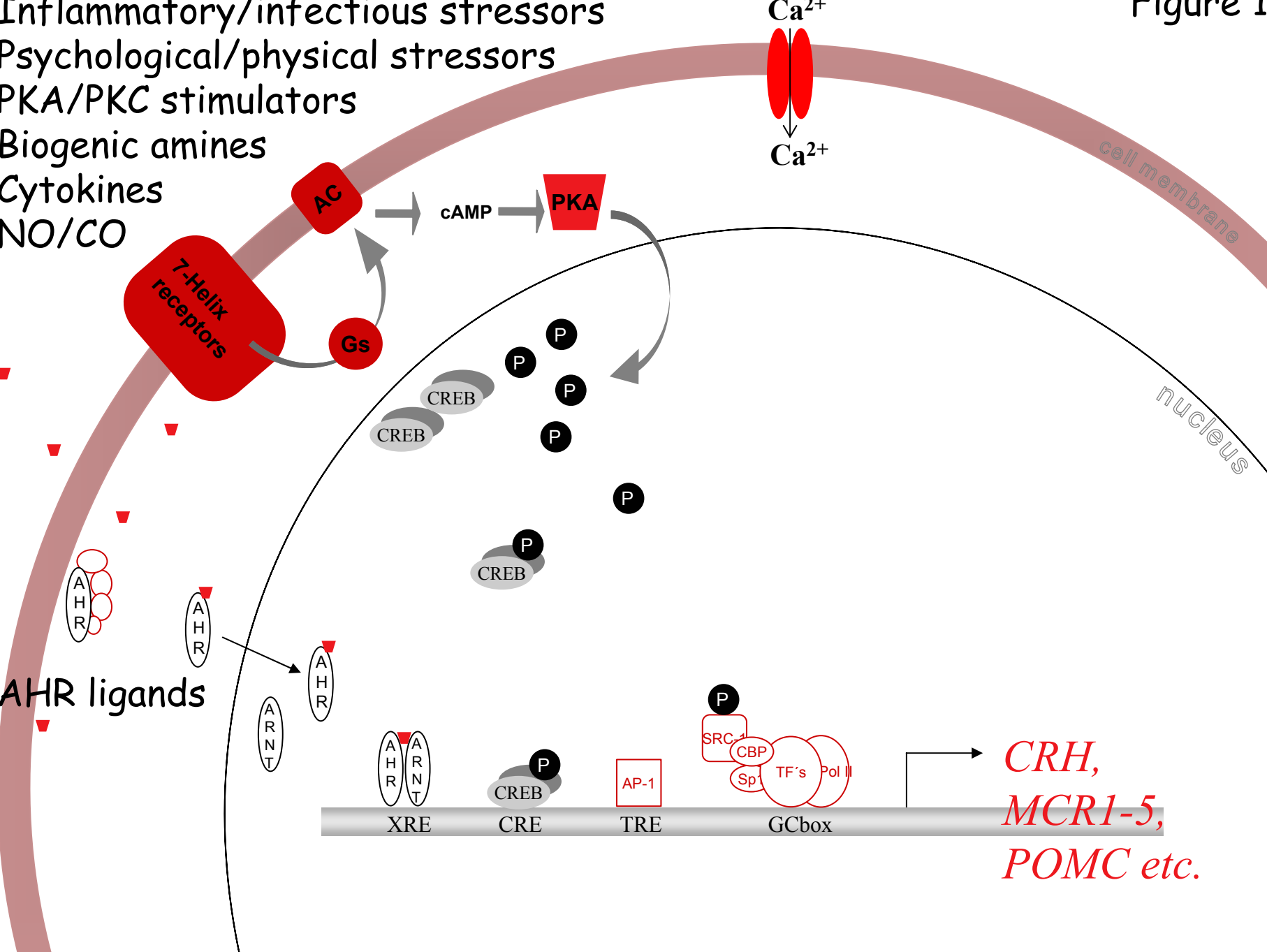
SPECIFIC OBJECTIVES AND EXPERIMENTAL PLAN

The project described in this application focuses on the role of AHR in transcriptional regulation of human CRH and human CRH-related genes *in vitro*. Possible interactions between responses to inflammatory mediators, such as cytokines and tumor necrosis factor alpha, via the cAMP activated pathways, and the AHR regulated responses will be studied. A transfection assay will be used in which reporter constructs fused to the 5' regulatory region of CRH will be utilized to demonstrate CRH gene expression.

The specific objectives are to:

- Characterize the CRH gene promoter region and construct a restriction fragment containing an approximately 5000 nucleotide long stretch including part of the 5' promoter region, exon 1 and the full intron 1 of the human CRH gene.
- Subclone the CRH fragment DNA into the promoterless firefly luciferase reporter vector pG3-Basic (Promega). Transfect AtT-20 cells using standard transfection methods.
- Study activation of CRH gene expression in the pituitary corticotroph cell line, AtT-20 after treatment with AHR-binding polycyclic aromatic compounds by measuring luciferase activity in cell extracts with a Luciferase Assay kit (Promega) on a luminometer.
- Study induction of POMC mRNA expression using competitive RT-PCR techniques according to our earlier protocols.
- Compare known AHR-binding compounds such as β -naphthoflavone, benzo(a)pyrene and 6-formylindolo[3,2-b]carbazol as transcriptional inducers.
- Study interactions using cAMP elevating treatments (forskolin plus isobutylmethyl-xanthine) together with AHR-binding compounds and intervention with CRH mRNA expression using AHR and cAMP antagonists .
- Prepare nuclear extracts of AtT20 cells and study electrophoretic mobility shift (EMSA) after treatment of cells with AHR-binding compounds. EMSAs will be performed using ³²P-labeled oligonucleotide probes generated from the XRE repeat region (Figure 2) of the human CRH gene promoter region.

PhD Maria Backlund has been recruited for a Post Doc position at IMM to work on the interaction between the aryl hydrocarbon receptor and the corticotropin system. She has experience in working with the experimental protocols to be used in this project. Also, a student doing a 20 p degree project in biochemistry will be working on the project. Experimental equipment and AtT-20 cells are available for performing the transfection studies.



CURRICULUM VITAE

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Education: PhD, 1984, Stockholm University, Wallenberg Laboratory,
Division of Genetic Toxicology. Title of thesis: *Genotoxic Hazards in the Rubber Industry: Application of Short-term Tests in Work Environment Analyses*
Docent, 1990, in Genetic Toxicology, Stockholm University, Wallenberg Laboratory,
Dept of Genetic and Cellular Toxicology

Present Position:

- Associate Professor, Institute of Environmental Medicine, Karolinska Institutet (January 1997 - present).

Previous Positions:

- Research Assistant, Division of Genetic Toxicology, Environmental Toxicology Unit, Wallenberg Laboratory, Stockholm University (1975-1984).
- Research Assistant, Department of Occupational Medicine, National Institute of Occupational Health (October, 1984 - November, 1986).
- Visiting Fellow, Department of Environmental Health Sciences, School of Medicine, Case Western Reserve University, Cleveland, Ohio, USA (August, 1985 - August, 1986)
- Assistant Professor, Department of Toxicology, National Institute of Occupational Health (November, 1986 - March, 1990).
- Associate Professor, Department of Toxicology, National Institute of Occupational Health/NIWL (March, 1990 - 1997).

Faculty appointed external examiner for:

- Sirkku T. Saarikoski, Helsinki, December 11, 1998
- Ragne Kristin Bentsen Farmen, Oslo, Jan 19, 2000

Presently supervising the following PhD-students:

- Linda Bergander, Emma Vincent

Degrees completed under my supervision:

- 2001: Yu-Dan Wei: "Tryptophan Photoproducts: Regulation of *CYP1A1* Transcription and Modulation of Cell Growth" PhD thesis, Stockholm university
- 2002: Rainer Tuominen: "Genetic and environmental factors behind differences in human susceptibility to PAH exposure" Licenciat thesis, Karolinska Institutet
- 2003: Anna-Karin Alexandrie: "Significance of polymorphisms in human xenobiotic metabolising enzymes" PhD thesis, Karolinska Institutet
- 2003: Ping Huang: "Constitutive and TCDD-induced expression of Ah receptor responsive genes with special focus on the brain and pituitary" PhD thesis, Karolinska Institutet

Publications and reports:

- Author or co-author of 63 published original scientific papers (24 published papers in 2000-2003), 7 book chapters and 9 scientific reports.

Relevant publications (referred to by numbers in the project proposal):

- Rannug, A., Rannug, U., Rosenkrantz, H.S., Winquist, L., Westerholm, R., Agurell, E. and Grafström, 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**, 15422-15427.
- Löfroth, G. and Rannug, A. (1988) Ah receptor ligands in tobacco smoke. *Toxicology Letters*, **42**, 131-136.
- Rannug, A. and Rannug, U. (1989) UV-irradiation of tryptophan gives rise to AHH-inducing compounds with very high affinity for the Ah receptor. *Chemosphere*, **18**, 1101-1106.

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5. Rannug, U., Agurell, E., Rannug, A. and Cederberg, H. (1992) Certain tryptophan photoproducts are inhibitors of cytochrome P450-dependent mutagenicity. *Environmental and Molecular Mutagenesis*, **20**, 289-296.
6. Rannug, U., Rannug, A., Sjöberg, U., Li, H., Westerholm, R. and Bergman, J. (1995) Structure elucidation of two tryptophan derived high affinity Ah-receptor ligands. *Chemistry and Biology*, **2**, 841-845.
7. 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. *Chemico-Biological Interactions*, **110**, 39-55.
8. Wei, Y.-D., Rannug U. and Rannug A. (1999) UV-induced CYP1A1 gene expression in human cells is mediated by tryptophan. *Chemico-Biological Interactions*, **118**, 127-140.
9. Wei, Y.-D., Bergander L., Rannug U. and Rannug A. (2000) Autoregulation of CYP1A1 transcription via the metabolism of the tryptophan derivative 6-formylindolo[3,2-b]carbazole. *Archives of Biochemistry and Biophysics*, **383**, 99-107.
10. Huang, P., Rannug A., Ahlbom E., Håkansson H. and Ceccatelli S. (2000) Effect of TCDD on the expression of CYP1A1, AHR and ARNT in rat brain and pituitary. *Toxicol Appl Pharmacol.*, **169**, 159-167.
11. Öberg, M., Wei, Y.D., Rannug, A. and Håkansson, H. (2001) Influence of light on the CYP1A1 activity in rat hepatoma cells. *Organohalogen compounds*, **53**, 408-410
12. 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.
13. Huang, P., Ceccatelli, S., Håkansson, H., Grandison, L. and Rannug, A. (2002) Constitutive and TCDD-induced expression of Ah receptor-responsive genes in the pituitary. *Neurotoxicology*, **129**, 1-11.
14. Bergander, L., Wahlström, N., Alsberg, T., Bergman, J., Rannug, A. and Rannug, U (2003) Characterization of in Vitro Metabolites of the Aryl Hydrocarbon Receptor Ligand 6-Formylindolo[3,2-b]carbazole by Liquid Chromatography-Mass Spectrometry and NMR. *Drug Metabol Dispos*, **31**, 233-41.
15. Tuominen, R., Warholm, M., Möller, L. and Rannug, A. (2003) Constitutive CYP1B1 mRNA expression in human blood mononuclear cells in relation to gender, genotype and environmental factors. *Environmental Research*, **93**, 138-148
16. Huang, P., Ceccatelli, S., Hoegberg, P., Håkansson, H. and 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. *Toxicol Appl Pharmacol.*, Article online
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