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(Far) Outside the box:

Genomic Approach to Acute Porphyria

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Summary

If I were living in Caucasus I would be writing fairy tales there
Chekov, 1888

The question of the reasons for the extreme variation in morbidity among the gene carriers of acute porphyria and the great diversity of the precipitating factors are approached by the aid of a model of interacting genomic circuits. It is based on the current paradigm of the acute porphyric attack as a result of a toxic proximal overload of the enzyme-deficient heme-biosynthetic patway. Porphyrogenic influx of precursors is seen as a consequence of uncontrolled induction of its gate-keeping enzyme, ubiquitous 5-aminolevulinate synthase (ALAS1), due to attenuated post-translational control of the enzyme combined with activated gene transcription. Focus is directed on the genomic control of the master-regulator of ALAS1-transcription, the nuclear receptor pair constitutively active receptor (CAR) and pregnane xenobiotic receptor (PXR). On activation by their ligands, i.e. lipophilic drugs, solvents, alcohols, hormonal steroids and biocides, these DNA-binding proteins transform xenobiotic or steroid stimuli to coordinated activations of gene transcription-programs for ALAS1 and apo-cytochromes P450 (apo-CYPs), thus effecting the formation of xenobiotic-metabolizing cytochrome P450 enzymes. The potency of the CAR/PXR-transduction axis is enhanced by co-activators generated in at least four other genomic circuits, each triggered by different external and internal stimuli clinically experienced to be porphyrogenic, and each controlled by co-activating and co-repressing modulators. The expressions of the genes for CAR and PXR are thus augmented by binding glucocorticoid receptor (GR) activated by a steroid hormone, e.g, cortisol generated in fasting, infection or different forms of stress. The promotor regions of ALAS1 and apoCYPs contain binding sites for at least three co-activating transcription factors enhancing CAR/PXR transduction: i.e. the ligand-independent growth hormone (GH)-pulse controlled hepatocyte nuclear factor 4 (HNF4), the insulin-responsive forkhead box class O-(FOXO) protein pathway activated in stress and infection, and the proliferator-activated receptor gamma co-activator 1 alpha (PGC-1alpha) circuit responding to glucagon liberated in fasting. Many interactions and cross-talk take place within the tangle of genomic circuits that control ALAS1-transcription, which may explain the extreme inter- and intra-individual variability in morbidity in acute porphyria. Reasons for gender-differences are found in sex-dependent control of HPA- and GH-activity as well as in direct, or GR-mediated effects on CAR/PCR activation. Constitutional differences in individual porphyric morbidity may be discussed along lines of mutations or duplications of genes for co-activating or co-repressing nuclear proteins active at different levels within the circuits.

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Introduction

A severe new disease condition, precipitated by drugs and engaging the nervous system, was observed during the 1890s. A few decades later it was clear that the disease, later named *acute porphyria*, depended on inherited disposition and that the symptoms generally were precipitated by environmental agents. More than one form of acute porphyria were eventually recognized and found to be associated with mutations in genes for diferent enzymes taking part in the biosynthesis of heme (Fig. 1). Toxic overload of the enzyme-deficient pathway due to induction of its gate-keeping enzyme, ubiqutous 5-aminolevulinate synthase (ALAS1), is presently seen as the immediate cause for the phenotypic manifestations of the underlying genetic disorder, but despite the thorough elucidation of the direct genomic correlates to the enzyme deficiencies that has taken place during the last two decades, the basic mechanisms making manifest these toxicogenetic dispositions have remained obscure in several respects.

One mechanism for control of ALAS1 activity has, during the last few years, been traced to specific intranuclear DNA-binding proteins able to transform external and metabolic stimuli to gene transcription programs. In the present review the explanatory potential of a polygenic approach to ALAS1 induction via different environmental and metabolic stimuli, is tested. The old questions regarding the reasons for the extreme variation in morbidity among the gene carriers, and the great diversity of agents able to precipitate the symptoms, are approached.

The attack of acute porphyria

The syndrome

The acute porphyric attack is an intermittently presenting syndrome of clinical manifestations of inborn enzyme deficiences within the heme biosynthetic pathway (see e.g. Kappas *et al.* 1983, Elder *et al.* 1997, Thunell 2000, Kauppinen 2005, Hift and Meissner 2005).

It makes its appearance in the four forms of acute porphyria, i.e. as acute intermittent porphyria (AIP) in porphobilinogen deaminase (PBGD)-deficiency, as hereditary coproporphyria (HCP) in coproporphyrinogen oxidase (CPGO)- deficiency, as variegate porphyria (VP) in protoporphyrinogen oxidase (PPGO)- deficiency, and as ALAD-deficieny porphyria (ADP) in the homozygous or double heterozygous deficiency of 5-aminolevulinic acid dehydratase (ALAD).

There may be prodromal symptoms to the attack in the form of stubborn obstipation, irritability, restlessness, insomnia or depressive moods. The acute crisis often starts with nausea and vomiting. Most often there are tachycardia and hypertension and generally abdominal pain or pain in the back or limbs. Motor neuropathy with muscle weakness sometimes progressing to tetraplegia may be present, occasionally also respiratory involvement, while sensory engagement is less common. The frequent psychiatric disturbances are typically fluctuating in character and range from depression and asthenia to severe agitation or frank psychosis with hallucinosis. Hyponatremia is a rather common feature, due to vomiting, iatrogenic overhydration or inappropriate pituary ADH secretion. Red urine from high concentrations of porphyrin precursors and porphyrins formed from these, is invariably present during an acute attack of any severity.

Precipitating agents

It is often possible to trace activation of the disease to recent exposure to agents known by experience to be able to precipitate the porphyric manifestations, i.e. many lipophilic drugs, organic solvents, components in red wine and coloured spirits including alcohols and congeners, cannabis, terpenes, biocides, steroid hormones, various kinds of stress possibly including oxidative stress, fluctuations in female sex hormone spectrum, major surgery, fasting, strenous excercise, or infection or other intercurrent illness (see e.g. Duret-Cosyns and Duret 1959, Eilenberg and Scobie 1960, Kappas *et al.* 1983, Bonkovsky *et al.* 1992, Kauppinen and Mustajoki 1992, Thunell *et al.* 1992, Moore and Hift 1997, De Siervi *et al.* 1999, Andersson and Harper 2002, Andersson *et al.* 2003, Hift and Meissner 2005)

Interindividual differences in morbidity

The form of acute porphyria in question plays

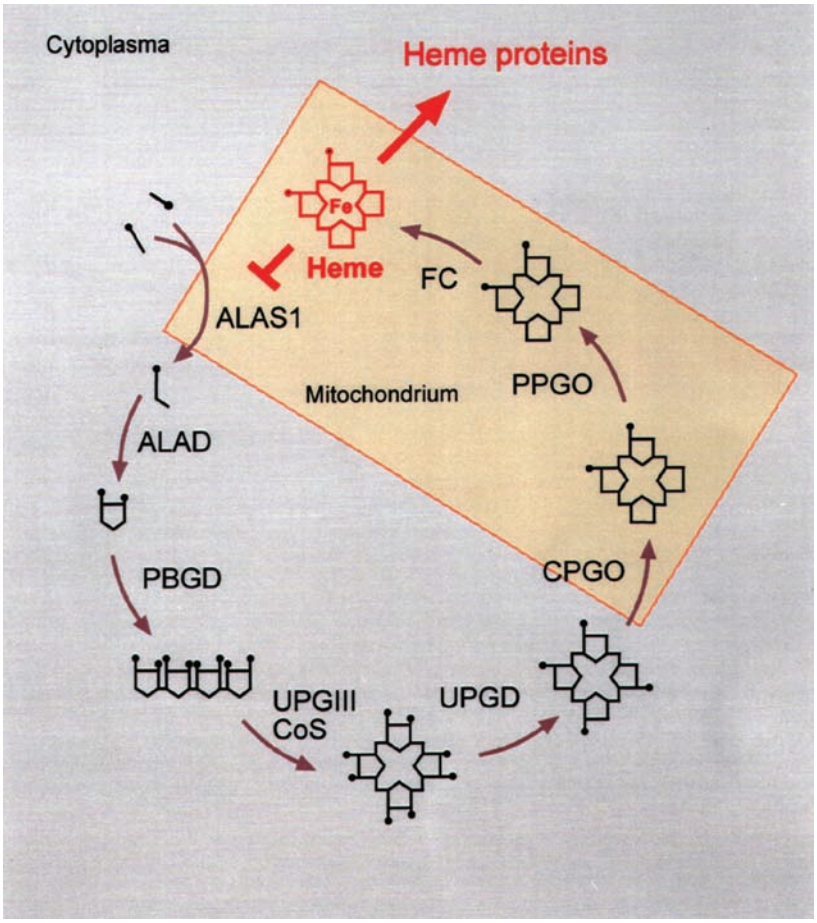

Fig. 1. "The box"
Dedicated heme biosynthesis follows an eight-step solbility gradient engaging four mitochondrial and four cytolasmatic enzymatic steps. Two hydrophilic aminoacids, glycine and activated succinate, are linked into the pathway by ALAS, condensing to form the linear five-carbon organic acid *ALA*. Dimerization of ALA catalysed by ALAD gives rise to the pyrrole *PBG*, and in one further condensation reaction 4 molecules PBG form the linear tetrapyrrole *preuroporphyrinogen*. This third step is catalysed by PBGD, the weakest but one link in the catalytic chain and further deficient in the acute porphyrias; in AIP secondary to mutated gene for the enzyme, in PV and HCP due to inhibition from porphyrins formed downstream. On enzymatic rearrangement of sidechains the tetrapyrrole ring-closes, forming the first porphyrinogen moiety in the pathway, the III-isomeric form of octacarboxylated *uroporphyrinogen*. By a sequence of decarboxylations four side chain acetate groups are removed from the molecule, giving rise to less water-soluble *coproporphyrinogen*. Two of the four remaining propionate side-chins are oxidatively decarboxylated to methyl groups and in a following reaction the hydrophobic *protoporphyrinogen* formed is oxidized to protoporphyrin by removal of hydrogen. In a final step one atom of divalent metal is incorporated, generally iron, forming the ferroprotoporphyrin *heme*, which is used for formation of catalytic heme-proteins or degarded.

In all tissues except erythroid the metabolic flux is controlled by the gate-keeping enzyme of the pathway, ubiquitious ALAS1, the activity of which is repressed by the end-product, i.e. heme. Each one of the eight catalytic steps, except the initial, may be impaired due to a mutation in the gene responsible for formation of the enzyme. The clinical conditions resulting from overload of a deficient enzyme within heme biosynthesis are termed *porphyrias*. Catalytic overload of one of the five enzymatic steps that handle porphyrins give rise to the *cutaneous porphyrias*, while accumulation of the porphyrin precursors proximally in the pathway are associated with the attackwise neuropsychiatric symptoms of the four forms of *acute porphyria*: AIP, VP, HCP and ADP (*see text*).

a role for morbidity in the acute attack. Homozygous or double heterozygous enzyme deficiencies, such as is the rule in ADP, are for obvious reasons associated with a more severe clinical exprsssion. Carriers of gene mutations for AIP are as a group more affected than those carrying genes for VP, and the susceptibility to individual porphyrogenic challenges may differ in the different forms of acute porphyria (Hift and Meissner 2005). Different mutations within the genes may affect the critical catalytic step in the heme-biosynthetic chain differently, helping to account for differences in clinical expression beween gene carriers within separate kindreds (Andersson *et al.* 2000, Floderus *et al.* 2002, von und zu Frauenberg 2005), even if on the whole, it has not proved possible to predict clinical phenotypes or severity based on known genetic differences.

As further discussed in the following, as a group women are more burdened by porphyric manisfestations than men, while attacks before puberty are extremely rare in girls as well as in boys. Within each category, the clinical presentation may, however, vary widely among the individuals with regard to frequency of occurrence as well as to range and severity of the symptoms. The variation in morbidity is partly, but by no means fully, explained by differences in exposure to porphyrogenic agents.

An exceptional metabolic event

Remarkably, the majority of individuals carrying a single mutation for acute porphyria seem to be more or less resistent to the every-day and principally trivial external or internal porphyrogenic challenges, while a few are burdened by repeated and severe attacks over years. Several gene carriers fall between these extremes, experiencing more severe acute porphyric manifestations only once or a few times during their lifetime (e.g. Moore *et al.* 1987). This points to the porphyric manifestation as an exceptional metabolic event and as the result of

occasional unfortunate combinations of more than one porphyrogenic factor working in union. Also, the large differences in porphyric morbidity among the gene carriers may suggest that basic endogenous factors are in play and that vulnerability to porphyrogenic challenge may have a polygenic background. Avenues to understanding of the irregular mechanisms behind the triggering of porphyric illness, may thus pass through the tangle of intranuclear receptor-directed networks that mediate impulses from the outside of the immediate porphyrin-metabolic box to the cruical enzyme within it, the hyperactivity of which gives rise to a toxic catalytic load, i.e *5-aminolevulinic acid synthase.*

The critical role of ubiquitous 5-amino-levulinic acid synthase (ALAS1)

The current hypothesis for the pathophysiology of acute porphyria is focused on the role of hepatic ubiquitous 5-aminolevulinic acid synthase (ALAS1), the inducible enzyme that initiates biosynthesis of heme by linking the two precursor substrates into the chain of seven other dedicated enzymes working in sequence. The ativity of ALAS1 is determined by the extent of current posttranslational and possibly also transcriptional control, as demonstrated in Leghorn male hepatoma cells, from the end-product of the biosynthetic chain, i.e. heme (Granick 1966, Kolluri *et al.* 2005), as well as by the regulatory DNA-binding nuclear proteins effecting its transcription.

In the carriers of AIP, HCP and VP, marked induction of ALAS1 may accelerate the traffic through the pathway to an extent to overload its third catalytic station, controlled by porphobilinogen deaminase (PBGD). This catalytic step, which is physiologically weak, is for genetic or other reasons further impaired in these forms of acute porphyria. As a result of the impaired trough-put of the pathway, porphobilinogen (PBG) and 5-aminolevulinic acid (ALA) accumulate, the PBGD-overload situation being signaled by increased exretion of the two porphyrin precursors, and red-colouring of the urine from oxidized and polymerized PBG. In ALAD-deficient porphyria the deficient enzyme operates the second station of the pathway, where a strangulation in the metabolic pipeline gives rise to accumulation only of ALA.

For unclear reasons the proximal metabolic stases in the heme-biosynthetic pathway are accompanied by the clinical manifestations of acute porphyria. The varying constellation of symptoms is principally common to all conditions accompanied by accumulation of ALA. It is thus present in all the four forms of acute porphyria as well as in conditions of ALAS-inhibition due to lead intoxication or to accumulation of succinylacetone in hereditary tyrosinemia type 1. This 5-carbon metabolite with a structure closely resembling that of the inhibitory neurotransmittor gamma-aminobutyric acid (GABA), has therefore been implicated in the pathogenesis of acute porphyric illness, however almost entirely via guilt-by-association (e.g. Kappas *et al.* 1983, Moore *et al.* 1987, Windebank *et al.* 1993, Meyer *et al.* 1998).

The superinductive ALAS1 response

As previously suggested, in view of the relative unusualness of clinical manifestations among gene carriers, it would seem that ALAS1 induction of sufficient magnitude to create a significant substrate overload in the proximal biosynthetic pathway to heme would be a rare event. Potent and simultaneous activations of both the transcriptional and the post-translational pathways for ALAS1-control in most cases probably have to take place for the disease to be triggered. Such a superinductive response to porphyrogenic challenge is only immediate when transcription of ALAS1 is potently triggered via its main transduction route, while the hepatocyte post-translational ALAS1-inhibitory mechanisms are inactive (Fig. 2).

The control of ALAS1 metabolic activity

Post-translational ALAS1 control

The dedicated heme biosynthetic pathway is initiated as well as terminated within the mitochondria of the cell, where ALAS1 is operative. The entry of ALAS1 into the mitochondrial compartment is inhibited by the end-product of the pathway it regulates, i.e. *heme*. This iron-containing porphyrin also excerts post-transcriptional feed-back control of the enzyme by reducing mRNA-stability (Srivastava *et al.* 1983, Drew and Ades 1989, Hamilton *et al.* 1991, May *et al.* 1995). Consequently, a decrease in the size of the hepatocyte regulatory free heme pool under a critical level – through drain during consumption for heme-protein synthesis, by trapping and destruction of heme by a cytochrome P450 (CYP) suicide substrate, or by intensified catabolism through activation of heme oxygenase – will give rise to

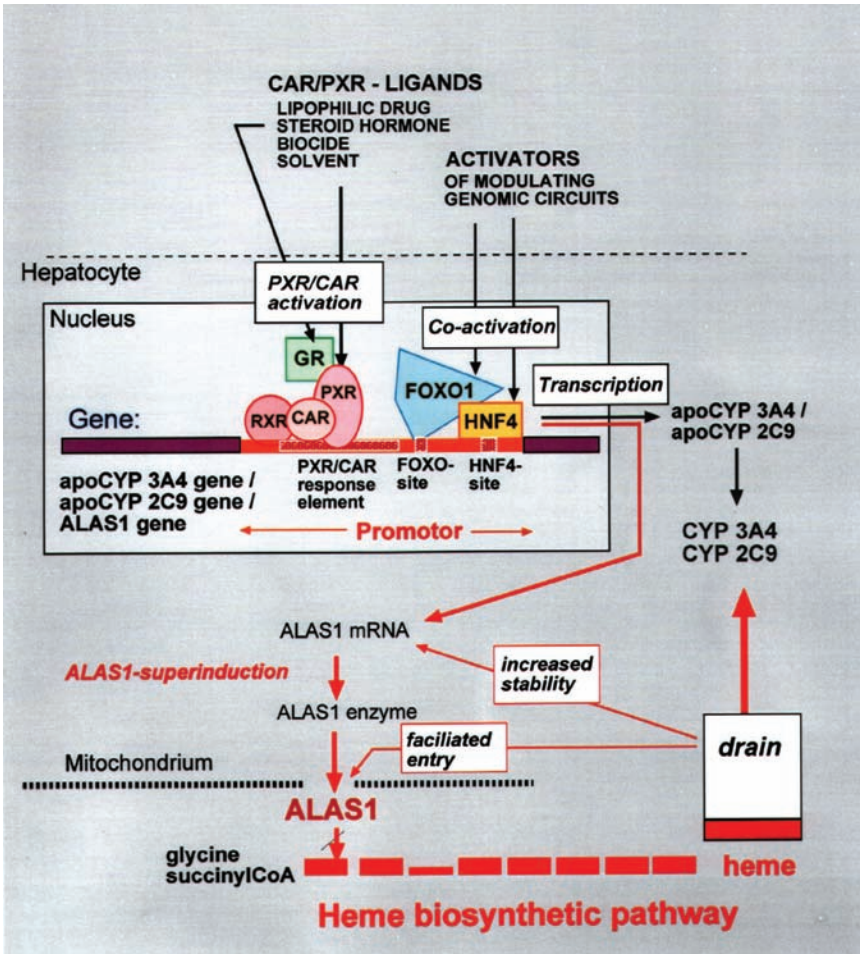

Fig. 2. The superinductive ALAS1-response (see abbreviations)
Heavy induction of hepatic ALAS1 takes place when the post-translational inhibitory control of the enzyme fails due to drain of the hepatocyte regulatory heme pool, on the same time as transcription of the gene for ALAS1 is enhanced by exogenous signals mediated by specific nuclear DNA-binding proteins.

Transcriptions of the genes for ALAS1 and apoCYP take place consertedly In answer to demands for cytochrome P450 (CYP) species handling oxido-reductive metabolic transformations in the liver, activation of the gene for the apo-CYP to be expressed takes place synchronously with the gene for the enzyme, ALAS1, which controls the production of heme needed for formation of the CYP-holoenzyme (In the figure CYPs 3A4 and 2C9, being most abundantly expressed in the liver, are given as example).

Nuclear receptors are activated by exogenous and endogenous ligands. The nuclear receptors PXR and CAR are activated on associating a variety of xenobiotics and steroid hormones. Steroids also bind to the glucocorticoid receptor (GR), augmenting the expression of the NR. By binding to specific NR-response elements within the ALAS1-promotor and together with RXR, the receptor pair CAR and PXR initiate transcription of ALAS1. Co-activation of the gene takes place on association of activated transcription factors FOXO1 and HNF4 to specific sites on the ALAS1-promotor. Activation of promotors for the apo-CYP genes follow the same schedule, presumably giving rise to equivalent formation of ALAS1- and apoCYP-mRNAs.

Co-activators to the NRs modulate the response of the target genes. The CAR/PXR-transduction is augmented by the transcription factors FOXO1 and HNF4 associating to the ALAS1 and apoCYP target gene. These co-activators operate via diffferent genomic circuits that are triggered by exogenous or endogenous stimuli and under control from co-activating or co-repressing modulators.

Post-translational ALAS1-activation is induced by heme-deficit. The volume of heme needed for formation of the CYPs induced, is secured by posttranslational ALAS1-induction taking place in response to the heme-drain resulting from the enhanced CYP-formation. The magnitude of the induction corresponds to the remaining size of the shrinking intracellular regulatory heme-pool. The induction mechanism operates by increasing the stability of ALAS1mRNA transcribed and by faciliating the entry of ALAS1 into the mitochondria where the enzyme is catalytically effective.

ALAS1-superinduction The increased stability of ALAS1mRNA and the augmented mitochondrial entry of the enzyme ad to the increased ALAS1-pressure on heme-biosyntheis built up by the intensified transcription tiggered by ligand-activated PXR/CAR.

Clinical consequences In the abscence of porphyrin-metabolic disorder the combined transcriptional and post-translational ALAS1-inductions represent a vitally important mechanism for adaption to demands for oxidoreductive cytochromes handling potentially noxious xenobiotics. In contrast, in a carrier of acute porphyria with impaired capacity for metabolic throughput at the third catalytic step of the pathway, ALAS1-superinduction may give rise to substrate overload in the proximal pathway and accumulation of presumably toxic porphyrin precursors. The actual clinical effect will depend on the affinity between CAR/PXR and their activating ligand, on the current activities of CAR/PXR-co-activators, and on the extent of heme-drain taking place, which is a function of the amount of CYP to be formed, i.e. the CYP-species in question and their inducibility in the situation at hand.

a compensatory influx of substrates into the pathway and subsequent acceleration of porphyrin synthesis for restoring heme in quantities large enough to reactivate the dormant ALAS1 de-inducing mechanisms. This chain of events is potentially porphyrogenic, but the post-translational ALAS1 induction given rise to is in itself probably generally not sufficient to overload the porphyric enzyme-deficient pathway in a significant way. If this were so, porphyric manifestations would likely be more common than they are.

The control of ALAS1 gene transcription

The bulk of hepatic ALAS1 induced is generated in answer to demands for heme for formation of catalytic

proteins, above all the cytochromes P450 (CYPs) engaged in phase 1 and phase 2 metabolism of xenobiotics, and espcially the CYP 2 and 3A4 species, which by far dominate the human hepatic hemoprotein pool (Guengerich 1995, Pelkonen and Breimer 1994, Pelkonen *et al.* 1998, Pelkonen *et al.* 2000).

Five different mechanisms are recognized for the induction of the xenobiotic-metabolizing CYPs (May *et al.* 1995, Fuhr 2000). One of these does not involve a CYP-gene transcriptional response but affects the lifetime of the holoenzyme formed. With this exception, CYP-inductions are due to increased rates of transcription of their genes above a basal level, mediated by intracellular receptor proteins operative within the hepatocyte nuclear compartment giving rise to increased levels of the enzyme protein within the cell. Beause their physiological, ”natural” ligands had, at first, been unknown, these DNA-binding proteins have been referred to as orphan nuclear receptors (NRs) (Kliewer *et al.* 1999). The NRs are activated on binding their receptor-specific ligands (Sueyoshi and Negishi 2001), i.e. a xenobiotic or a hormonal steroid. After associating one further nuclear receptor, the 9-cis retinoic acid xenobiotic receptor (RXR) the resulting heterodimerization complex attaches to recognition sites within enhancer sequences on their apoCYP and ALAS1 target genes, thus effecting concerted transcriptions of these (Frazer *et al.* 2002, Podovinek *et al.* 2004). The nuclear factors, which mediate or modulate the transcription of xenobiotic-metabolizing CYP genes, belong to the growth hormone-regulated network of liver transcription factors, and a role of the growth hormone system in sex dependent CYP gene regulation is probable (Lahuna *et al.* 2000, Wolbold *et al.* 2003). The NRs differ in specific ligand affinity as well as in regard to their main target response-elements within the CYP genes and to mechanisms employed for mediating their effects. In humans the constitutively active receptor (CAR) and the pregnane xenobiotic receptor (PXR) mediate the majority of xenbobiotic-induced CYP transcriptional responses, more than 50 per cent of the presently prescribed drugs being metabolized by their target genes. Few prescription drugs are ligands to a cytosolic helix-loop-helix protein, the aromatic hydrocarbon receptor (Ah-receptor), which is mainly activated by polycyclic hydrocarbons, or to the peroxisome proliferator activated family of receptors (PPARs), which play fundamental physiological roles in the regulation of energy balance. The CAR-PXR transduction mechanism is also responsive to steroid hormone-ligands.

The interactive mechanisms of the nuclear receptors PXR and CAR

With respect to pathways that modulate ALAS1 transduction, information on the interactive mechanisms of NRs is of interest, because these determine not only the identities and numbers of CYPs induced and thus the total volume of theALAS1 inductive response, but also the effects of endogenous and exogenous factors able to modulate it. The mechanisms involved have, during the last few years, been reviewed in several papers, in which also references to original work discussed below are found (Waxman 1999, Dussault *et al.* 2002, Honkakoski *et al.* 2003, Wang and LeCluyse 2003).

Binding of the ligand to the NR

Steroid hormones, as well as drugs and other xenobiotics bind to the *ligand-binding domain* on the NR. This event gives rise to activation of the receptor that initiates the transcriptional response to the exposure. In the nuclear receptors PXR and CAR the ligand-binding domain is highly hydrophobic and flexible, allowing lipid-soluble molecules of differing sizes to bind in multiple orientations with differing goodness of fit, and thus with different affinities and transactivating potencies. In these central receptors, the response to binding of ligand also includes an initial glucocorticoid receptor (GR)-effected increase in nuclear receptor expression, making the process two-staged. Many different proteins can bind at each element within the glucocorticoid responsive regions of the target gene promotors, modulating the response to the activated NR through co-activation or repression.

Because of the large volume and smooth shape of the ligand-binding pockets of CAR and PXR, a multitude of organic molecules fit in these. This is the reason for the CYP-inducing properties shared by the large number of substances with only lipophilicity as common feature. Although the ligand-NR interactions are largely promiscious, there are large differences among the the two individual NRs when it comes to affinity to a given ligand and thus its CYP-inducing ability, every inducer expressing its own pattern of induction and own inducing power.

Binding of the NR to the target gene

The organisation of the *DNA-binding domain* of

the NR determines its degree of fit to the specific drug response-elements within the main target gene, and thus the identity of it. Several different response elements are recognized in the CYP-genes, differing in NR-specificity as well as in response activity, explaining differences between inducing ligands with regard to the transcriptional response elicited.

Due to overlapping binding specificities there is a sharing between CAR and PXR activity in target genes. Even if there is considerable cross-talk between the two, their power to induce promotor activity in their common target genes may nevertheless differ substantially.

The genes for the most abundant inducible hepatic CYPs belong to the CYP2 and 3A families, activated by CAR/PXR via a concerted transcription of the apoCYP proteins and the ALAS1 enzyme needed for biosynthesis of the catalytic heme component of the holoenzyme to be formed.

The CYP2 genes contain a phenobarbital response element (PBREM) with two NR-binding sites based on a direct repeat (DR)-4 motif that binds drug-activated CAR heterodimerized to RXR. The CAR nuclear receptor is sequestred in the cell cytosol and normally excluded from the nucleous. Its CYP-inducing power is dependent on its degree of success in nuclear translocation as well as in its extent of activation by the ligand. The mechanism by which CAR translocates to the nucleous is not clear, but a prior binding to a ligand does not seem to be essential. It is recognized that a leucin-rich peptide near the C-terminus of the CAR molecule, designated the xenobiotic response signal (XRS), is needed for the translocation to take place. However, proteins preventing the translocation of CAR have also been identified, e.g the cytoplasmic CAR retention protein (CCRP). Different phosphorylations and dephosphorylations via drug-activated protein phosphatases are operative in preparing the receptor for its nuclear entry, and are thus decisive for its capcity to play a CYP-inductive role.

The CYP3A gene promotor-regions contain proximal DR-3 and ER-6 motifs as well as distal DR-3 and ER-6 motifs, within a xenobiotic-responsive enhancer molecule (XREM) on the gene. Ligand-activated PXR-RXR as well as CAR-RXR heterodimers will bind to these sites, enhancing transcription. In contrast to CAR, the PXR protein constitutively resides in the nucleous and a translocation within the cell is not necessary for its access to the target gene. The rate-limiting step in its activation by PXR is thus essentially the rate of ligand-binding, which depends on the affinity between ligand and receptor and is determined by the goodness of fit of ligand molecule in the NR ligand pocket as well as on the intranuclear concentration of the ligand.

ApoCYP4A is highly inducible but not abundant. The apoCYP 4A gene contains direct repeats (DR-1) NR response elements that bind the peroxisome proliferator-activated receptor alpha (PPAR)-RXR heterodimer, activating its transcription. As is the case with PXR/CAR, this receptor is activated by structurally diverse spectrum of ligand agonists, mostly acidic in nature, but few drugs are included among these (Hankinson 1995).

The ALAS1 gene contains a transcriptional enhancer element, the aminolevulinic acid synthase drug-responsive enhancer sequence (ADRES) that employs DR4 recognition sites for PXR and CAR (Podovinec *et al.* 2004). The ADRES elements respond to a wide range of drugs and account for a majority of the transcriptional activity of the gene *in vivo*. The transcription of the ALAS1 enzyme is thus stimulated by the same NRs that induce transcription of the major apoCYP genes engaged in drug metabolism, allowing coordinated induction in response to xenobiotic or steroid challenge.

Until recently the ADRES elements were the only squences in the ALAS1 gene recognized to be able to effect drug-induced transcription. However, an additional region, much closer to the transcription starting point (-0.3 – -3.5 kb) has recently been described (Kolluri *et al.* 2005). It is not yet known whether this more proximal site for drug-dependent up-regulation binds to NRs or not.

As further discussed in the following, the ALAS1 promotor region also contains binding sites for coactivating transcription factors induced under fasting, the activation of which seemingly does not proceed via ligand-activated CAR/PXR (Handchin *et al.* 2005).

PXR/CAR activation-function domains

Co-activators and co-repressors binding to the *activation-function domains* (AF)1 and 2 in the PXR protein, and AF2 in the CAR protein, are engaged in the activation of these nuclear receptors and are thus in control of the transactivation response of their target gene. Sites for NR-linked transcriptional modulation, i.e. AF and COUP- and the CCAAT/enhancer binding protein alpha (C/EBPalpha)-binding sites, are also present in apoCYP promotors and in ALAS1-gene ADRES elements.

The transcription factor C/EBPalpha regulates a number of liver stress-response genes and participates in the upregulation of the CYP3A-genes by glucocorticoids. Point mutations of nucleotides within either of the two C/EBPalpha binding sites in the CYP3A promotors thus abolishes their transactivation potential (Rodrigues *et al.* 2003, Podovinec *et al.* 2004).

In the case of PXR, in the abscence of activating ligand, transactivation is kept under control by the co-repressor silencing mediator of retinoid and thyroid hormone receptor (SMRT). The interaction is mediated through the ligand-binding domain of PXR and the ID2 domain of SMRT, co-expression resulting in co-localisation of the two receptors at discrete nuclear foci. Ligands to PXR silence SMRT by exchange of the co-repressor with the p160 co-activator RAC3, thus enhancing CYP expression (Johnson *et al.* 2005).

Modulation of PXR/CAR transactivtion

Modulation of the PXR/CAR-induced transcription of ALAS1 may take place along at least four different avenues, i.e. via the growth hormone (GH)-pulse controlled hepatocyte nuclear factor 4 (HNF4), via the glucocorticoid receptor (GR) responding to hypothalamic-pituary-adrenal (HPA) axis signaling, via insulin responsive Forkhead box class O (FOXO) protein activity, and via the non-insulin dependent PGC-1alpha pathway activated by glucagon (Fig. 3).

The HNF-4 pathway

Hepatocyte nuclear factor 4 (HNF4) is a ligand-independent activator of several hepatic genes. It is under control by the growth hormone (GH)-pulse dependent signal transducer and activator of transcription 5 (STAT 5) that belongs to a group of seven factors that act as signaling components between the plasma membrane and the nucleous, and as transcription factors with specific DNA binding ability in the nucleous (Wittig and Groner 2005). HNF4 is a mediator of sex-dependent expression of liver CYPs and follows a GH-dependent highly pulsative activity in males and a more steady level in females, where the daily GH production during fertile years are three times higher than in men (Wiwi and Waxman 2004). Liberation of GH takes place in answer to various forms of stress, e.g. trauma, surgery, infections and mental stress, as well as in infection and in rapidly ensuing hypoglycemia. It takes part in transcriptions of CYPs 2 and 3A4 in synergy with ligand-activated PXR (Tirona *et al.* 2003, Ferguson *et al.* 2005).

The co-activators GRIP1, steroid receptor co-activator -1 (SRC1) and P300/ cEBP enhance the transactivation potential of HNF4 (Wang *et al.* 1998), as does FOXO activated in fasting (Handchin *et al.* 2005). HNF4 is a critical target for cysteine-nitrosylation by nitrous oxide produced on activation of nitric oxide synthase (NOS), thus attenuating CYP-production and drug CYP metabolism during NOS-activation e.g. in inflammation (Vossen and Erard 2002). A complex between HNF4 and PGC-1alpha controls gluconeogenesis also under conditions of insulin signaling via transcription of the genes for phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (Glc6P). Ligand-activated PXR splits the HNF4 / PGC-1alpha complex, quenching hepatic glucose production even under fasting (Rubins *et al.* 2005).

By co-activating PXR, HNF4 also controls bile acid biosynthesis via transcription of CYP7A1 (cholesterol 7alpha hydroxylase) and CYP8B1 (sterol 12alpha hydroxylase), the rate limiting enzymes in the production of bile acids from cholesterol (Bhalla *et al.* 2004). Through feed-back control, bile acid precursors downregulate PXR via activation of the farnesoid xenobiotic receptor (FXR), a nuclear receptor that upregulates the PXR transactivation-repressor small heterodimer partner (SHP) (Orulin *et al.* 2003, Eloranta *et al.* 2005).

The FOXO avenues

Under different forms of stress induction of ALAS1 is effected via activation of transcription factors belonging to the FOXO family of proteins, which translate environmental and metabolic stimuli into changes in gene expression programs.

The divergent functions of FOXO are regulated by signal-induced post-translational modifications of the protein, controlled through phosphorylations and acetylations, which individually determine the direction of its nuclear-cytoplasmatic shuttling, binding of cognate DNA, protein-protein interactions and selection of transcription programs. Targets for FOXOs are genes controlling hepatic generation of glucose as well as production of ALAS1 (Bartel *et al.* 2005). Outside the nuclear compartment, FOXO proteins are transcriptionally inactive and degraded. Their intracellular localisation and activation are directed by the key hormones in glucose homeostasis, i.e.

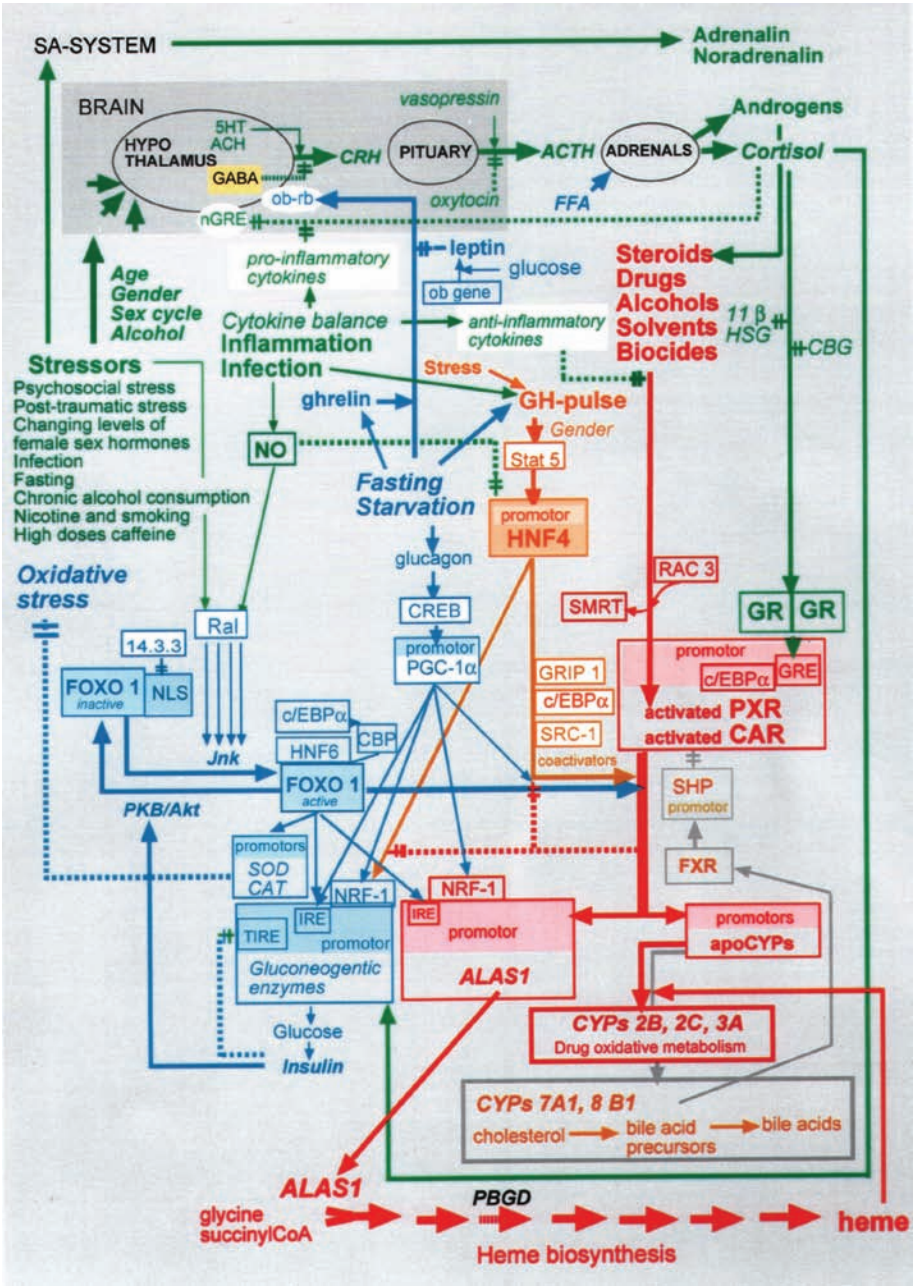

Fig. 3. Modulation of CAR/PXR-mediated ALAS1-transcription

Generation of cytochrome P450 (CYP)-species takes place via concerted transcriptions of the genes for ALAS1- and apoCYPs effected by the nuclear receptor pair PXR/CAR. These transcription factors are activated on binding a ligand xenobiotic, or steroid-hormone e.g. generated on engagement of the hypothalamic-pituary-adrenal axis in stress, inflammation or fasting. The main stress hormone, cortisol, increases the expression of the nuclear receptors on binding to the glucocorticoid receptor (GR) on their promotors. Bile acid synthesis interferes with CAR/PXR-activity via precursors activating the SHP repressor.

The CAR/PXR potential is enhanced when the transduction axis is supported by signals from three pathways transmitting exogenomic impulses. The enzyme Jnk thus reacts to various forms of stress by activating the transcriprtion factor FOXO1, while the HNF4 is activated by pulses of growth hormone (GH) triggered e.g. in fasting, infection or traumatic stress, both circuits co-activating CAR/PXR transduction. Under the glucagon-response to fasting PGC-1alpha enhances HNF-4-mediated co-activation of CAR/PXR, and also triggers ALAS1-expression directly.

As evident from the infrequent occurrence of clinical manifestations in acute porphyria the balance within the genomic system as a rule, and in most individuals permanently, favours forces counteracting significant ALAS1-induction. Most probably these are found in the hepatic generation of glucose that accompanies activation of each one of the circuits in control of ALAS1-transcription, except the CAR/PXR axis. Cortisol, FOXO1, PGC1alpha and HNF4 are thus activators of genes for gluconeogenic enzymes, the latter circuit inhibited by ligand-activated CAR/PXR. Glucose, and the insulin response, thus interrupts the ghrelin-stimulated activation of the HPA-axis that takes place in fasting, and incrase the expression of the gene for leptin that inhibits it. It also quences the stimulatory effect of fasting on the GH-pulse, and activates the PKB/Akt enzyme system that exports activated FOXO1 for extranuclear destruction.

The activities of the CAR/PXR co-activating circuits are modulated by different co-activators and co-repressors (*see text*). Constitutional differences among these may explain differences between individuals in susceptibility to porphyrogenic challenge, while occasional variations in their activity levels may be one reason for individually fluctuating vulnerability.

glucagon, which opens a pathway to hepatic glucose liberation and ALAS1-transcription during fasting, and insulin which closes them down (Perrot *et al.* 2005).

The FOXO-shuttle

Under insulin signaling, i.e. during normo- and hyperglycemic conditions, the insulin-activated enzyme phosphatidyl inositol 3-kinase-protein kinase B (PKB/Akt) effects phosphorylations of FOXO1 at threonine and serine residues, which give rise to nuclear export of the FOXO-protein to cytoplasm in inactive form and vulnerable to degradation by the ubiquitin-proteasome pathway. FOXO-proteins mutated at these Akt-phosphorylated sites show 3-5 fold higher

transcriptional activity than does wild type (Vogt *et al.* 2005). The 14-3-3 proteins, which belong to a family of regulatory signaling molecules, shift the equilibrium of FOXO towards cytoplasm by reducing the flexibility of its nuclear localisation sequence (NLS) in a way to inhibit interaction between FOXO and the nuclear importing machinery (Obsilova *et al.* 2005).

In the abscence of insulin, cytoplasmatic FOXO is imported to, and retained in the nucleous (Matsuzaki *et al.* 2005) by the action of stress-responsive Jun-N-terminal kinases (Jnk) (Matsumoto and Accilli 2005), which are activated by the small Ras-like (Ral) GTP-ase (Feig 2003) in reponse to factors such as nitric oxide (Mittar *et al.* 2004), oxidative stress and the proinflammatory cytokine TNFalpha. These agents are also implicated in FOXO-induced transcription of the antioxidant enzymes Mn superoxide dismutase (MnSOD) and catalase (Essens *et al.* 2004).

The phosphorylations of the protein imposed by Jnk may override the inactivating insuline-responsive Akt-phophorylations behind nuclear exclusion of FOXO1 and increase the expressions of its main target genes (Frescas *et al.* 2005).

FOXO activation

FOXO phosphorylated by insulin responsive Akt is transcriptionally inactive dependent on reduced capacity to bind target DNA and due to interference with the binding of acetylating co-activator (Vogt *et al.* 2005).

Within the nucleus, reversible acetylations of FOXO effected by the histone acyltransferase cAMP response element-binding protein (CREB)-binding protein (CBP) and the NAD-dependent histone deacetylase silent information regulator 2, control the transcriptional activity of the FOXO protein, which is dependent on free lysines at positions 242, 245, and 262. Acetylations at these sites neutralize the positive lysine charges neccessary for it to bind cognate DNA sequence. They also increase the sensitivity of the FOXO protein to phosphorylation via the PKB/Akt pathway that translocate it back to cytoplasm (Matsuzaki *et al.* 2005). In contrast, under conditions of close-down of insulin signaling, lysine acetylations of FOXO resulting from carboxyterminal acyltransfers by p300/CBP enhances the FOXO-controlled expression of hepatic gluconeogenic genes. This co-activator is recruited to FOXO by HNF6 in transcriptional synergy with CCAAT/enhancer binding protein alpha (C/EBPPalpha) (Youshida *et al.* 2006).

FOXO target gene transcription

During fasting, i.e.the absence of insulin-signaling and activated by Jnk, FOXO1 is engaged in activity-modulating crosstalk with CAR/PXR, which explains the observations that induction of drug-metabolizing CYPs is inhibited by insulin, while gluconeogenesis is repressed by CYP-inducing drugs. FOXO is thus a co-activator in the transcriptional induction of ALAS1 and apoCYPs triggered by CAR/PXR. Conversly, FOXO-induced transcription of the genes for PEPCK and Glc6P are interrupted by ligand-actived CAR/PXR (Kodama *et al.* 2004).

During the insulin-response to glycemia, the FOXO / CAR/PXR interaction is closed-down by the Akt-dependent inactivation of FOXO and subsequent nuclear export and destruction of the protein. However, in fasting FOXO1 is reported to take part also in pathwys directly engaging the promotors of ALAS1 and gluconeogenic enzymes and which are critcally dependent on the participation of the transcriptional co-activator proliferator-activated receptor gamma co-activator 1alpha (PGC-1alpha). This pathway has recently been further elucidated and throughly reviewed (Handchin *et al.* 2005). At least in mice, this control of ALAS1-transcription seems not to be mediated via the CAR/PXR system, an intriguing finding in view of the question of the destination of heme generated outside concerted transcriptions of ALAS1 and CYP apoproteins, unless it would represent a device for topping up a shrinking ALAS1-regulatory heme-pool.

The glucagon-controlled PGC-1alpha circuits

As previously discussed PGC-1alpha is co-activator of several nuclear receptors and other transcription factors. It is induced in the liver during fasting and strenous excercice, effecting co-regulated transcriptions of the genes for ALAS1 and the gluconeogenic enzymes PEPCK and Glc6P. It is induced by glucagon, which together with glucocorticoids respond to reductions in plasma glucose level. Transcription of the gene is triggered by CREB binding directly to the promotor. It also takes part in HNF4-effected gluconeogenesis, a pathway controlled by growth hormone. This mechanism is more active in women than in men and independent of nutritional state except under conditions of rapidly ensuing hypoglycemia.

PGC-1 alpha is recruited to the target genes by the nuclear respiratory factor-1 (NRF1), a transcription factor that increases expression of nuclear encoded

mitochondrial genes. This pathway to hepatic glucose liberation as well as to isolated induction of ALAS1 is not primarily sensitive to insulin. In contrast, the parallell PGC-1alpha pathway to hepatic gluconeogenesis and ALAS1-transcription proceeding via FOXO1 recruited to an insulin responsive IRE-element within the promotors of the target genes, is open only in fasting. The resulting release of glucose from the liver eventually inhibits glucagon signaling, thus closing the PGC-1alpha-FOXO1 pathway.

One further mechanism for insulin-directed endstage interruption of hepatic glucose production directly involves the expression of the genes for PEPCK and Glc6P. It is operative through regulation of a DNA-element present in the promotors of these two enzymes, i.e. the thymine-rich insulin response element (TIRE). Overexpression of the phosphorylating enzyme glycogen synthase kinase 3 (GSK-3) reduces the inhibitory effect of insulin on PEPCK and Glc6P transcriptions, while inhibition of GSK- 3, e.g. by the action of phosphatidyl inositol 3-kinase (Pl-3), increases the repressive insulin effect on hepatic glucose production (Finlay *et al.* 2004).

The stress-activated sympathicoadrenal (SA) system and hypothalamic-pituary-adrenal (HPA)-axis

Stress

In view of the complex hormonal response to physical, metabolic or psychosocial stress, it is not suprising that stress is found among the precipitating factors in acute porphyria (e.g. Andersson and Harper 2002). The concept of stress is not unambigous but is usually applied to the reactions of the organism to stressors giving rise to hormonal responses engaging the sympatico-adrenal (SA) system and the hypothalamic-pituary-adrenal (HPA)-axis.

In stress there are thus rapid reactions of the SA-system, the HPA-axis and, in inflammation, the immune system. Hormonal effects of stress are the increased release of the catecholamines adrenalin and noradrenalin via the autonomous system, and release of glucocorticoides and androgens from the adrenal cortex. Also cytokines are included in the endocrine stress-signal, responding by increased plasma levels as well as by fluctuating ratios between pro- and antiinflammatory species. (e.g McEwen 2000, Tsigos and Chrousos 2002).
A significance of the hormonal stress-response in the system of modulatory genomic circuits that control hepatic ALAS1 induction, is suggested by the several stressors observed to be clinically porphyrogenic. Also thought-provoking is that HPA-overactivity is a finding in insomnia and depression (Dinan and Scott 2005), i.e in two common manisfestations of acute porphyria, and that the sympaticoadrenal manifestations irritability, tachycardia and hypertension invariably are present during the acute porphyric attack (Kappas *et al.* 1983).

The glucocorticoid response

Glucocorticoids generated on activation of the HPA-axis constitute one important class of mediators of the metabolic reponse to stress. They effect transcription of many genes through activation of their glucocorticoid receptor (GR) (Pascussi *et al.* 2000, Pascussi *et al.* 2003, Wang *et al.* 2003), which belongs to the steroid hormone receptor subclass controlling physiological processes by activation or repression of a host of target genes. The transactivation, via ligand-activated CAR/PXR, of genes programming for CYP2 and 3A4 is thus enhanced by GR. The liver transcription factor C/EBPalpha, known to regulate a number of other stress-respone genes, takes part in the transcriptional control of CYPs via GR (Rodrigues *et al.* 2002).

In dimeric form GR initiates transcription of the target gene by binding to specific DNA glucocorticoid response-elements (GREs) in the promotor regions. Glucocorticoid-mediated repression of genes, on the other hand, is effected by trans-repression of other transcription factors, or via the action of negative GREs (nGREs), such as e.g. take part in the feed-back regulation of the HPA-pathway on the hypothalamic and pituary levels (Dostert and Heinzel 2004), a negative modulation disturbed by proinflammatory cytokines (Schepers *et al.* 2005).

To judge from the widespread clinical porphyrogenicity among stresssors acting on the HPA-axis, one of the major avenues to ALAS1 induction may pass through this circuit. As there are several points of attack for pharmacologic interference within this pathway, closer consideration of its control may be of interest in attempts to find ways for intervenention in acute porphyria.

Glucocorticoid PXR/CAR activation

Glucocorticoids play a dual role in CYP3A4 induction. Under physiological conditions, i.e. in submicromolar concentrations, a xenobiotic-independent, low amplitude CYP3A4 induction is produced through transcription of PXR/CAR initiated by GR. As previously

discussed the trancription factor C/EBPalpha is needed for the GR-triggered transcription.

In supra-micromolar concentrations, i.e.under chronic bolus- or stress conditions, glucocorticoids give rise to a high amplitude CYP3A4 induction through direct ligand-activation of PXR (Pascussi *et al.* 2001, Wang *et al.* 2003). The C21 steroids, e.g. the pregnane metabolite of progesterone, are the most potent steroid activators of PXR, but also corticosteroids and estrogens activate PXR albeit in higher concentrations. In contrast, the transcriptional activity of CAR may be blocked by association of a steroid ligand. The testosterone androstanol metabolite thus dissociates this receptor from the steroid receptor co-activator protein (SRC1) (Forman *et al.* 1998), which is a general transducer between DNA-bound NRs and the basal transcriptional machinery of their target gene, and required for transcriptional stimulation by many NRs including GR (Edwards 1999).

Cortisol liberation

The HPA-axis constitutes the pathway of control of the glucocorticoid response to neurogenic and metabolic stress (Herman *et al.* 1996). Its main effector is the gluconeogenic hormone cortisol. It is activated by sets of neurons in the hypothalamic paraventricular nucleous (PVN), which are stimulated from multiple sources including brainstem aminergic-peptidergic afferents, blood-borne signals, input from the limbic system and local-circuit interactions with the preoptic hypothalamic continuum. Projections from different parts of the brain terminate in the immediate surround of PVN, an area heavily populated by GABA-ergic neurons. Different stressors employ different stress circuits, the ensuing response being dependent on the specific pathway employed by the stimulus.

The HPA-axis is activated by the corticotropin-releasing hormone (CRH), a hypothlamic peptide secreted into the pituary porta-system in response to stress, stimulating a pituary release of adrenocorticotropin (ACTH), which effects adrenalglucocorticoid synthesis. The secretion of CRH is regulated by stimulatory neurotransmittor pathways in the immediate brain region, operative via 5-hydroxytryptamine (5HT) and acetylcholine (ACH), and subjected local inhibitory actions of norepinephrine (NE) and gamma aminobutyric acid (GABA) signaling circuits (Herman *et al.* 1996, Herman *et al.* 2002).

In view of the constancy of ALA-accumulation in acute porhyric illness and the importance of inhibitory GABA-signaling within the HPA-axis, it is conceivable that accumulated ALA competing for neuronal sites with its structural neurotransmittor analogue GABA may de-inhibit HPA-axis hyperactivity in ALAS1-inducing direction (McGillon *et al.* 1975).

Stress-induced epinephrin delivered to the gland via the blood stream, and vasopressin, which is co-produced in increasing amounts when the hypothalamic PV neurons become chronically activated, strongly potentiate CRH and ACTH release, while oxytocin inhibits it. The latter, ”well-being hormone” may contribute to a relative immunity to post-partum stress during the lactation period of the mother. Prostaglandins and nitrous oxide take part in the regulation of the HPA-axis by enhancing and attenuating, repectively, the stimulating effect of vasopressin (Bugajski *et al.* 2004).

In the adrenal cortex ACTH stimulates the conversion of cholesterol, taken up from plasma in the form of low density lipoprotein, to a steroid, which subjected to a series of CYP-catalyzed oxido-reductive reactions passing via porphyrogenic 17-alpha-hydroxy progesteron, gives rise to *cortisol*, the main stress hormone. In a feed-back fashion cortisol inhibits the hypothalamic release of corticotropin, a control mechanism attenuated during chronic stress (Helmreich *et al.* 2005).

Modulation of systemic cortisol effect

The effects of stress-induced glucocorticoids ulimately depend on the bioavailability of the steroid produced and on target sensitivity as determined by GR function. The systemic activity of cortisol is modulated by restrictions in bioavailaility via association to cortisol-binding globulin (CBG) and by reversible conversion to the inactive metabilite cortisone by the 11beta-hydroxysteroid-dehydrogenase (11beta-HSD) system.

Glucocorticoid sensitivity varies among different target tissues and show large individual differences dependent on age and sex hormone status of the individual. It can be enhanced in times of acute stress, e.g in response to excercise or psychosocial stress, and downregulated under chronic stress. Downregulation is also found in patients with diverse somatic and psychiatric diseases, e.g. depression (Rohleder *et al.* 2003, Ising *et al.* 2005).

Stressors of the HPA-axis

Stressors of several kinds activate the HPA-axis: e.g. experimental stress (Helmreich *et al.* 2005),

pharmacological stress (Rocca *et al.* 2005), changing levels of sex hormones as e.g. in the premenstrual period, ante-and postpartum, during transition phase to menopause and during use of oral contraceptives (Elenkov and Chrousos 2002, Swaab *et al.* 2005), psychosocial stress (Rohleder *et al.* 2003, Kudielka *et al.* 2004), sleep deprivation (Sgoifo *et al.* 2006), fasting (Fichter and Pirke 1986), loud noise (Ptaz *et al.* 2005), posttraumatic stress (Vanitallie 2002, de Kloet *et al.* 2005), percieved stress (Goldman *et al.* 2005), chronic alcohol consumption (Gianoulakis *et al.* 2003), high doses caffeine (Patz *et al.* 2005), nicotine and smoking (Rohleder and Kirchbaum 2005), infection and cytokines (Silverman *et al.* 2005). Many of the HPA-axis stressors are clinically recognized to be porphyrogenic.

Effects of fasting on the HPA-axis

The HPA-axis plays a pivotal role in restoring energy homeostsis in different allostatic states. It responds rapidly to changes in nutritional state and reduced caloric intake and catabolic state have powerful stimulating effects on HPA activity in experimental animals as well as in humans (Fichter and Pirke 1986). Food intake stimulates transcription of the gene (ob gene) responsible for the production of the hormone *leptin*, which suppresses the HPA-response to stress stimuli. Feeding also reverses overexpression of the hypothalamic leptin receptor (Ob-Rb) induced under fasting (Giovambattista *et al.* 2000). *Ghrelin,* a peptide predominantly produced in the stomach under conditions of energy restriction has significant neuroendocrine effects, stimulating corticotrop, lactotrop secretion as well as liberation of GH. The effects are abolished in feeding and by glucose and insulin (Giordano *et al.* 2004).

Virtually all metabolic disorder accompanied by elevated plasma levels of free fatty acids (FFA), e.g. starvation, obesity and diabetes, are associated with hypercorticoidism. In rat adrenocortical cells it is observed to be due to direct stimulation of the adrenal cortex by unsaturated FFA amounting to about 50 per cent of that effected by ACTH. The mechanism of stimulation differs from that of ACTH, yet still requiring active protein synthesis (Sarel and Widmaier 1995).

Effects of infection and inflammation on HPA activity

The marked impairment of hepatic drug metabolism during inflammation and infection has been known for may years. The mechanisms behind this effect have not been fully elucidated but may be due to downregulation of CYPs by proinflammatory cytokines such as e.g. IL-6 (Teng and Piquette-Miller 2005). On the other hand, infection is a well-known trigger of acute porphyria, i.e. as a potent inducer of ALAS1. The porphyrogenic effect may be secondary to the changes in cytokine balance to which infections give rise. In infection and inflammation the stress system is thus activated for protection against excessive immune response. It generates catecholamines and glucocorticoids that inhibit the formation of proinflammatory cytokines such as Il1, Il-6, IL-8, TNFalpha, IFNgamma, and stimulates production of antiinflammatory cytokines, e.g. IL-10, IL-4, TGFbeta. Changes in the activity of the stress system, as may take place e.g. in acute or chronic stress, cessation of chronic stress, severe exercise, pregnancy and in the post-partum period, may be accompanied by oscillations in cytokine balance that modulate the activity of the HPA-axis in inhibiting as well as in stimulating direction (Elenkov *et al.* 2002). In certain phases of infection a predominance of proinflammatory cytokines disturbs the negative feedback exerted by cortisol on the HPA-axis.

Also, nitrous oxide generated by endothelial nitrous oxide synthase (eNOS) plays a cruical role in cytokine-induced HPA-axis activation (Gadek-Michalska and Bugajski 2005). Further, in the cross-talk between the HPA-axis and the cytokine system the secretion of vasopressin by magnocellular neurons is enhanced, also with activating effect on the HPA-axis. This effect may play a role in the emergence of the syndrome of inappropriate antidiuretic hormone secretion (Mastorakos *et al.* 1994, Mastorakis 2003, Gionis *et al.* 2003), a complication encountered in about one third of acute porphyric attacks (Hift and Meissner 2003).

As will be discussed the context of FOXO proteins and HNF4 (below), infection and inflammation may also employ other pathways to co-activation of CAR/PXR transduction.

Relative significances of the different CAR/PXR co-activating genomic circuits

There is a complex interplay among the different regulatory pathways controlling the transcription of ALAS1. It encompasses a host of genomic interactions, the individual relative significances and potencies of which being difficult to survey in whole and problematic to assess on theoretical grounds. Also, in view of the

sporadic nature of the clinical manifestations, the system gives the impression of most of the time being under control of repressive forces, and that the acute porphyric attack would be the result of an occasionally upset balance favoring stimulatory circuits.

It would, however, seem that by keeping track of the relative occurrence, among gene carriers of acute porphyria, of specific attack-precipitating factors acting within the network, it would be possible to gain some appreciation regarding the relative importance of each one of the ALAS1-inducing genomic circuits.

Thus, Kappas *et al.* (1983), assuming a number of unknown predisposing factors, stated that endogenous hormones probably are the most important attack-precipitation agents in acute porphyria, while drugs and low caloric intake were referred to as common causes for acute porphyric symptoms, and infection, intercurrent illness and major surgery named as other agents able to trigger the condition.

Kauppinen and Mustajoki (1992) investigated the cause of attacks in a group of patients with acute porphyria, including 195 persons with AIP and 73 with VP. The frequencies of different precipitating factors were: menstruation 30 %, infection 29 %, alcohol 25 %, fasting 12 %, and prescription drugs 10 %.

In the material of Hift and Meissner (2005), which included 15 patients with in all 69 attacks of AIP, almost all attacks were related to the menstrual cycle, while infection or pregnancy was implicated in only one and two cases, respectively, and other triggering factors not recognized. In contrast, in 21 attacks of VP alcohol, cannabis or other drugs were implicated in 64 per cent, and in no case was any menstrual relation found. The most probable explanation for the difference between the two forms of acute porphyria with regard to gonadal origin of the attacks, probably is the vast female preponderance in the AIP-group studied (13 women, 2 men), as compared to the VP-group (5 women, 5 men), and that, as is known from many studies, in AIP women are more afflicted with porphyric morbidity than men. Andersson and Harper (2003) found the following spectrum of attack-precipitating factors among carriers of the W198X AIP gene mutation, (figures calculated on numbers of individuals reporting a specific precipitating factor): menstruation 32 %, stress 30 %, prescription drugs 19 %, fasting 18 %, alcohol 14 %, infection 14 %, physical excercise12 %, occupational environment 8 % and pregnancy 6 %.

An increasingly important role of stress may be suggested from observations made by the Swedish Porphyria Patient´s Association, which is in close individual contact with the majority of the acute porphyria gene carriers in the country, about one thousand. Its chancellery thus claims, albeit without statistics, that the favorable trend in acute porphyric morbidity observed the last four decades seems to have reversed. For example, more 25-35 y women with AIP report having noticed red urine and experienced clinical symptoms. Even if increased exposure to environmental estrogens in the form of phyto-estrogens may play a role, one major reason for such development could be the increasing strain that this category of gene carriers are exposed to, these days often without support handling home, children, social life and professional career.

All the porphyrogenic exposures noted in the studies are thus found to play well-defined roles along the different pathways that regulate ALAS1-transcription (Fig. 3), but with the exception of an evidently constitutional female readiness for a porphyrogenic ALAS1-induction via gonadal factors, the results do not permit any quantitative conclusions regarding the relative importance of each one of the genomic pathwys to ALAS1 induction. Neither, is there any help in the circumstance that there is a considerable difference between AIP and VP with regard to porphyric morbidity, which has earlier been pointed out (Hift *et al.* 1993) and found also in the present study by Hift and Meissner, where there is a 14-fold increase in risk for acute attack in AIP as compared to VP (Hift and Meissner 2005). The reason for this is rather be sought outside the genomic interplay leading to ALAS1 transcription, depending as they probably are on differeces in the balance between transcriptional and post-translational ALAS1-induction. In AIP the activity of PBGD thus is invariably severly impaired, especially in gene mutations giving rise to totally abolished allelic production of the enzyme. In contrast, in VP the activity of PBGD would depend on the actual extent of its inhibition exerted by porphyrinogen species accumulated downstream and which evidently in most instances leaves a higher capacity for metabolic throughput in the part of the pathway controlled by PBGD. As a consequence, in AIP the fertile women would be more vulnerable than VP females to the hormonal fluctuations within the sex cycle or to effects of exogenous sex hormones affecting genomic circuits that control ALAS1 gene transcription. Under conditions of equal ALAS1-transcription pressure, the degree of post-translational induction of the enzyme

elicited by a trigger consequently becomes more important in VP than in AIP for setting up a heavy enough substrate load on PBGD to cause accumulation of porphyrin precursors and emergence of symptoms of acute porphyria. The mechanism of ALAS1-superinduction, such as e.g. takes place after exposure to a CYP-inducing drug with accompanying drain of the hepatic regulatory heme-pool, would therfore be of higher relative importance in VP as compared to AIP.

To conclude, these and other population-based studies validate the genomic model for the precipitating mechanisms in acute porphyrias but give only few clues to the relative importances of the different circuits engaged.

Modulating interferences

Stress and fasting

As discussed, various forms of physical, traumatic, mental or psychosocial strain affects ALAS1-transcription via the CAR/PXR-pathway, by stimulation of the HPA-axis, by liberation of GH or by activation of the stress-responsive Ral-Jnk system for FOXO1 enhanced by cytokines and nitous oxide produced in infection and inflammation. Under stress the FOXO1-pathway may be at least partly open not only in fasting, but the nutritional state of the individual is probably still one of the one most important factors controlling ALAS1-transcription, e.g. weight reduction by reduced caloric intake, irregular and insufficient meals, or strenous excercise without intake of food strongly enhancing porphyrogenic effects of other agents.

Thus, during caloric deprivation the HPA-axis is permanently stimulated dependent on liberation of ghrelin and due to abolished inhibitory action of leptin, and the glucagon-stimulated PGC-1alpha pathways to direct as well as CAR/PXR-mediated ALAS1-induction are active as well. Furthermore, fasting gives rise to pituitary liberation of growth hormone releasing hormone, which by maintaining a GH-pulse stimulates HNF4 co-activating PXR/CAR. Food restriction may also counteract insulin-dependent inhibition of FOXO1 activated in infection or in answer to various forms of stress.

Glucose

All the genomic circuits that co-activate CAR/PXR are also active in maintaining energy homeostasis by initiating hepatic generation of glucose, while the activated CAR/PXR-axis in itself rather inhibits such glucose generation. Gluconeogenic activity is thus found to be repressed under treatment with certain drugs, while induction of ALAS1 by drugs is known to be inhibited by insulin (Kodama *et al.* 2004). The relatively uncommon occurrence, in carriers of acute porphyria, of a clinical reaction to potentially ALAS1-inducing agents may be partly understood via this mechanism.

Glucose is an effective posttranslational ALAS1 inhibitor and is in addition a central repressor of ALAS1-transcription via insulin-mediated interruption of FOXO-signaling, by way of inhibition of ghrelin-release, via transcription of HPA-inhibiting leptin and by inhibiting GH-release induced in fasting. It is generated on the activation of gluconeogenic enzymes that takes place via activation of glucagon-dependent PGC-1alpha circuits, by GH-pulse triggered HNF4-activity supported by PGC-1alpha, by the action of the gluconeogenic stress hormone cortisol, and by FOXO1 activated by the stress-responsive Jnk system. Thus, triggering of each one of the four CAR/PXR co-activating genomic circuits is accompanied by generation of glucose with a potentially dampening effect on ALAS1-transcription. A balance between the two coupled, but counteracting courses of events, which under most circumstances, in most carriers of acute porphyria and most of the time, seems to favour the anti-porphyrogenic gluconeogenic mechanism, would explain the relatively uncommon occurrence of the acute porphyric attack. When the anti-gluconeogenic CAR/PXR axis is primarily activated by sex hormone or a xenobiotic ligand of potent enough impact, the hepatic production of glucose triggered by co-activating circuits will be less able to quench a significant ALAS-transcriptional response. The dominance of gonadal influences and of alcohol and drugs among clinical inducers of the acute porphyric attack would point in this direction. The fact that also agents acting only via the circuits ouside the CAR/PXR-axis sometimes trigger porphyrogenic induction of ALAS, implies that a full effect of gluconeogenic damping may be more or less temporary, possibly due to the insulin response elicited that represses further hepatic production of glucose and is able to block only the FOXO-pathway to co-activation of CAR/PXR.

Insulin

Where Jnk-activating stressors are operative, or where infection and inflammation generates proinflammatory cytokines and nitric oxide able to

activate these kinases that induce nuclear import and activation of FOXO, insulin may help counteract the process by inactivating and exporting FOXO for subsequent extrcellular destruction. A FOXO-shuttle overbalanced to the intranuclear compartment of the liver cell, may be a more common mechanism for maintaining ALAS1-transcription than previously recognized, as suggested from the observation (Andersson *et al.* 1999) that diabetes mellitus may be beneficial for individuals with frequent clinical manifestations of AIP.

Gonadal influence

There is a strong gonadal influence on morbidity in acute porphyria, women being about twice as frequently symptomatic as men (e.g. Waldenström 1937, Kauppinen and Mustajoki 1992, Andersson *et al.* 2003, Hift and Meissner 2005). As discussed above the difference is more pronounced in AIP than in VP. Several factors may contribute to the difference between the sexes with regard to porphyric susceptibility. The liver, which is the main organ effecting a porphyrogenic response to ALAS1-inducing stimuli, is a sexually dimorphic organ in many species including man (Wiwi and Waxman 2004). The impact of this fact on gene expression is mainly dictated by the temporal pattern of the GH excretion, which is intermittent and highly pulsative in males, while stimulated by estrogens and more constant in females, who in fertil age have a GH-production three times that of men. There is a sexual dimorphism also within the central cholinergic systems, affecting vasopressin and its effect of the activity of the HPA-axis, the control being more responsive to stress and other stimuli in women than men (Rhodes and Rubin 1999). Some differences noted between women and men are dependent on the nature of the stressor, but the responses from the sympathicoadrenal system as well as from the HPA–system show marked and consistent sex differences. The HPA-axis is subject to strong gonadal influence, but also is target tissue sensitivity dependent on the age and sex hormone status of the individual (Rogleder *et al.* 2003, Swaab et a l2005, Kajantie and Philips 2006). There is a direct inhibitory action of androgens on CRH expression, mediated by a potential androgen-responsive element in the human CRH promotor (Bao *et al.* 2006). In women the responsivness changes with the phase of the menstrual cycle, menopausal status and preganancy being strong determinants. There is no difference in basal cortisol levels in pre-early pubertal girls and boys, but they are higher in mid-postpubertal girls as compared to mid-pubertal boys (Netherton *et al.* 2004). In young males there is heightened hypothalamic drive that decreases with age, while young females exhibit a greater adrenal cortex sensitivity to ACTH (Kudielka *et al.* 2004, Broadbear *et al.* 2005). During the fertile years women usually show lower HPA- and SA- response than men of the same age, but there are significant differences in HPA-circuitry activity during their hormonal cycle with attenuation during ovulation and augmentation of activity during early follocular phase (Kajantie and Philips 2006, Goldstein *et al.* 2005). In pregnancy the HPA-and SA-responsiveness to stress is attenuated, probably as a result of estrogen exposure, but in the case of the HPA-cortisol system probably also due to modulations by vasopressin and CPG activity. The relationship between the level of percieved stress and physiological response is stronger for women than men (Goldman *et al.* 2005), but under induced hypogonadal conditions, cortisol response to excercise are greater in men than in women (Roca *et al.* 2005). Several diseases associated with HPA-axis hyperactivity and strong cortisol outflow, including depression and neurodegeneration, are more common in women than men (Swaab *et al.* 2005).

Mutations and gene duplications

Mutations or duplications of genes programming for factors in control of modulators of PXR/CAR-activity may explain differences in individual porphyric susceptibility. Effects on ALAS1-transcription by such types of change in gene activity are suggested e.g. from observations of augmented transcriptional activity of FOXO proteins mutated at Akt-phosphorylated sites (Matsusaki *et al.* 2005), from mutations within the ob gene effecting leptin transcription and HPA-axis activity (Giovambattista *et al.* 2000), from the effect of mutations in the c/EBPalpha binding-sites, and conceivably several others within the genomic network controlling ALAS1-transcription.

As touched upon above (“Modulation of PXR/CAR transactivation”) the transcription factor STAT5 belongs to a small family of factors with dual functions, effecting signal transductions as well as gene transcriptions. An interesting fact is that this co-activator of CAR/PXR-induced ALAS1-transcription contributes to the survival and proliferation of malignant cells (see e.g. Wittig and Groner 2005). Hepatic over-expression of the gene for STAT5 would thus make way not only for over-morbidity in acute porphyria but also for

development of primary hepatocellular cancer, i.e. two phenomenons coupled in acute intermittent porphyria (e.g. Andersson *et al.* 1995).

Bile acid biosynthesis

The PXR/CAR nuclear receptors are active in the GH-controlled HNF4-mediated transcription of ALAS1 and of apoCYP genes for production of oxidative enzymes taking part in bile acid biosynthesis, i.e. the apoCYPs 7A1 and 8B1. In this role the activity of PXR/CAR is repressed in a feed-back way by bile acid precursors acting on the farnesoid nuclear receptor (FXR), which activates transcription of the CAR/PXR co-repressor small heterodimerization partner (SHP). The potency of HNF-4 is enhanced by PGC-1alpha, but PXR interferes with the gluconeogenic and bile-acid mediated signaling by targeting the common co-activator PGC1alpha, dissociating it from its complex with HNF4.

Clinical rationales

Recognition of the specific nuclear pathway or pathways engaged in an attack of acute porphyria may provides basis for selection of specific means to terminate the ALAS1 inductive process. In cases where the intensified transcription of the ALAS1 gene has been initiated directly via activation of the PXR/CAR system, careful interrogation of the patient will usually disclose exposure to a potent ligand to these NRs. Often the attack relates to recent anesthesia or current porphyrogenic medication, perhaps in connection with infection, to recent exposure to alcohol, to exposure to organic solvents in e.g. painting or cleaning, or to use of biocide for extermination of weeds or insects. The obvious action to take is to dicontinuate the exposure.

Also where the attack is enhanced by HPA-axis hyperactivity, the patient in most cases is able provide the clue to the pathogenesis. It is often found in a demanding life-style with psychosocial stress, sleep-deficit, irregular and insufficient caloric intake, high doses caffeine and nicotine and chronic alcohol intake. In woman the condition may have been precipitated by extra HPA-axis strain from currently fluctuating sex hormone levels, and in both sexes by acute or chronic physical stress, typically in connection with performance or training for long-distance athletics. Hyperactivity of the HPA-axis can be assessed by analysis of salivary cortisol. Specific pharmacological intervention would be theoretically possible by use of drugs that attenuate HPA-axis signaling.

The FOXO- and PGC-1alpha pathways to ALAS1 induction are activated under conditions where insufficient caloric intake, closes down insulin-signaling in favour of glucagon. The patient may have had major surgery or X-ray necessating fasting, or has attempted weight reduction by way of caloric restriction. In specific cases the question of anorexia may be actualized. Other causes for induction of a ketogenic energy metabolism should also be considered, e.g. strenous excercise without nutrition or diabetes mellitus out of control.

The often unavoidable porphyrogenic risk associated with infection or neccessary ALAS1-inducing medication are lessened by regular meals, sufficient sleep and avoidance of alcohol and other psychostimulants. In acute situations, parenteral or peroral administration of glucose, in severe cases preferentially supported by insulin, will rapidly block most pathways to ALAS1-induction, transcriptional as well as post-translational. Adhrence to illness-preventive life-style regimens is especially important for women in fertile years, during pregnancy or the post partum period, but every gene carrier for acute porphyria should be informed on the activating mechanisms, exposures to avoid and the possibility for self-medication by intake of carbohydrate, such as e.g. provided by the Swedish Porphyria Association (Thunell *et al.* 2005), the European Porphyria Initiative (www.porphyria-europe.org), or the American Porphyria Foundation (www.apf.org).

The pathogenic model proposed for acute porphyria may, in combination with pharamcokinetic data, provide a basis for prediction of drug porphyrogenicity in individual cases. The capacity of the substance in question for ALAS1-induction will thus be a function of of its CAR/PXR-activating capacity and of its extent of access to the hepatocyte nuclear compartment, while the susceptibility of the porphyria gene carrier to its action largely will depend on his or her current activity of co-activators to CAR/PXR and their modulators. Work is in progress to exploit this potential of the model.

Abbreviations

ACH	acetylcholine
ACTH	adrenocorticotropin
ADH	antidiuretic hormone
ADP	ALAD-deficieny porphyria
ADRES	aminolevulinic acid synthase drug-responsive enhancer sequence

AF-domain	activation-function domain
Ah-receptor	the aromatic hydrocarbon receptor
AIP	acute intermittent porphyria
ALA	5-aminolevulinic acid synthase
ALAD	5-aminolevulinic acid dehydratase
ALAS1	ubiquitous 5-aminolevulinate synthase
11beta-HSD	11beta-hydroxysteroid-dehydrogenase
CAR	constitutively active receptor
CBG	cortisol-binding globulin
CBP	histone acyltransferase cAMP response element-binding protein (CREB)-binding protein
CCRP	cytoplasmic CAR retention protein
C/EBPalpha	CCAAT/enhancer binding protein alpha
CPGO	coproporphyrinogen oxidase
CREB	cAMP response element-binding protein
CRH	corticotropin-releasing hormone
CYP	cytochrome P450
DR	direct repeat
eNOS	endothelial nitrogen oxide synthase
FOXO	Forkhead box class O
FXR	farnesoid xenobiotic receptor
GABA	gamma amino butyric acid
GH	growth hormone
GHRH	growth hormone releasing hormone
Glc6P	glucose-6-phosphatase
GR	glucocorticoid receptor
GREs	glucocorticoid response-elements
GSK-3	glycogen synthase kinase 3
HCC	hepatocellular cancer
HCP	hereditary coproporphyria
HNF	hepatocyte nuclear factor
HPA axis	hypothalamic-pituary-adrenal axis
5HT	5-hydroxytryptamine
IL	interleukin
IRE	insulin-responsive element
Jnk	Jun-N-terminal kinases
MnSOD	Mn superoxide dismutase
NE	norepinephrine
nGREs	negative GREs
NLS	nuclear localisation sequence
NOS	nitric oxide synthase
NR	nuclear receptor
NRF1	nuclear respiratory factor-1
PBGD	porphobilinogen deaminase
PBREM	phenobarbital response element
p300	NAD-dependent histone deacetylase silent information regulator 2
PEPCK	phosphoenolpyruvate carboxykinase
PGC-1alpha	proliferator-activated receptor gamma co-activator-1 alpha
PKB/Akt	phosphatidyl inositol 3-kinase-protein kinase B
PPAR	peroxisome proliferator activated family of receptor
PPGO	protoporphyrinogen oxidase
PXR	pregnane xenobiotic receptor
RAC3	p160 co-activator
RAL	Ras like
RXR	9-cis retinoic acid xenobiotic receptor
SHP	small heterodimer partner
SMRT	silencing mediator of retinoid and thyroid hormone receptor
SRC1	steroid receptor co-activator protein-1
STAT 5	signal transducer and activator of transcription 5
TIRE	thymine-rich insulin response element
VP	variegate porphyria
XRS	xenobiotic response signal

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References

ANDERSSON C, BJERSING G, LITHNER F. The epidemiology of hepatocellular carcinoma in patients with acute intermittent porphyria. *J Intern Med* **240**: 195-201, 1996.

ANDERSSON C, BYLESJÖ I, LITHNER F. Effects of diabetes mellitus on patients with acute intermittent porphyria. *J Int Med* **245:** 193-197, 1999.

ANDERSSON C, HARPER P: *Porfyri.* In: Internmedicin. ASPLUND K, BERGLUND G, LINDGREN S, LINDBLOM N (eds), Liber AB Stockholm 2002, 9, pp 547-556.

ANDERSSON C, INNALA A, BÄCKSTRÖM T: Acute intermittent porphyria in women: clinical expression, use and experience of exogenous sex hormones. A population-based study in northern Sweden. *J Int Med* **254**: 176-183, 2003.

BARTHEL A, SCHMOLL D, UNTERMAN TG: FoxO proteins in insulin action and metabolism. *Trends Endocrinol Metab* **16**: 183-189, 2005.

BAO AM, FISCHER DF, WU YH, HOLE M, BALESAR R, UNMEHOPA UA, ZHOU JN, SWAAB DF: A direct androgenic involvement in the expression of human corticotropin-releasing hormone. *Mol Psychiatry* Jan 31 (Epub ahead of print), 2006.

BHALLA S, OZALP C, FANG S, XIANG L, KEMPER JK: Ligand-activated pregane X receptor interferes with HNF4 signaling by targeting a common coactivator PGC-1alpha. Functional implications in hepatic cholesterol metabolism. *J Biol Chem* **279**: 45139-45147, 2004.

BONKOVSKY HL, CABLE EE, CABLE JW, DONOHUE SE, WHITE EC, LAMBRECHT RW, SRIVASTAVA KK, ARNOLD WN: Porphyrogenic properties of terpenes camphor, pinene, and thujone (with a note on historic implications for absinth and the illness of van Gogh). *Biochem Pharmacol* **43**: 2359-2368, 1992.

BROADBEAR JH, PIERCE BN, CLARKE IJ, CANNY BJ: Role of sex and sex steroids in mediating pituary-adrenal responses to acute buspirone treatment in sheep. *J Endoneuroendocrinol* **17**: 804-810, 2005.

BUGAJSKI J, GADEK-MICHALSKA A, BUGAJSKI AJ: Nitric oxide and prostaglandin systems in the stimulation of hypothalamic-pituary-adrenal axis by neurotransmittors and neurohormones. *J Physiol Pharmacol* **55**: 679-703, 2004.

DE KLOET CS, VERMETTEN E, GEUZE E, LAVELARS A, HEIJNEN CJ, WESTENBERG HG: Assessment of HPA-axis function in postraumatic stress disorder: Pharmalogical and non-pharmacological challenge tests, a review. *J Psychiatr Res* (Epub ahead of print), 2005.

DE SIERVI A, ROSETTI MV, PARERA VE, MENDEZ M, VARELA LS, DEL C, BATLE AM: Acute intermittent porphyria: biochemical and clinical analysis in the Argentinean population. *Clin Chim Acta* **288**: 63-71, 1999.

DOSTERT A, HEINZEL T. Negative glucocorticoid receptor response elements and their role in glucocorticoid action. *Curr Pharm Des* **10**: 2807-2816, 2004.

DREW PD, ADES IZ: Regulation of the stability of chicken embryo liver delta-aminolevulinate synthase mRNA by hemin. *Biochem Biophys Res Commun* **162**: 102-107, 1989.

DURET-COSYNS S, DURET RL: Étude psychiatrique de la porphyrie essentielle. *Ann Med Psychol* **2**: 193-211, 1959.

DUSSAULT I, FORMAN BR. The nuclear receptor PXR: a master regulator of "homeland" defence. *Crit Rev Eucaryot Gene Expr* **12**: 53-64, 2002.

EDWARDS DP: Coregulatory proteins in nuclear hormone receptor action. *Vitam Horm* **55**: 165-218, 1999.

EILENBERG MD, SCOBIE BA: Prolonged neuropsychiatric disability and cardiomyopathy in acute intermittent porphyria. *The Lancet* **1**: 858, 1969.

ELENKOV IJ, CHROUSOS GP: Stress hormones, proinflammatory and antiinflammatory cytoines, and autoimmunity. *Ann N Y Sci* **966**: 290-303, 2002.

ELORANTA JJ, KULLAK-UBLIK GA: Coordinate transcriptional regulation of bile acid homeostasis and drug metabolism. *Arch Biochem Biophys* **433**: 397-412, 2005.

ESSERS MA, WEIJZEN S, DE VRIES-SMITS AM, SAARLOOS I, DE RUITER ND, BOS JL, BURGERING BM: FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J* **23**: 4802-4812, 2004.

FEIG LA: Ral-GTPases: approaching their 15 minutes of fame. *Trends Cell Biol* **13**: 419-425, 2003.

FERGUSON SS, CHEN Y, LECLUYSE EL, NEGISHI M, GOLDSTEIN JA: Human CYP2C8 is transcriptionally regulated by the nuclear receptors constitutive androstane receptor, pregnane X receptor, glucocorticoid receptor and hepatic nuclear factor 4alpha. *Mol Pharmacol* **68**: 747-757, 2005.

FICHTER MM, PIRKE KM. Effect of experimental and pathological weight loss upon the hypothalamo-pituary-adrenal axis. *Psychoneuroendocrinology* **11**: 295-305, 1986.

FINLAY D, PATEL S, DICKINSON LM, SHPIRO N, MARQUES R, RHODES CJ, SUHERLAND C: Glycogen synthase kinase-3 regulates IGFBP-1 gene transcription through the thymine rich insulin response element. *BMC Mol Biol* **5**: 15, 2004.

FRESCAS D, VELENTI L, ACCILLI D: Nuclear trapping of the forkhead transcription factor FOXO 1 via Sirt-dependent deacetylation promotes expression of glucogenetic genes. *J Biol Chem* **280**: 20589-20595, 2005.

FLODERUS Y, SHOOLINGIN–JORDAN P, HARPER P. Acute intermittent porphyria in Sweden. Molecular, functional and clinical consequences of some new mutations found in the porphobilinogen deminase gene. *Clin Genet* **62**: 288-297, 2002.

FORMAN BM, TZAMELI I, CHOI HS, CHEN J, SIMHA D, SEOL W, EVANS RM, MOORE DD: Androstane metabolites bind to and deacivate the nuclear receptor CAR-beta. *Nature* **395**: 612-615, 1998.

FUR U. Induction of drug-metabolizing enzymes: pharmacokinetic and toxcological consequenses in humans. *Clin Pharmacokinet* **38**: 493-504, 2000.

GADEK-MICHALSKA A, BUGAJSKI J: Nitric oxide mediates interleukin-1beta- and nicotine-induced hypothalamic-pituary-adrenocortical respose in social stress. *J Physiol Pharamcol* **56**: 491-503, 2005.

GIONIS D, ILIAS I, MOUSTAKI M, MANTZOS E, PAPADATOS I, KOUTRAS DA, MASTORAKOS G: Hypothalamic-pituary-adrenal axis and interleukin-6 activity in children with head trauma and syndrome of inappropriate secretion of antidiuretic hormone. *J Pediatr Endocrinol Metab* **16**: 49-54, 2003.

GIANOULAKIS C, DAI X, BROWN T,. Effect of chronic alcohol consumption on the activity of the hypothalamic-adrenal axis and pituary beta-endorphin as a function of alcohol intake, age and gender. *Alcohol Clin Res* **27**: 410-423, 2003.

GIOVAMBATTISTA A, CHISARI AN, GAILLARD RC, SPINEDI E: Food intake-induced secretion modulates hypothalamo-pituary-adrenal axis response and hypothalamic Ob-Rb expressiion to insulin administration. *Neuroendocrinology* **72**: 341-349, 2000.

GOLDMAN N, GLEI DA, SEPLAKI C, LIU IW, WEINSTEIN M: Percieved stress and physiological dysregulation in older adults. *Stress* **8**: 95-105, 2005.

GOLDSTEIN JM, JERRAM M, POLDRACK R, AHERN T, KENNEDY DN, SEIDMAN LJ, MAKRIS N: Hormonal cycle modulates arousal circuity in women using functional magnetic resonance imaging. *J Neurosci* **25**: 9309-9316, 2005.

GRANICK S: The induction in vitro of the synthesis of delta-aminolevulinic acid synthetase in chemical porphyria: a response to certain drugs, sex hormones and foreign chemicals. *J Biol Chem* **241**: 1359-1375, 1966.

GUENGERICH FP: Human cytochrome enzymes. In: *Cytochrome P450: Structure, Mechanism and Biochemistry.* (Second edition). PR ORTITZ DE MONANELLO (ed). Plenum Press NY. 1995, pp 473-509.

HAMILTON JW, BEMENT WJ, SINCLAIR PR, SINCLAIR JP, ALCEDO JA, WETTERHAHN KE: Heme regulates hepatic 5-aminolevulinate synthase mRNA expression by decreasing mRNA half life and not by altering its rate of transcription. *Arch Biochem Biophys Res Commun* **289**: 387-392, 1991.

HANDCHIN C, LIN J, RHEE J. PEYER A-K, CHIN S, WU P-H, MEYER UA, SPIEGELMAN BM: Nutritional regulation of hepatic heme biosynthesis and porphyria through PGC-1alpha. *Cell* **122**: 505-515, 2005.

HANKINSON O: The aryl hydrocarbon receptor complex. *Annu Rev Pharmacol Toxicol* **35**: 307-340, 1995.

HELMREICH DL, PARFITT DB, LU XY, AKIL H, WATSON SJ: Relation between the hypothalamic-pituary-thyroid axis and the hypothalamic-pituary-adrenal (HPA) axis during repeated stress. *Neuroendocrinology* **81**: 183-192, 2005.

HERMAN JP, PREWITT CM, CULLINAN WE: Neuronal circuit regulation of the hypothalamo-pituary-adrenocortical axis. *Crit Rev Neurobiol* **10**: 371-94, 2005.

HERMAN JP, TASKER JG, ZIEGLER DR, CULLINAN WE: Local circuit regulation of paraventricular stress integration: glutamate-GABA connections. *Pharmacol Biochem Behav* **71**: 457-468, 2005.

HIFT R, MEISSNER P: An analysis of 112 acute porphyric attacks in Cape Town, South Africa. *Medicine* **84**: 48-60, 2005.

HIFT RJ, MEISSNER PM, SUTHERLAND J, KIRCH RE:. Relative severity of acute intermittent porphyria and variegate porphyria; a four year experience with the acute attack in Cape Town. *Neth J Med* **42**: 42-48, 1993.

HONKAKOSKI P, SUEIOSHI T, NEGISHI M: Drug activated nuclear receptors CAR and PXR. *Ann Med* **35**: 172-182, 2003.

ISING M, KUNZEL HE, BINDER EB, NICKEL T, MODELL S, HOLSBOER F: The combined dexamethasone/CRH test as a potential surrogate marker in depression. Prog Neuropsychopharmacol *Biol Psychiatry* **29**: 1085-1093, 2005.

JOHNSON DR, LI CW, GHOCH JC, CHEN LY, CHEN JD: Regulation and binding of pregnane X receptor by nuclear receptor corepressor SMRT. *Mol Pharmacol* Oct 11, (Epub ahead of print), 2005.

KAJANTIE E, PHILIPS DI: The effects of sex and hormonal status of the physiological response to acute psychosocial stress. *Psychoneuroendocrinology* **31**: 151-178, 2006.

KAPPAS A, SASSA S, ANDERSON KE: The Porphyrias. In*: The Metabolic Basis of Inherited Disease* (Fifth Edidition). STANBURY JB, WYNGAARDEN JB, FREDRICKSON DS, GOLDSTEIN JL, BROWN MS (eds), McGraw-Hill Book Company, New York, 1983, pp 1301-1384.

KAUPPINEN R: Porphyrias. *The Lancet* **365**: 241-252, 2005.

KAUPPINEN R, MUSTAJOKI P: Prognosis of acute porphyria: occurrence of acute attacks, preciptating factors, and associated diseases. *Medicine* (Baltimore) **71**: 1-13, 1992.

KLIEWER SA, LEHMAN JR, WILLSON TM: Signal transduction review. Orphan nuclear receptors: Shifting endocrinology into reverse. *Science* **284**: 757-760, 1999.

KODAMA S, KOIKE C, NEGISHI M, YAMAMOTO Y: Nuclear receptors CAR and PXR cross-talk with FOXO1 to regulate genes that encode drug-metabolizing and gluconeogenic enzymes. *Mol Cell Biol* **24**: 7931-7940, 2004.

KOLLURI S, SADLON TJ, MAY BK, BONKOVSKY HL: Haem repression of the housekeeping 5-aminolevulinic acid synthase gene in the hepatoma cell line LMH. *Biochem J* **15**: 173-180, 2005.

KUDIELKA BM, BUSKE-KIRCHBAUM A, HELLHAMMER DH, KIRCHBAUM C: HPA-axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. *Psychoneuroendocrinology* **29**: 83-98, 2004,

LAHUNA O, RASTEGAR M, MAITER D, THISSEN JP, LEMAIGRE FP, ROSSEAU GG: Involvement of STAT5 (signal transducer and activator of transcription 5) and HNF-4 (hepatocyte nuclear factor 4) in the transcriptional control of the hnf6 gene by growth hormone. *Mol Endocrionol* **14**: 285-294, 2000.

MASTORAKOS G, WEBER JS, MAGIAKOU MA, GUNN H, CHROUSOS GP: Hypothalamic-pituary-adrenal axis activation and stimulation of systemic vasopressin secretion by recombinant interleukin-6 in humans: potential implications for the syndrome of inappropriate vasopressin secretion. *J Endocrinol Metab* **79**: 932-933, 1994.

MATSUZAKI H, DAITOKU H, HATTA M, AOYAMA H, YOSHIMOCHI K, FUKAMIZO A: Acetylation of FOXO 1 alters its DNA-binding ability and sensitivity to phosphorylation. *Proc Natl Acad Sci USA* **102**: 11278-11283, 2005.

MATSUZAKI H, ICHINO A, HAYASHI T, YAMAMOTO T, KIKKAWA U: Regulation of intracellular localization and transcriptional activity of FOXO4 by protein kinase B through phosphorylation at the motif sites conserved among the FOXO family. *J Bichem (Tokyo)* **138**: 485-491, 2005.

MAY BK, DOGRA SC, SADLON TJ, BHASKER CR, COX TC, BOTTOMLY SS: Molecular regulation of heme biosynthesis in higher vertebrates. *Prog Nucleic Acids Res Mol Biol* **51**: 1-51, 1995.

MEYER UA, SCHURMANS MM, LINDBERG RL: Acute porphyria: pathogenesis of neurological manifestations. *Semin Liver Dis* **18**: 43-52, 1998.

MCEWEN BS: The neurobiology of stress: from serendipidy to clinical relevance. *Brain Res* **886**: 172-189, 2000.

McGILLON FB, THOMPSON GG, GOLDBERG A: Tissue uptake of delta-aminolevulinic acid. *Biochem Pharmacol* **24**: 299-301, 1975.

MITTAR D, SEHAJPAL PK, LANDER HM: Nitric oxide activates Rap1 and Ral in Ras-independent manner. *Biochem Biophys Res Commun* **10**: 203-209, 2004.

MOORE MR, HIFT RJ: Drugs in the acute porphyrias – toxicogenetic diseases. *Cell Mol Biol (Noicy-le-grand*) **43**: 89-94, 1997.

MOORE MR, MCCOLL KE, RIMMINGTON C, GOLDBERG A: Pathogenesis of acute porphyria. In: *Disorders of porphyrin metabolism*. Plenum Medical Book Company, New York and London 1987, pp 119-137.

NETHERTON C, GOODYER I, TAMPLIN A, HERBERT J: Salivary cortisol and dehydroepiandrosterone in relation to pubertty and gender. *Psychoneuroendocrinology* **29**: 125-140, 2004.

OBSILOVA VC, VECER J, HERMAN P, PABIANOVA A, SULC M, TESINGER J, BOURA E, OBSIL T: 14-3-3 protein interacts with nuclear localization sequence of forkhead transcription factor FoxO4, *Biochemistry* **30**: 11608-11617, 2005.

ORULIN JC, LASSERRE F, PINEAU T, FABRE JM, SA-CUNHA A, MAUREL P, VILAREM MJ, PASCUSSI JM: The small heterodimer partner interacts with the pregnane X receptor and represses its transcriptional activity. *Mol Endocrinol* **17**: 1693-1703, 2003.

PASCUSSI JM, DROCOURT L, FABRE JM, MAUREL P, VILAREM MJ: Dexamethasone induces pregnane X receptor and retinoid X receptor-alpha in human hepatocytes: synergistic increase in CYP3A4 induction by pregnane X receptor activators. *Mol Pharmacol* **58**: 361-372, 2000.

PASCUSSI JM, DROUCOURT L, GERBAL-CHALOIN S, FABRE JM, MAUREL P, VILAREM MJ: Dual effect of dexamehasone on CYP3A4 gene expression in human hepatocytes. Sequentiual role of glucocorticoid receptor and pregane X receptor. *Eur J Biochem* **268**: 6346-6358, 2001.

PASCUSSI JM, GERBAL-CHALOIN S, DROCOURT, MAUREL P, VILREM MJ: The expression of CYP2B6, CYP2C9 and CYP3A4 genes: a tangle of networks of nuclear and steroid receptors. *Biochim Biophys Acta* **17**: 243-253, 2003.

PATZ MD, DAY HE, BUROW A, CAMPEAU S: Modulation of the hypothalamo-pituary-adrenocortical axis by caffeine. *Psychoneuroendocrinology* Dec 31 (Epub ahead of print), 2005.

PELKONEN O, BREIMER DD: Role of environmental factors in the pharmacokinetics of drugs: Considerations with respect to animal models, P450 enzymes, and probe drugs. In: *Handbook of Experimental Pharmacology*. WELLING PG and BALANTALANT LP (eds). 1994, pp 289-332.

PELKONEN O, MÄENPÄÄ J, TAAVITSAINEN P, RAUTIO A, RAUNIO H: Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotica* **28**: 1203-1253, 1998.

PELKONEN O, RAUTIO A, RAUNIO H, PASANEN M: CYP 2A6: a human coumarin 7-hydroxylase. *Toxicology* **144**: 139-147, 2000.

PERROT V, RECHLER MM: The coactivator p300 directly acetylates the forkhead transcription factor Foxo1 and stimulates FOXO 1-induced transcription. *Mol Endocrinol* **19**: 2283-2298, 2005.

PODOVINEC M, HANDSCHIN C, LOOSER R, MEYER UA: Identification of the xenosensors regulating human 5-aminolevulinate synthase. *Proc Nat Acad Sci* **101**: 9127-9132, 2004.

RHODES ME, RUBIN RT: Functional sex differences (´sexual diergism´) of central nervous system cholinergic systems, vasopressin, and hypothalamic-pituary-adrenal axis activity in mammals: a selective review. *Brain Res Brain Res Rev* **30**: 135-152, 1999.

ROCA CA, SCHMIDT PJ, DEUSTER PA, DANACEAU MA, ALTEMUS M, PUTNAM K, CHROUSOS GP, NIEMAN LK, RUBINOW DR: Sex-related differences in stimulated hypothalamic-pituitary-adrenal axis during induced gonadal suppression. *J Clin Enocrinol Metab* **90**: 4224-4231, 2005.

RODRIGUES E, VILAREM MJ, RIBEIRO V, MAUREL P, LECHNER MC: Two CCAAT/enhancer binding protein sites in the cytochrome P4593A1 locus. Potential role in the glucocorticoid response. *Eur J Biochem* **270**: 556-564, 2002.

ROHLEDER N, KIRCHBAUM C: The hypothalamic-pituary-adrenal axis in habitual smokers. *Int J Psychophysiol* dec1 (Epub ahead of print), 2005.

ROHLEDER N, WOLF JM, KIRCHBAUM C: Glucocorticoid sensitivity in humans – interindividual differences and acute stress effects. *Stress* **6**: 207-222, 2003.

RUBINS NE, FRIEDMAN JR, LE PP, ZHANG L, BRESTELLI J, KAESTNER KH: Transcriptionl networks in the liver: hepatocyte nuclear factor 6 is largely independent on Foxa2. *Mol Cell Biol* **25**: 7069-7077, 2005.

SAREL I, WIDMAIER EP: Stimulation of steroidogenesis in cultured rat adrenocortical cells by unsaturated fatty acids. *Am J Physiol* **268**; R1484-90, 1995.

SCHEPERS OJ, WICHERS MC, MAES M: Cytokines and major depression. *Prog Neuropsychopharmacol Biol Psychiatry* **29:** 201-217, 2005.

SILVERMAN MN, PEARCE BD, BIRON CA, MILLER AH: Immune modulation of the hypothalamic-pituary-adrenal axis during viral infection. *Viral Immunol* **18**: 41-78, 2005.

SGOIFO A, BUWALDA B, ROOS M, COSTOLI T, MERATI G, MEERLO P: Effects of sleep deprivation on cardiac autonomic and pituary-adrenocortical stress reactivity in rats. *Psychoneuroendocrinology* **31**: 197-208, 2006.

SUEYOSHI T, NEGISHI M: Penobarbital response elements of cytochrome P450 genes and nuclear receptors. *Annu Rev Pharmacol Toxicol* **41**: 123-143, 2001.

SRIVASTAVA, G, BORTHWICK IA, BROOKER JD, WALLACE JC, ELLIOT WH: Hemin inhibits transfer of pre-delta-aminolevulinate synthase into chick embryo liver mitochondria. *Biohem Biophys Res Commun* **117**: 344-349, 1983.

SWAAB DF, BAU AM, LUCASSEN PJ The stress system in the human brain in depression and neurodegeneration. *Ageing Res Rev* **4**: 141-194, 2005.

TENG S, PIQUETTE-MILLER M:. The involvement of the pregane X receptor in hepatic gene regulation during inflammation in mice. *J Pharmacol Exp Ther* **312**: 841-848, 2005.

THUNELL S: Porphyrins, porphyrin metabolism and porphyrias I. *Scand J Clin Lab Invest* **60**: 509-540, 2000.

THUNELL S, FLODERUS Y, HENRICHSON A, MOORE MR, MEISSNER P, SINCLAIR J: Alcoholic beverages i acute porphyria. *J Stud Alcohol* **53**: 272-276, 1992.

THUNELL S, BJERNEVIK K, THUNELL G, HARPER S: *Patients´s and Doctor´s Guide to Acute Porphyria 2005*. Swedish Porphyria Association, Stockholm 2005, pp 1-72.

TIRONA RG, LEE W, LEAKE BF, LAN LB, CLINE CB, LAMBA V, PARVIZ F, DUNCAN SA, INOUE Y, GONZALES FJ, SCHUETZ EG, KIM RB: The orphan nuclear receptor HNF4alpha determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. *Nat Med* **9**: 220-224, 2003.

TSIGOS C, CHROUSOS GP: Hypotlamic-pituary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res* **53**: 865-871, 2002.

VANITALLIE TB:. Stress: a risk factor for serious illness. *Metabolism* **51**: 40-45, 2002.

VOGT PK, JIANG H, AOKI M: Triple layer control: phosphorylation, acetylation and ubiquitination of FOXO proteins. *Cell Cycle* **4**: 908-913, 2005.

VON UND ZU FRAUNBERG M, PISCHIK E, UDD L, KAUPPINEN R: Clinical and biochemical characteristics and genotype-ghenotype correlation in 143 Finnish and Russian patients with acute intermittent porphyria. *Medicine* **84**: 35-47, 2005.

VOSSEN C, ERARD M: Down-regulation of nuclear receptor DNA-binding activity by nitric oxide – HNF4 as a model system. *Med Sci Monit* **8**: RA217-220, 2002.

WALDENSTRÖM J: *Studien über Porphyrie*. *Acta Med Scand* **82** (Suppl): 1-254, 1937.

WANG H, FAUCETTE SR, GILBERT D, JOLLEY SL, SUEYOSHI T, NEGISHI M, LECLUYSE EL: Glucocorticoid receptor enhancement of pregnane X receptor-mediated CYP2B6 regulation in primary human hepatocytes. *Drug Metab Dispos* **31**: 620-630, 2003.

WANG H, LECLUYSE EL: Role of orphan nuclear receptors in the regulation of drug-metabolizing enzymes. *Clin Pharmacokinet* **42**: 1331-1357, 2003.

WANG JC, STAFFORD JM, GRANNER DK: SRC-1 and GRIP coactivate transcription with hepatocyte nuclear factor 4. *J Biol Chem* **273**: 30847-308450, 1998.

WAXMAN D: P450 gene induction by structurally diverse xenochemicals: Central role of nuclear receptors CAR, PXR and PPAR. *Arch Biochem Biophys* **369**: 11-23, 1999.

WINDEBANK AJ, BONKOVSKY HL: Porphyrical neuropathy In: *Periperal neuropathy*. DYCK PJ, THOMAS PK, GRIFFIN JW, LOW PA, PODULSO J. (eds), WB Saunders, Philadelphia 1993, pp 1161-1168.

WITTIG I, GRONER B: Signal transducer and activator of transcription 5 (Stat5), a cruical regulator of immune and cancer cells. *Curr Drug Targets Immune Endocr Metabol Disord* **5**: 449-463, 2005.

WIWI CA, WAXMAN DJ: Role of hepatocyte nuclear factors in growth hormone-regulated, sexually dimorphic expression of liver cytochromes P450. *Growth Factors* **22**: 79-88, 2004.

WOLBOLD R, KLEIN K, BURK O, NUSSLER AK, NEUHAUS P, EICHELBAMUM M, SCHWAB M, ZANGER UM: Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology* **38**: 978-988, 2003.

YOUSHIDA Y, HUGHES DE, RAUSA FM 3RD, KIM IM, TAN Y, DARLINGTON GJ, COSTA RH: C/EBP alpha and HNF6 protein complex stimulates HNF6-dependent transcription by CPB coactivator recruitment in HepG2 cells. *Hepatology* **43**: 276-286, 2006.

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