Pharmacology, Biochemistry and Behavior 93 (2009) 230–236

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Review

Gene–environment interactions resulting in risk alcohol drinking behaviour are mediated by CRF and CRF1

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ARTICLE INFO

Available online 3 May 2009

Keywords:
CRF
CRF1
Stress
Alcoholism
HPA-axis
Norepinephrine
Noradrenaline
Substance abuse

ABSTRACT

Both genetic and environmental influences are known to influence an individuals' vulnerability to the misuse of alcohol. One of the most relevant environmental risk factors for alcoholism is that of stress. This review aims to examine the role of the biological stress systems in the etiology of alcoholism, with a focus on corticotrophin releasing factor (CRF) and its receptor CRF1. CRF is reviewed in the context of the biological stress systems within which it acts such as the HPA-axis, the noradrenergic system and the central and medial amygdale. These systems are examined in more detail by reviewing their genetic and molecular components in both humans and animals. It is concluded from the findings of the studies discussed in this review that CRF has a central role in the modulation of the stress response and that genetic variations in CRF or CRF1 may confer a susceptibility to alcoholism which is, in part, mediated by life stressors. Together these neurobiological, animal and human data suggest a role for CRF when developing treatment modalities for alcoholism alongside a pharmacogenetic approach to identify subtypes of patients which would benefit from these treatments or interventions.

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1. Stress response systems

There are many environmental stressors that we encounter in our everyday lives, from those of occupational stress and family responsibilities to more severe stressors such as traumatic accidents, emergency situations or bereavement. What is common to each of these forms of stress is that they will all, to some degree, result in the activation of our stress response systems. The stress response involves the activation of a number of integrated physiological responses which cause alterations in the autonomic, endocrine, immune, circadian, reproductive and cardiovascular systems which lead to behavioural and homeostatic changes within an organism.

The main endocrine system mediating the stress response is the Hypothalamic–Pituitary–Adrenal (HPA) axis. Activation of the HPA axis leads to an increase in circulating cortisol (Bjorntorp, 2001) which then activates a multitude of physiological reactions to allow the body to cope with stress. Additionally the locus-coeruleus (LC), densely innervated by noradrenergic neurons, has been shown to be activated in response to stress (Korf et al., 1973) which in turn leads to the rapid increase in turnover of norepinephrine (Valentino et al., 1993). Norepinephrine (NE) is a catecholamine which has roles in arousal and alerting functions (Robbins et al., 1998) and is known to

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doi:10.1016/j.pbb.2009.04.014

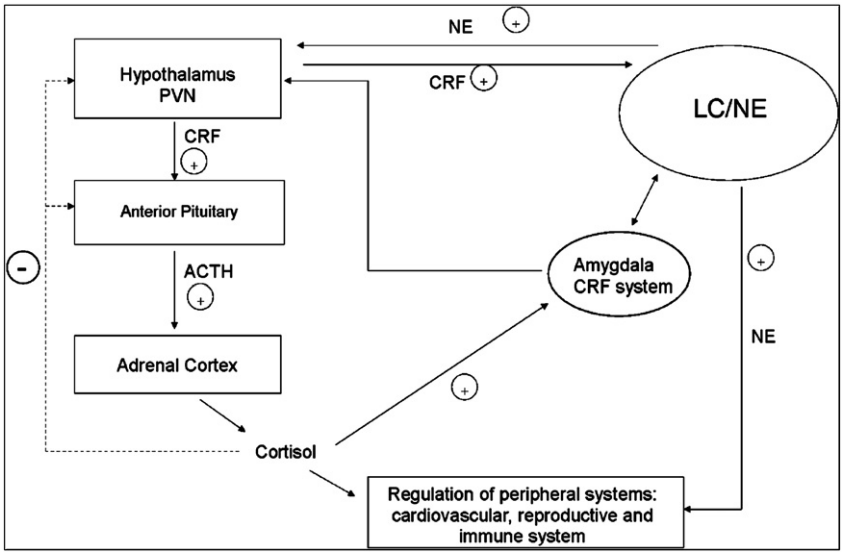

Fig. 1. A schematic diagram of the stress response, comprising the HPA-axis, the LC/NE system, and the extended amygdala CRF system, and their interactions. Full black arrow = activation/interaction, dotted arrow = inhibition.

initiate the peripheral responses to stress such as increasing heart rate, glucose metabolism and the blood flow to skeletal muscle.

Stress activates a number of biological systems in order to appropriate a coordinated physiological and behavioural response. Two important biological stress systems, the HPA-axis and the LC/NE stress response are by no means distinct, and interact to exert their co-ordination of the appropriate behavioural response. Corticotrophin releasing factor (CRF) is also implicated in the release of cortisol during HPA-axis stimulation, has been found to be expressed in the LC (Cummings et al., 1983b) suggesting that it may modulate neuronal activity in this region. CRF-mediated pathways have been shown to link the amygdala and the LC (Curtis et al., 2002b) and LC neurons in turn have been shown to provide noradrenergic stimulation of the PVN leading to HPA-axis activation (Fig. 1) (Commons et al., 2003; Curtis et al., 2002a).

There is evidence to show that there are heritable components to the baseline activity of stress response systems and that genetic polymorphisms in genes which are central to these pathways, will affect the functioning of the stress reaction and result in an altered biological output. Baseline levels of HPA axis activity (commonly measured by blood levels of HPA axis hormones cortisol, adrenocorticotrophin [ACTH] and arginine-vasopressin [AVP]) between individuals have been shown to be highly variable (Wüst et al., 2000). Studies of twin pairs found evidence to show that this variability is partly a result of genetic factors (Linkowski et al., 1993; Meikle et al., 1988). It has also been demonstrated that the response of the HPA-axis to psychosocial stress has a genetic component (Kirschbaum et al., 1992).

Table 1 gives a summary (exemplary rather than exhaustive) of studies which have looked at the genetic basis of differential stress responses. Commonly, the stress response is defined by measuring hormones of the HPA-axis such as cortisol or ACTH after exposure to psychosocial or physiological stressors. Additionally, sympathetic and autonomic stress responses are measured by observing changes in vasodilation, heart rate and blood pressure. It should be noted that the majority of these studies are reporting only the associations of gene polymorphisms and altered stress reactions, with only one demonstrating that the effects seen are a result of a functional polymorphism, as verified by in vitro genetic analysis.

The findings summarised in Table 1 are reflected at the behavioural level: it is readily discerned that individuals cope with stress differently. Aberrant coping mechanisms in response to stress have been linked to a wide range of psychiatric disorders such as depression (Camron, 2006), anxiety (Abelson et al., 2005), post-traumatic stress disorder and importantly for this review, substance abuse.

2. Stress and substance abuse

The differential effects of stress on behaviour are increasingly relevant for addiction. Two environmental factors which have been shown to have a key role in the development and facilitation of alcoholism and substance abuse disorders are those of drug-related cues and stress (Sinha, 2001). Drug-related cues are those which a substance abuser has repeatedly paired with drug taking and therefore come to elicit craving for the drug when presented alone. Following alcohol detoxification and abstinence, relapse into alcoholism is often induced by exposure to stress and cues and this is readily acknowledged by patients and clinicians alike (Sinha, 2001). Studies into alcohol relapse have shown that detoxified inpatients display an attenuated salivary cortisol response upon cue-exposure when compared to social drinkers and that this was associated with early relapse (Junghanns et al., 2005).

Self-reported drug craving has also been linked to increased stress and Sinha et al. found that an increase in stress was associated with a shorter time to cocaine relapse in treatment seeking cocaine dependent individuals (Sinha et al., 2006). They also found that when subjects were grouped into high and low CRF responders (CRF response to stress), the high CRF responders used increased amounts of cocaine per occasion during their 90 day follow up from an inpatient unit. These findings suggest that some specific element of the stress response is implicated in a behavioural component of relapse and that CRF specifically is related to the amount of drug consumed during relapse.

Dysregulation of the noradrenergic stress system has been implicated in substance abuse. Central noradrenergic stimulation is related to alcohol stimulation and both central and peripheral noradrenergic neurons are implicated in withdrawal (Hawley et al., 1985). There is evidence to suggest that Disulfiram, a drug commonly used to treat alcoholism by producing aversive effects upon ethanol ingestion, may be exerting its pharmacological effects via alterations in the noradrenaline system (by inhibiting the synthesis of NE) (Amit et al., 1976). Animal models provide a direct link between stress, NE and alcohol consumption by demonstrating that stress-induced reinstatement of alcohol seeking and self-administration in rats is attenuated by administration of an ADRA2A agonist (Lofexidine), which effectively inhibits NE transmission (Le et al., 2005). Yohimbine, which is an ADRA2A antagonist, has been shown to induce stress

Table 1
Summary of studies examining the genetic modulation of the stress response, focusing on genes of both the noradrenergic stress system and the HPA-axis.

Genes involved in the noradrenergic stress response				
Gene/polymorphism	Role of gene/s	Study design	Biological output	Reference
Tyrosine hydroxylase (TH)/4 SNP haplotype in the promoter region	Rate limiting enzyme in catecholamine biosynthesis	Measured noradrenergic stress response to environmental (cold) stress in 80 individuals from the most extreme diastolic blood pressure percentiles in the population.	Increased urinary catecholamine excretion, as well as blood pressure response to stress wereexcretion, as well as blood pressure response to stress was associated with the promoter haplotype.	Rao et al. (2008)
CYB561, CHGB/Intron 1, Exon 4	Catecholamine synthesis and storage	Vascular responses to tyramine (an indirect presynaptic norepinephrine releaser) injected into dorsal hand veins of 49 normotensive men and women of 5 ethnicities were measured	Both polymorphisms were associated with an increased vascular response to tyramine.	Fung et al. (2008)
ADRA2A, SNP in 3′UTR	ADRA2A involved in the inhibition of NE release.	Coriolis stress, a strong stimulus that induces motion sickness in humans, was measured in 194 healthy individuals	Increased autonomic stress response (as measured by heart rate and blood pressure) was shown to be genotype specific.	Finley et al. (2004)
ADRA2A, ADRB2/ 1291C–>G, Arg16Gly	ADRA2A/ADRB2 are involved both pre and post-synaptically in the regulation of NE release.	A dexamethasone/CRH test examined cortisol and ACTH levels in 189 depressed patients.	Males showed pre-treatment HPA-axis activity dependent on ADRA2A genotype, and females with the ADR2B genotype.	Haefner et al. (2008)

Genes involved in HPA-axis regulation				
Gene	Role of gene/s	Study design	Biological output	Reference
GR/Bcl1 and N363S	Glucocorticoid receptor is involved in regulation of gene expression upon its activation by glucocorticoids, including cortisol.	A dexamethasone test for negative feedback inhibition at the level of the pituitary was carried out.	Polymorphisms found to be associated with increased suppression of cortisol levels in the morning, N363S association supported by in vitro data.	Stevens et al. (2004); Panarelli et al. (1998); van Rossum and Lamberts (2004); Russcher et al. (2005)
GR/Bcl1 and N363S	As above	The Tier Social Stress Test (TSST) was administered with ACTH to induce stress and subsequently, increases in salivary and plasma cortisol were measured.	The N363S group had the highest cortisol responses to TSST and ACTH when compared to wild type. Bcl1 genotypes, however, were still higher than wild type.	Wüst et al. (2004)
MR/I180V	Mineralcorticoid receptor is activated by glucocorticoids and mineralocorticoids.	Using TSST, and ACTH to induce stress subsequent increases in salivary and plasma cortisol were measured.	I180V is associated with an increased salivary and plasma cortisol but also with increased autonomic output as measured by heart rate.	DeRijk et al. (2006)
FKBP5/ rs4713916, rs1360780 and rs3800737	FKBP5 is a co-chaperone protein for the GR, which is necessary for the correct co-localisation and activation of the GR.	The cortisol response to psychosocial stress was analysed in 64 healthy volunteers	MA homozygotes showed insufficient cortisol recovery and increased self reported anxiety	Ising et al. (2008)

and anxiety responses in humans and lab animals. In rats yohimbine administration is accompanied by an increase in operant alcohol self-administration and alcohol reinstatement. However, the effects of yohimbine have been shown to be attenuated by the administration of a CRF1 antagonist, antlarmin (Marinelli et al., 2007), demonstrating that the CRF and NE systems are interacting to influence alcohol drinking during exposure to stress.

The HPA-axis is also known to be dysregulated in response to substance abuse (Kreek et al., 1984) and it has been shown that severely elevated or reduced HPA-axis activity is associated with a greater risk of developing substance abuse in later life (Huizink et al., 2006). Prenatally stressed rats show and increased vulnerability to drug abuse in later life and display and also present a dysregulated HPA-axis (Deminiere et al., 1992). In humans, Sinha et al. (2003) analysed the brain responses to psychosocial stress and drug cues in cocaine addicts and found that both their HPA-axis and noradrenergic pathways were dysregulated when exposed to these environmental stressors, compared to neutral imagery (Sinha et al., 2003).

Zimmermann et al. (2004) showed that dysregulation of the HPA-axis may be partially heritable by studying 29 individuals (prior to the onset of alcohol dependence) with a positive family history of alcoholism (PHA) and 23 individuals with no family history of alcoholism (FHN) (Zimmermann et al., 2004). They were then subjected to psychosocial stress both with and without the administration of alcohol and subsequent HPA axis activity was measured by monitoring ACTH levels. They found that the PHA individuals had a significantly greater response to psychosocial stress when compared to the FHN and this was attenuated by the administration of alcohol. This suggests that there is a genetic component to elevated stress responses and in those genetically predisposed to alcoholism alcohol has a greater 'dampening' effect.

However, exactly how alcohol and stress can cause long term neurochemical changes which lead to the development of an alcoholic state remains to be elucidated. Whilst we know that acute stress causes an increase in circulating cortisol, there are long term deficiencies in HPA-axis activity seen in alcoholics and into protracted abstinence (Junghanns et al., 2007). Interestingly, research in field of epigenetics is beginning to demonstrate how environmental influences exert their effects on gene expression. The term epigenetics refers to long term reprogramming of genes without any change to the nucleotide sequence (Szyf et al., 2007).The mechanisms involved range from DNA methylation (Razin, 1998), histone acetylation status and chromatin remodelling (Henikoff et al., 2004); all of which regulate the accessibility of genes to transcriptional machinery, thus regulating gene expression.

Pandey et al. found that the regulation of histone deacetylase (HDAC) was altered in the amygdala of ethanol exposed rats, with acute ethanol exposure downregulating HDAC activity and thus increasing acetylation, whereas, during withdrawal from ethanol the opposite effect was seen (Pandey et al., 2008). Levels of histone deacetylation affect the chromatin structure of chromosomes and consequently have profound effects on gene expression. It is notable that these modifications were observed in the amygdala, which is the location of the CRF system which projects to the LC. Weaver et al. (2004) showed that early life experience could lead to changes in gene expression that persist into adulthood. They demonstrated that there are methylation differences in a transcription response element (NGFA) in the promoter of the glucorticoid receptor (GR), which are dependent upon exposure to a high licking and grooming (LG) mothers (as compared to low LG mothers) (Weaver et al., 2004), resulting in altered GR gene expression and differential HPA-axis activation in response to stress. The studies by Pandey and Weaver et

al. neatly demonstrate how both alcohol exposure and early life stress can mediate gene expression in brain regions relevant for CRF and alcoholism, resulting in an altered stress response in later life.

3. The role of CRF

CRF is a 41 amino acid peptide which is secreted from the paraventricular nucleus (PVN) in response to stress and triggers the release of ACTH from the anterior pituitary and leads to the eventual increase in peripheral cortisol (Tsigos and Chrousos, 1994). It exerts its effects on cells by the binding of the CRF receptors, CRF1 and CRF2 (Chen et al., 1993; Lovenberg et al., 1995) which are members of the 7 transmembrane G-protein coupled receptor family. CRF1-receptors are found at high levels in the cerebral cortex, cerebellum, amygdala, hippocampus and olfactory bulb (Van et al., 2000). Cortisol mediates positive effects on the CRF system in the amygdala (Makino et al., 2002) whereas it has a negative feedback effect on the CRF neurons in the PVN (Fig. 1). The amygdala CRF system and the PVN CRF system have been shown to be differentially regulated in response to a variety of stressful situations (Makino et al., 1994a; Makino et al., 1994b). CRF has been shown to exhibit anxiogenic properties and is known to be responsible for the co-ordination of the stress response in mouse models (Stenzel-Poore et al., 1992). This is supported by the observations that CRF1 null mice show decreased anxiety like behaviour and display an impaired stress response (Smith et al., 1998). The role of the CRF2 receptor still remains controversial as there is evidence from animal models suggesting CRF2 antagonists have an anxiolytic effect (Takahashi et al., 2001), however CRF2 null mice display an anxiogenic phenotype (Bale et al., 2000).

CRF also exerts its effects outside of the central HPA-axis, and these interactions are also important for co-ordinating the response to stress. Britton et al. showed that the anxiogenic effects of CRF are not inhibited by dexamethasone suppression of the HPA-axis (Britton et al., 1986), and this was confirmed by the observation that hypophysectomised rats still display a behavioural response to CRF (Eaves et al., 1985). Indeed, Valentino et al. were able to show directly that CRF administration in mice increases LC discharge activity and causes the release of NE (Valentino et al., 1991). This finding, together with the evidence that CRF immunoreactive fibers are found projecting into the LC in rats (Cummings et al., 1983a), suggest that CRF has a central role in the stress response. The extended amygdala also contains high amounts of CRF containing nerve terminals (Merchenthaler et al., 1982) and these also project into the LC (Berridge and Waterhouse, 2003). Therefore, CRF appears to link two of the body's main stress response systems, namely the HPA-axis and the LC/NE system. This suggests a central role for CRF in coordinating and controlling many of the different afferents of the biological stress pathways and their role in substance abuse.

4. The role of CRF in alcohol abuse: evidence from animal models

CRF has been shown to be expressed in neuroanatomical regions of the brain which are relevant for substance abuse and reward such as the central amygdala (CeA) (Gray and Bingaman, 1996), the locus coeruleus (LC) (Valentino and Wehby, 1988) and the hypothalamus (Menzaghi et al., 1994). Levels of CRF have been shown to be affected by ethanol, as in animal experiments an immediate sharp increase of (CRF) release was induced by ethanol as an acute response of the HPA axis to alcohol exposure. This increase in CRF is maintained at significantly higher levels for the duration of ethanol exposure and CRF release was found to be returned to basal levels within 10 min following removal of ethanol (de Waele and Gianoulakis, 1993). However, long-term exposure to alcohol may result in some loss of pituitary responsiveness accompanying the increase of CRF release (Sarnyai et al., 2001). In rat, the acute administration of alcohol induces dose-related increases in plasma ACTH levels (Ogilvie et al., 1997), which suggests that alcohol induced release of ACTH is likely to depend ultimately on the presence of endogenous CRF (Rivier et al., 1996).

Alcohol withdrawal can act itself as a stressor and mimic many of the anxiety-like responses induced by CRF administration. Rats maintained on a liquid diet of ethanol for 3 weeks show increased anxiety responses in elevated plus mazes in response to restraint stress when compared to controls. This suggests that alcohol causes a hyperactive response to stress. Interestingly administration of a CRF antagonist suppresses this increased anxiety suggesting that CRF is the mechanistic cause of this behaviour (Valdez et al., 2003). Alcohol withdrawal has also been shown to activate the extra-hypothalamic CRF systems as increased CRF expression is shown in the bed-nucleus striatal terminalis (BNST) and central amygdala (CeA) in dependent rats undergoing withdrawal (Funk et al., 2006; Zorrilla and Koob, 2004).

One animal study, by Hansson et al., attempted to examine the molecular basis for stress induced alcohol drinking by examining the alcohol drinking behaviour of Marchigian–Sardinian Preferring (mSP) rats (genetically selected for high alcohol preference) after exposure to stress (Hansson et al., 2006). It was found that mSP rats have a lower threshold for reinstatement of alcohol seeking behaviour when using foot-shock as a stressor. In order to elucidate the molecular underpinnings of this msP phenotype in situ hybridization was conducted across a panel of 20 stress-related genes in 16 brain regions to look for differential gene expression. A robust up-regulation of the corticotrophin releasing hormone receptor 1 (CRF1) transcript was found on msP rats across several brain regions. Further analysis of the CRF1 gene region found that the up-regulation was probably linked to two marker haplotype encompassing the CRF1 promoter that is unique to the msP rat. That an increase in levels of CRF1 was the driving force of this behaviour was confirmed with the use of a CRF1 antagonist, Antlarmin, which blocked stress-induced reinstatement of alcohol drinking in msP rats, but not in control Wistar rats. Another study by Hansson et al. showed that given two weeks ad lib access to alcohol, msP rats show a down-regulation of CRF1 transcripts in brain regions which are relevant for motivation such as the central and medial amygdala (CeA, MeA) and the nucleus accumbens. This suggests that alcohol is voluntarily consumed in part for its ability to reduce CRF1 levels in these regions (Hansson et al., 2007).

These animal data further confirm the role of CRF in stress-related alcohol drinking as it would appear that the elevation of CRF1 in these rats causes them to be more sensitive to stress-induced alcohol drinking. Genetic polymorphisms in the promoter region of the CRF1 gene are believed to be the reason for the over-expression of the CRF1 transcript, and pharmacologically blocking the CRF1 receptor rescues the hypersensitive stress response in msP rats. It became increasingly apparent then, that CRF has a key role in the facilitation of stress-related alcohol abuse. A more detailed summary of the work conducted on animals to examine the role of CRF in alcohol abuse and stress can be found in Table 2, or as reviewed by Heilig and Koob (Heilig and Koob, 2007).

5. The role of CRF in alcohol abuse: human experimental evidence

This evidence that CRF mediates stress-induced drinking via genetic modulations in the CRF receptors is supported by data linking CRF1 to drinking behaviour in humans. Treutlein et al. studied 14 SNPs across the CRF1 gene and identified two tagging SNPs (htSNPs) which identified between haplotypes with a frequency of $\geq$0.7% (Treutlein et al., 2006). These 2 htSNPs were analysed in a cohort of adolescents with little previous exposure to alcohol and a cohort of alcohol-dependent adults. In the adolescent sample significant genotype differences were found when measuring binge-drinking, lifetime prevalence of alcohol intake and lifetime prevalence of drunkenness. In the adult alcohol dependent sample significant associations with a high amount of alcohol drinking were seen. These were the first data

Table 2
Summary of animal experiments studying the role of the CRF systems in stress and alcohol abuse.

Reference	Stress system affected	Animal model/experimental design	Results
Valentino and Wehby (1988)	CRF system in the locus coruleus (LC)	Discharge rates of neurons in the LC were compared in mice subject to hemodynamic stress and CRF administration.	Hemodynamic stress produced identical effects on LC discharge as CRF, and the effect was blocked by a CRF antagonist, showing that CRF functions in the LC during the initiation of the stress response.
Valdez et al. (2003)	CRF system, non-specific	Examined the role of CRF in protracted abstinence by examining the behaviour of ethanol dependent rats in the elevated plus-maze after subjection to restraint stress.	Dependent rats displayed significantly higher levels of anxiety after restrain stress, and this behaviour was attenuated by a CRF antagonist, suggesting a role for CRF in increased stress vulnerability in alcoholics.
Funk et al. (2006)	CRF in extended amygdala	Ethanol self-administration was compared in ethanol dependent rats compared to non-dependent controls, during withdrawal. This was re-examined after the administration of a CRF antagonist.	Ethanol dependent rats showed an increase in ethanol self-administration compared to controls, and this was attenuated by a CRF antagonist, which had no effect in control rats. This response was specific to the central amygdala (CeA).
Hansson et al. (2006)	CRF system, non-specific	Ethanol consumption in msP rats (selected for high alcohol preference) was measured before and after stress. In situ hybridization across a panel of stress-related genes was carried out to determine the molecular basis for the msP phenotype	It was found that msP rats have a lower threshold for ethanol-induced reinstatement after exposure to foot-shock stress and this is accompanied by an up-regulation of CRHR1 across several brain regions.
Hansson et al. (2007)	CRF/CRHR1 in CeA and medial amygdala (MeA) and nucleus acc.	Levels of CRHR1 in msP rat brains were measured after 2 weeks ad lib exposure to alcohol	A down-regulation of CRHR1 transcripts were observed in the CeA, MeA and nucleus acc. when rats were given ad lib exposure to alcohol. These are areas which had previously seen an up-regulation of CRF during withdrawal, suggesting that alcohol is consumed, in part, to regulate CRF/CRHR1 activity in this region.

to link genetic polymorphisms in CRF1 to both risky-drinking behaviour in adolescents and specific patterns of drinking in adults.

In order to replicate the findings of the animal studies which showed that polymorphisms in the promoter of CRF1 caused differences in the amount of stress-induced drinking (Hansson et al., 2006), Blomeyer et al. measured the effects of negative life events on the amount of drinking in the same cohort of adolescents which had been genotyped for CRF1 htSNPs by Treutlein et al. (Blomeyer et al., submitted for publication; Treutlein et al., 2006). They found that the C allele of htSNP rs1876831 was found to be associated with drinking more alcohol per occasion and increased lifetime rates of heavy drinking, and this was mediated by the number of negative life events experienced in the last 3 years. Blomeyer et al. were the first group to show that CRF1 moderates the impact of stress on heavy drinking, and provided a direct translation for the findings of Hansson et al. in msP rats.

6. Therapeutic implications

Neurobiological and animal and human genetic findings point towards the relevance of CRF1 for alcohol abuse and have resulted in the study of several CRF1 antagonists which could be used to prevent relapse into alcoholism (Chu et al., 2007; Sabino et al., 2006). To date these studies have been conducted on animals, but have interesting translational benefits when considering treating humans. Recently, Sommer et al. found that the increased voluntary alcohol intake and sensitivity to stress seen in post-dependent rodents (paralleled by an increase in CRF1 expression in the amygdala) could be reduced by the CRF1 antagonist, MTIP (Sommer et al., 2008). Another study by Gilpin et al. showed that in alcohol preferring (P) rats, the administration of the CRF1 antagonist MPZP, attenuated dependence induced increase in alcohol self administration, with no effect on alcohol consumption in non-dependent rats (Gilpin et al., 2008). The administration of naltrexone attenuated alcohol administration in both dependent and non-dependent rats. An increase of alcohol intake and a sensitivity to stress is also observed in the post-dependent state in humans which lends credence to the use of CRF1 antagonists for the treatment of alcoholism. As the effects of CRF1 antagonism seem to be only relevant to animals in the dependent and post dependent state this enhances its potential as a therapy for the treatment of alcoholism.

Hansson et al. showed that a CRF1 antagonist blocked stress induced reinstatement of ethanol drinking in mSP rats, but not in Wistar rats. They then showed that this was due to a polymorphism in the promoter region of CRF1 which was unique to the mSP rat strain (Hansson et al., 2006). Yang et al. looked at the effect of CRF1 anatagonism on stress induced increases in alcohol uptake, and found no effect in the mouse strain (129SVEV). Furthermore they were not able to produce a stress induced increase of alcohol uptake in the C57BL/6J mouse strain (Yang et al., 2008). Therefore, it seems likely that a pharmacogenetic approach could be employed in humans to identify subtypes of alcoholic patients especially amenable to treatment with CRF1 antagonists. Evidence from animals has shown that there are strain dependent effects of CRF1 antagonism on alcohol drinking, and also stress induced drinking and these are likely due to genetic differences across different rat and mouse strains.

It is possible that the CRF1 polymorphisms found to be associated with adolescent drinking patterns and increased alcohol drinking in adults (Treutlein et al., 2006) will be predictive of response to CRF1 antagonist treatment for alcoholism. Also, a number of the genetic polymorphisms summarised in Table 1 may be predictive of efficacy to treatment by CRF1 antagonist as these have been shown to be associated with altered HPA-axis activity, however further work needs to be done to test these hypotheses.

7. Conclusions

There is now a wealth of evidence to demonstrate that acute alcohol-induced change of all hormones of the HPA-axis probably takes place at the level of CRF-secreting neurons (Sarnyai et al., 2001), and the studies reviewed here lend support to this theory. However, it is also clear that CRF has extra-hypothalamic effects which exert its behavioural response to stress, probably through activation of the LC/NE stress system and the extended amygdala CRF system.

It was proposed by Koob et al., that the alcohol induced changes of CRF activity over time contribute towards the negative reinforcing affects of alcohol drinking. The basis for this hypothesis draws from the notion that there are anti-reward systems in place in the brain which are responsible for limiting reward. As alcohol dependence develops these anti-reward systems are recruited and at the same time there is a decrease in reward function (Koob, 2008a; Koob and Le Moal, 2008). CRF is believed to have a central facilitating role in these adaptations in the brain. As CRF expression is linked to anxiety and fear, they reasoned that the hyperactivity of the CRF systems observed after prolonged exposure to alcohol, could contribute to the development of aversive emotional states which then drive compulsive drinking behaviour (Koob, 2008b). Recruitment of the CRF system may also underlie the vulnerability to stress which is observed in abstinent alcohol dependents.

To summarise the data of these studies on CRF1 and alcohol drinking behaviour, it can be clearly seen that genetic variations in CRF1 moderates the impact of stress on heavy drinking, revealing an effect of negative life events ,only among individuals carrying a particular genotypic variant of this gene. This work suggests an important role of CRF1in integrating gene–environment effects in humans and implies genotypic variations in the moderation of the individual's response to stress exposure.

8. Future work

It is clear now that CRF1 and CRF have key roles in the modulation of stress-related drinking behaviour, but questions remain as to which other genes involved in the physiological and behavioural response to stress will be related to alcohol abuse. Although CRF1 has proven to be firmly associated with alterations in alcohol drinking behaviour by the mediation of the stress response, the other biological systems with which it interacts to affect this response remain largely unstudied.

This could in part be answered by whole genome analysis (WGA) of population based cohorts with good phenotypic characterization of both pre-natal and lifetime stressors, combined with detailed assessments of drinking and substance abuse behaviour. This hypothesis-free approach would surely allow the elucidation of more genes which are involved in gene–environment interactions and alcohol abuse. Despite the huge benefits of analysing the whole genome in a large population based cohort, the power to detect gene–environment interactions is greatly reduced when we consider the fact although DNA sequences are resolute from birth; the environment has the ability to switch these genes on and off. One way of acknowledging these drawbacks is to enrich the results of whole genome scans with empirical transcriptional data from animals. By employing strategic approaches similar to those described by Hansson et al. (2006, 2007) whole-genome association data could be enriched with transcriptional data from low vs high stress-responding animals and their biological responses to alcohol. It is also important to consider the epigenetic effects that the environment confers upon gene expression as the studies by Pandey and Weaver et al. show us. CRF overexpression (Sommer et al., 2008) and HPA-axis dysregulation (Junghanns et al., 2007) persist into protracted abstinence and the modification of epigenetic patterns on stress-related genes could be partially responsible for this. This could even be specific to an individual, dependent on genotype, as some DNA sequences are more readily epigenetically modified than others. Transcriptional and epigenetic data would help to elucidate the genes which are switched on and off during stress and further genetic analysis of these genes in humans could help to explain the disparate responses of individuals to stress and their subsequent drinking habits. Combining sets of genes from transcriptional animal data and human WGA data could lead to bioinformatics' functional characterization in order to provide information about whole networks of genes which could be deregulated prior to or as a result of alcohol drinking and/or stress and give insight into how these pathways interact to provide very specific and individual responses to stress and how eventually, they could, confer vulnerability to alcoholism.

Acknowledgements

This work was supported by the UK department of Health NIHR-Biomedical Research Centre 'Mental Health' and the European Comission FP6 Integrated Project "IMAGEN".

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