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REVIEW

Neuropeptides: implications for alcoholism

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Abstract

The role of neuromodulatory peptides in the aetiology of alcoholism has been relatively under-explored; however, the development of selective ligands for neuropeptide receptors, the characterization and cloning of receptors, and the development of transgenic mouse models have greatly facilitated this analysis. The present review considers the most recent preclinical evidence obtained from animal models for the role of two of the opioid peptides, namely β-endorphin and enkephalin; corticotropin-releasing factor (CRF), urocortin I and neuropeptide Y (NPY) in deleterious and excessive alcohol consumption, focussing on specific brain regions, in particular the central nucleus of the amygdala, that appear to be implicated in the pathophysiology of alcoholism. The review also outlines potential directions for further research to clarify neuropeptide involvement in neuromodulation within discrete brain nuclei pertinent to behavioural patterns.

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Alcohol causes as much, if not more death and disability as measles, malaria, tobacco or illegal drugs (World Health Organization, 2001) . In economic terms, alcohol abuse has been estimated at US$167 billion per year; however, 'in human terms, the costs are incalculable' (National Institute on Alcohol Abuse and Alcoholism, 2001). For these reasons there has been extensive research into the pathophysiology underlying alcoholism; however, current therapeutic approaches cannot be regarded as a comprehensive solution to this extensive problem (Graham *et al*. 2002). A number of brain regions have been identified as being significantly involved in the reinforcing (and ultimately addictive) properties of alcohol, including the ventral tegmental area, the nucleus accumbens and the amygdala (Fig. 1; see Koob *et al*. 1998). In particular, as will be emphasised in this review, the central nucleus of the amygdala (CeA) appears to have a significant role in this regard. Several neurotransmitters have now been implicated in the pathophysiology of alcoholism, including dopamine, serotonin, GABA (Koob *et al*. 1998) and glutamatergic-mediated neurotransmission (Tsai and Coyle 1998). Until relatively recently however, the role of neuromodulatory peptides in the aetiology of alcoholism has been somewhat under-explored (with the possible exception of opioid peptides), due in part to the lack of selective ligands for neuropeptide receptors, the late characterization and cloning of the receptors themselves, and the relatively recent development of other tools (such as transgenic/knockout mice and genetic analysis of trait loci) that can facilitate this analysis. However, drugs that interact with neuropeptide systems have great potential in the pharmacotherapy of alcoholism: witness the widespread (although somewhat less than satisfactory) use of the opioid antagonist naltrexone in the treatment of alcoholism (O'Malley *et al*. 1992; Volpicelli *et al*. 1992), identified prior to clinical use via a large body of preclinical data (see Cowen and Lawrence 1999). Based on the preclinical and other data, several other neuropeptides may have a significant role in the aetiology of alcoholism; these are discussed herein.

Opioid peptides

A reasonable body of evidence now indicates that ethanol, at reward-relevant doses, causes the release of β-endorphin from both neuronal (hypothalamic) and non-neuronal (i.e. the anterior pituitary) sources. As indicated in Table 1, people at high risk for the development of alcoholism (strong family history) show a significantly greater ethanol-induced pituitary release of β-endorphin (reflected in serum levels)

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Abbreviations used: CeA, Central nucleus of the amygdala; CRF, corticotropin-releasing factor; i.c.v., intracerebroventricular; i.p., intraperitioneal; NPY, neuropeptide Y.

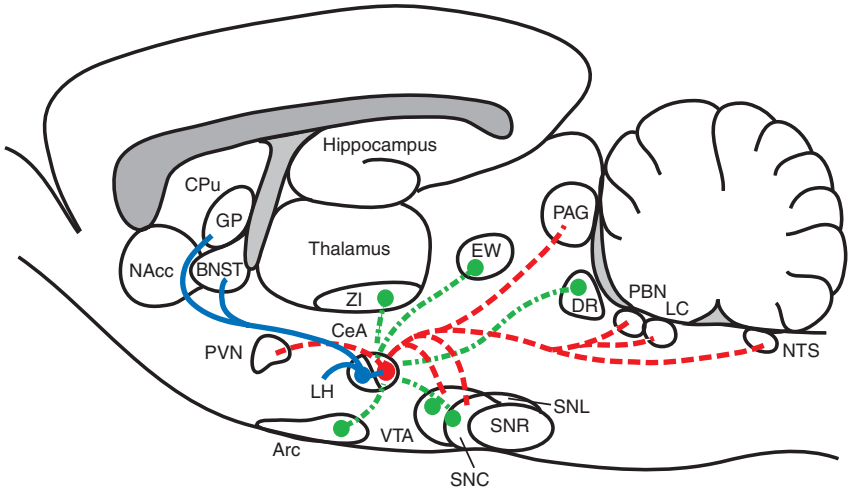

Fig. 1 Schematic diagram of the rat brain with particular reference to the neuroanatomical connections of the central nucleus of the amygdala (CeA). Projections predominantly arising from the lateral CeA are shown in blue (solid line); projections predominantly arising from the medial CeA are shown in red (long dashes). Projections to the CeA are shown in green (long-short dashes). Note this diagram is not a comprehensive schematic. The CeA receives dopaminergic input from the medial substantia nigra/dorsolateral ventral tegmental area (SN/VTA; Fallon and Moore 1978; Loughlin and Fallon 1983), as well as the A13 group in the medial zona incerta (ZI; Eaton *et al.* 1994; Wagner *et al.* 1995), a serotonergic projection presumably from the dorsal raphe nucleus (DR; Yoshimoto *et al.* 2000), β-endorphin-immunoreactive terminals from the arcuate nucleus (Arc; Finley *et al.* 1981; Gray *et al.* 1984) and urocortin-immunoreactive terminals (Bittencourt *et al.* 1999) that may arise from neurons within the Edinger-Westphal nucleus (EW), but may also arise from other urocortin-expressing neuronal populations. The primary neuronal origin of NPY-immunoreactive terminals within the CeA is not yet clear. GABAergic neurons from the lateral CeA project to the medial CeA (Sun and Cassell 1993; Sun *et al.* 1994). Enkephalin and corticotropin-releasing factor occur in the lateral portion of the CeA where they are coexpressed with GABA but rarely with each other (Veinante *et al.* 1997). Enkephalin- and CRF-expressing neurons contribute to the projection from the lateral CeA to the bed nucleus of the stria terminalis (BNST; Uhl *et al.* 1978; Sakanaka *et al.* 1986); CRF to the projection to the lateral hypothalamus (LH; Fellmann *et al.* 1982; Sakanaka *et al.* 1986) and enkephalin in the projection to the globus pallidus (GP; Arluison *et al.* 1990). Neurons from the medial CeA project to a number of brain regions (with minor projections from the lateral CeA) including the nucleus of the solitary tract (NTS), the parabrachial nucleus of the pons (PBN), the locus coeruleus (LC), the periaqueductal gray (PAG), the substantia nigra pars compacta/ventral tegmental area (SNC/VTA) and the paraventricular nucleus of the hypothalamus (PVN; Veening *et al.* 1984; Cassell *et al.* 1986; Gray *et al.* 1989; Wallace *et al.* 1989; Gray and Magnuson 1992; Wallace *et al.* 1992). CPu, caudate-putamen; NAcc, nucleus accumbens.

compared with people at low risk for the development of alcoholism. Paralleling the human situation, alcohol-preferring strains of rats and mice show a significantly greater release of β-endorphin from the hypothalamus compared to alcohol-non-preferring strains of rat and mice. Although the parallels between the animal models with the human situation are intriguing, the role of the release of β-endorphin from the anterior pituitary in the reinforcing properties of ethanol is unclear, although there remains the possibility of retrograde transport to the median eminence and hypothalamus (Gianoulakis *et al.* 1989, 1996). Serum β-endorphin levels may simply be a reflection of the ethanol-induced synthesis and release of CRF (and vasopressin) from the paraventricular nucleus of the hypothalamus (Rivier *et al.* 1984; Ogilvie *et al.* 1997), which causes the release of adrenocorticotropic hormone (ACTH) and β-endorphin from the anterior pituitary (Rivier *et al.* 1982; Jackson *et al.* 1984). Given the generally recognised association of the nucleus accumbens with reward processes (Koob 1992; Koob *et al.* 1998), the release of β-endorphin within the nucleus accumbens in response to ethanol, as well in response to the psychostimulants cocaine and D-amphetamine (Olive *et al.* 2001) would appear to be significant. Since the effect of ethanol on β-endorphin release in the nucleus accumbens may occur via stimulation of the endorphinergic cell bodies within the hypothalamus (de Waele *et al.* 1992; de Waele *et al.* 1994), ethanol may induce release of β-endorphin from other terminal regions.

We also posit a role for enkephalinergic neurons within the central nucleus of the amygdala (see Fig. 1) in the reinforcing properties of ethanol. Induction of c-Fos protein occurs in the central nucleus of the amygdala (CeA) following either acute injection of alcohol (Chang *et al.* 1995; Ryabinin *et al.* 1997) in rats or during the acquisition of alcohol self-administration by C57BL/6 J mice (Ryabinin *et al.* 2001), but not once the self-administration is established (Weitemier *et al.* 2001; Ryabinin *et al.* 2003). However, we have recently demonstrated that chronic free-choice ethanol consumption by alcohol-preferring Fawn-Hooded rats led to an up-regulation in preproenkephalin mRNA in the CeA

Table 1 Release of β-endorphin-like immunoreactivity induced by ethanol

Study	Model	Dose/Concentration of Ethanol	Release characteristics
(Gianoulakis *et al.* 1989)	Serum levels of β-endorphin (human) 15, 45 & 120 min postdrink, β-endorphin measured by radioimmunoassay (RIA) and gel filtration	0.5 g/kg	Greater in those with a family history of alcoholism (high-risk) vs. those with no family history of alcoholism (low-risk); predominant peptide was β-lipotropin
(de Waele *et al.* 1992)	Perifusion of dissected hypothalami (mouse), β-endorphin measured by RIA & high pressure liquid chromatography (HPLC)	10, 20, 25, 30 & 60 mM	Concentration-dependent release; greater from the hypothalami of alcohol-preferring C57BL/6 mice compared with alcohol-non-preferring DBA/2 mice
(de Waele *et al.* 1994)	Perifusion of dissected hypothalami (rat), β-endorphin measured by RIA & HPLC	10, 20, 30 & 60 mM	Concentration-dependent release; release of bioactive form (nonacetylated β-endorphin 1–31) was greater from the hypothalami of alcohol-preferring AA rats compared with alcohol-non-preferring ANA rats
(Gianoulakis *et al.* 1996)	Serum levels of β-endorphin 15,45, 120 & 180 min postdrink (human), β-endorphin measured by RIA and gel filtration	0, 0.25, 0.5 & 0.75 g/kg	Dose-dependent release in those with a family history of alcoholism (high-risk) but not in those with no family history of alcoholism (low-risk); predominant peptide was β-lipotropin
(Olive *et al.* 2001)	Microdialysis measurement of neurotransmitter release from the nucleus accumbens of conscious, freely moving rats; β-endorphin measured by RIA	2 × 2 g/kg i.p.	
(Marinelli *et al.* 2003)	Microdialysis measurement of the nucleus accumbens of conscious, freely moving rats; β-endorphin measured by RIA	0.8, 1.6 & 2.4 g/kg i.p	Dose-dependent release

(Cowen and Lawrence 2001) and Criado and Morales (2000) have shown that following an acute injection of ethanol (2 g/kg, i.p) in Sprague-Dawley rats, 94% of Fos-immunoreactive cells in the CeA are colocalised with preproenkephalin mRNA. Interestingly, administration of the opioid antagonist methylnaloxonium directly into the CeA decreased ethanol self-administration at doses slightly lower than those necessary when delivered directly into the nucleus accumbens (Heyser *et al*. 1999). Although not yet demonstrated, we speculate that ethanol consumption facilitates the release of enkephalin within the CeA, although since β-endorphin terminals are also present in the (medial) CeA (Finley *et al*. 1981; Gray *et al*. 1984), the effect of methylnaloxonium in the CeA may be directed towards β-endorphin-mediated neuromodulation.

Ethanol consumption by mice lacking the preproenkephalin gene (Table 2; Koenig and Olive 2002) or by mice expressing a truncated form of the *Pomc* gene such that β-endorphin is not expressed (Table 2; Grisel *et al*. 1999; Grahame *et al*. 2000) is not significantly greater than that of wild-type mice; under certain paradigms, mice expressing the truncated form of the *Pomc* gene actually drank more alcohol (Grisel *et al*. 1999; Grahame *et al*. 2000). Such data are difficult to interpret, given the now-recognized phenomenon of phenotypic drift (Sur *et al*. 2001; Cowen *et al*. 2003); however, the data may indicate redundancy in alcohol-induced opioid peptide-mediated signalling. This would appear to be confirmed by the fact that the opioid antagonist naltrexone decreased alcohol consumption under limited access conditions by both wild-type and β-endorphin-deficient mice (Grahame *et al*. 2000). Ultimately, a double enkephalin/endorphin null mouse (if viable), or a more temporally restricted inhibition of gene expression, for example using RNA interference (Fire *et al*. 1998), may get to the heart of this issue. In contrast to the equivocal results with the neuropeptide-deficient mouse models, the diminished level of alcohol self-administration by μ-opioid receptor deficient mice (Roberts *et al*. 2000b) would appear to clearly indicate that this receptor (and neuromodulation mediated by this receptor) is significant in the reinforcing properties of alcohol, regardless of the endogenous peptide that may be mediating the effect. Whereas the δ-opioid receptor knockout mouse demonstrated enhanced ethanol consumption subsequent to operant self-administration

Table 2 'Knockout' mice and ethanol consumption

Study	Mouse model	Effect on ethanol consumption
(Koenig and Olive 2002)	Preproenkephalin deficient mice	No effect
(Grahame *et al.* 1998; Grisel *et al.* 1999; Grahame *et al.* 2000)	Truncated form of Pomc gene; no expression of β-endorphin	No effect; increase in certain paradigms
(Roberts *et al.* 2000b)	μ-opioid receptor deficient mice	Decreased ethanol consumption
(Roberts *et al.* 2001)	δ-opioid receptor deficient mice	Increased ethanol consumption
(Olive *et al.* 2003)	CRF-deficient mice	Increased ethanol consumption
(Sillaber *et al.* 2002)	CRF_1 receptor deficient mice	No difference to wild-type mice under basal conditions, marked post-stress increase in ethanol consumption
(Thiele *et al.* 1998; Thiele *et al.* 2000	Neuropeptide Y deficient mice	Increased ethanol consumption
(Thiele *et al.* 2002)	NPY Y1 receptor deficient mice	Increased ethanol consumption
(Pandey *et al.* 2003a)	NPY Y2 receptor deficient mice	Decreased ethanol consumption
(Thiele *et al.* 2000)	NPY Y5 receptor deficient mice	No effect on ethanol consumption; increased sensitivity to sedative/hypnotic effects

training (Roberts *et al*. 2001), the authors suggested that this consumption was in fact related to an anxiolytic effect of ethanol, since these mice demonstrated an anxiety-related phenotype that was reversed by ethanol consumption.

The data with respect to alcohol and opioid peptides clearly concurs with the use of the opioid antagonist naltrexone in the clinical treatment of alcoholism (O'Malley *et al*. 1992; Volpicelli *et al*. 1992), as noted in the introduction. However, the latest Cochrane review has suggested limited effectiveness for naltrexone in the treatment of alcoholism (Srisurapanont and Jarusuraisin 2003). Thus far, naltrexone would appear to be most effective in combination with coping skills training, with continued use over the medium term rather than a short initial intervention (O'Malley *et al*. 1992; O'Malley *et al*. 1996a; Heinala *et al*. 2001). There has been some discussion as to whether naltrexone is more effective in blocking craving or the rewarding effects of alcohol per se (Sinclair 1998; O'Malley *et al*. 2002); opioid-mediated signalling appears to have a role in both. Subjective rewarding responses to alcohol (Volpicelli *et al*. 1995) have been reported to be blocked by concurrent self-administration of naltrexone in alcoholics; naltrexone has also been reported to decrease self-measured craving in alcoholics, both in those seeking treatment (O'Malley *et al*. 1996b) and those not seeking treatment (O'Malley *et al*. 2002). The preclinical data presented above, for example on β-endorphin release induced by alcohol, could support a role for opioid-mediated signalling in the rewarding properties of alcohol; however, in one of the few preclinical animal studies that may adequately model craving, Liu and Weiss (2002) recently demonstrated that reinstatement of alcohol consumption, induced by an olfactory cue previously associated with the availability of alcohol, was prevented by naltrexone. Naltrexone was ineffective, however, on reinstatement of alcohol seeking behaviour induced by footshock stress (Liu and Weiss 2002). The clinical studies clearly indicate that in spite of treatment with naltrexone, relapse is a recurring problem (O'Malley *et al*. 1996a; Heinala *et al*. 2001); in the study by Heinala *et al*. (2001) approximately 70% of patients treated with naltrexone/coping skills had relapsed at the end of the study period (the best outcome of the four treatment groups), although a relatively high proportion of these had only one relapse episode. We would argue that this result indicates that strong motivational factors for relapse remain, and that a combination pharmacotherapy approach (e.g. Rezvani *et al*. 2000) is most likely to produce the greatest long-term benefit.

Corticotropin-releasing factor (CRF)

A number of recent studies have demonstrated that CRF antagonists prevent or diminish alcohol consumption or alcohol-seeking behaviour in several quite varied models associated with states of dysphoria (see Table 3). Thus, footshock stress-induced reinstatement of alcohol-seeking behaviour (Le *et al*. 2000; Liu and Weiss 2002), ethanol withdrawal-induced operant responding for alcohol (Valdez *et al*. 2002b) and anxiety-related alcohol consumption (Lodge and Lawrence 2003) are diminished by the use of the non-selective CRF antagonist d-Phe-CRF (Le *et al*. 2000; Liu and Weiss 2002; Valdez *et al*. 2002b) or selective CRF_1 receptor antagonists (Le *et al*. 2000; Lodge and Lawrence 2003). Although there appears to be a common pharmacological pathway, different neuroanatomical locations may account for the effects of these CRF antagonists on alcohol-seeking behaviour or alcohol consumption. Thus, Le *et al*. (2002) have demonstrated that delivery of the CRF antagonist d-Phe-CRF within the median raphe nucleus blocks

Table 3 CRF antagonists decrease alcohol consumption/alcohol-seeking behaviour

Study	Antagonist/dose	Effect/Model
(Le *et al.* 2000)	d-Phe-CRF_{12-41} (0, 0.3 & 1 μg i.c.v) CP-154 526 (selective CRF_1 antagonist) (0, 15, 30 & 45 mg/kg i.p.)	Dose-dependent decrease in footshock-induced reinstatement of extinguished responding for alcohol.
(Le *et al.* 2002)	d-Phe-CRF_{12-41} (0 & 50 ng, intramedian raphe nucleus)	Blocked footshock-induced reinstatement of extinguished responding for alcohol.
(Liu and Weiss 2002)	d-Phe-CRF_{12-41} (0, 1 & 10 μg i.c.v.)	Dose-dependent decrease in footshock-induced reinstatement of extinguished responding for alcohol, but no effect on conditioned stimulus-induced reinstatement.
(Valdez *et al.* 2002b)	d-Phe-CRF_{12-41} (0, 1, 5 & 10 μg i.c.v.)	Dose-dependent decrease in responding for alcohol in rats that had undergone withdrawal from chronic ethanol vapour 2 h and 3–5 weeks prior to operant session, but not in control rats.
(Lodge and Lawrence 2003)	Antalarmin (selective CRF_1 antagonist) (20 mg/kg i.p. bi-daily)	Prevented the acquisition and reduced established ethanol consumption by isolation-reared Fawn-Hooded rats.

stress-induced reinstatement of alcohol-seeking behaviour, indicating a role for this nucleus in stress-induced alcohol-seeking behaviour. Whether the primary source of CRF in the median raphe nucleus is the CRF-expressing neurons within this nucleus (Sakanaka *et al*. 1987) or from some other distal nucleus such as the dorsal raphe nucleus (Sakanaka *et al*. 1987) is unclear. However, ethanol withdrawal in rats has also been shown to cause a marked release of CRF in the CeA (Fig. 1) as measured by microdialysis, reaching a maximum at 10–12 h from the beginning of withdrawal (Merlo Pich *et al*. 1995). Further, direct application of a CRF receptor antagonist, α-helical-CRF, into the CeA caused a decrease in the anxiogenic effects of withdrawal from ethanol (Rassnick *et al*. 1993). Finally, basal levels of CRF release in the CeA as measured by microdialysis were shown to be elevated in the alcohol-preferring sP (Sardinian-preferring) rat strain compared with the sNP (Sardinian-non-preferring) rat strain (Richter *et al*. 2000); the elevated release of CRF from the CeA of sP rats was suggested to be related to their greater levels of anxiety, as measured by the elevated plus maze (Richter *et al*. 2000). Thus the data would appear to suggest that a primary locus of the effects of CRF antagonists on withdrawal-related alcohol consumption may be the CeA, and this nucleus may also have a more generalized role in anxiety-related alcohol consumption.

That 'dysphoria'-induced relapse has a significant role in the clinical situation has been emphasised by Koob and colleagues recently (Roberts *et al*. 2000a; Valdez *et al*. 2002b). Thus in a sample of 100 alcoholic patients, 96 had experienced depression and 94 anxiety in the 28 days prior to interview; 80 of the 100 patients identified anxiety or depression or both as having provoked drinking (Hershon 1977). In another study (Marlatt and Gordon 1980), 39% of alcoholics indicated negative emotional states were the primary reason for relapse (in contrast, 11% indicated urges and temptations were the primary motivation). However, the potential of selective CRF_1 receptor antagonists, such as antalarmin, as therapeutic drugs appears to be in conflict with data demonstrating that CRF-deficient mice drank approximately twice as much as their wild-type counterparts when ethanol (at concentrations above the taste threshold) and water were available in a two-bottle preference test (Olive *et al*. 2003; Table 2), although it has been suggested (Bachtell *et al*. 2003; Olive *et al*. 2003) that this may be due to a compensatory increase in urocortin I expression (see below) in these mice (Weninger *et al*. 2000). More problematic was the demonstration that a series of stressful episodes lead to a marked and prolonged increase in ethanol consumption in CRF_1-receptor deficient but not wild-type mice (Sillaber *et al*. 2002), even though initially there were no significant differences in alcohol consumption between CRF_1-receptor deficient and wild-type mice. These data seem to suggest that the CRF_1 receptor is involved in adaptive responses to stress that are impaired in these mice, as well as the acute response to stressors (Timpl *et al*. 1998). One of the few clinical studies published thus far examining the effect of a CRF_1 receptor antagonist (Zobel *et al*. 2000) indicated the drug (R121919) was well tolerated and produced significant improvements in measures of anxiety and depression over a period of 30 days. While the conflicting data may simply represent species differences in terms of CRF signalling within the central nervous system, the data may argue against a blanket, chronic use of CRF antagonists in the treatment of alcoholism; clearly further study is needed.

Urocortin

c-Fos immunoreactivity is induced in the Edinger-Westphal nucleus by ethanol in a range of experimental paradigms (see

Table 4 Induction of c-Fos immunoreactivity by ethanol in the Edinger-Westphal nucleus

Study	Model
(Chang *et al*. 1995)	Acute injection of ethanol (3 g/kg i.p), Sprague-Dawley rats
	Chronic injection of ethanol (3 g/kg i.p. bi-daily, 17–24 days), Sprague-Dawley rats
(Ryabinin *et al*. 1997)	Acute injection of ethanol (0.5 & 1.5 g/kg i.p), Sprague-Dawley rats
(Topple *et al*. 1998)	Limited-access beer consumption, Wistar rats
Bachtell *et al*. 1999)	Limited-access ethanol/sucrose consumption, C57BL/6 J mice
(Ryabinin *et al*. 2001)	Initiation of ethanol/sucrose consumption, C57BL/6 J mice
(Weitemier *et al*. 2001)	Operant responding for 10% ethanol by alcohol-preferring AA rats
(Bachtell *et al*. 2002a)	Acute injection of ethanol (2.4 g/kg i.p), C57BL/6 J mice
(Bachtell *et al*. 2002b)	Acute injection of ethanol (0, 0.6–4.8 g/kg i.p), C57Bl/6 J & DBA/2 J mice
	Chronic injection of ethanol (2.4 g/kg i.p. daily, 14 days), C57Bl/6 J & DBA/2 J mice
(Ryabinin *et al*. 2003)	Dark-phase limited-access ethanol consumption, C57BL/6 J mice
(Bachtell *et al*. 2003)	Limited-access ethanol/sucrose consumption, C57BL/6 J & DBA/2 J mice

Table 5 Relationship between ethanol consumption, hypothermic effects of ethanol and urocortin in the Edinger-Westphal (EW) and lateral septal nucleus (LSN)

Mouse strain	Hypothermic response/number of urocortin-positive cells (EW)	Ethanol consumption/number of urocortin positive cells (EW)	Ethanol consumption/urocortin in the lateral septal nucleus
Heterogenous B6D2 F2 mice	Positive correlation between hypothermic response and urocortin positive cells (Bachtell *et al*. 2002b)	Positive correlation between ethanol consumption (limited access; not continual access) and urocortin positive cells (Bachtell *et al*. 2003).	Negative correlation between ethanol consumption and urocortin-immunoreactive processes in the LSN (Bachtell *et al*. 2003).
COLD/HOT mice	COLD mice – greater hypothermic response than HOT mice (Crabbe *et al*. 1987) and higher number of urocortin positive cells (Bachtell *et al*. 2002b)	COLD mice drink more ethanol at concentrations above 5% (Cunningham *et al*. 1991); higher number of urocortin positive cells (Bachtell *et al*. 2002b)	
C57BL/6 J mice/ DBA/2 J mice		C57BL/6 J consume more ethanol (Belknap *et al*. 1993) and have a higher number of urocortin positive cells (Bachtell *et al*. 2002b).	
HAP/LAP mice		HAP mice consume more alcohol than LAP mice (Grahame *et al*. 1999) and have a higher number of urocortin positive cells (Bachtell *et al*. 2003).	HAP mice – Replicate 1: greater number of urocortin immunoreactive processes (Bachtell *et al*. 2003). Replicate 2: No difference in urocortin immunoreactive processes (Bachtell *et al*. 2003).

Table 4); unlike the effect of ethanol in the CeA and the paraventricular nucleus of the hypothalamus, this Fos response still occurs with chronic experimenter-administered ethanol (Chang *et al*. 1995; Bachtell *et al*. 2002b) or chronic self-administration (Topple *et al*. 1998; Weitemier *et al*. 2001). The neuropeptide urocortin I is predominantly expressed within the Edinger-Westphal nucleus (Vaughan *et al*. 1995), and as such efforts have concentrated on determining the relationship between the effects of alcohol and urocortin I expression in this nucleus (summarised in Table 5). A positive correlation between the hypothermic response to alcohol (3.6 g/kg i.p) and the number of urocortin I-expressing cells in heterogenous B6D2 F2 was determined (Bachtell *et al*. 2002b). Similarly COLD mice, which are relatively sensitive to the hypothermic effects of alcohol, have a higher number of urocortin I-expressing cells than HOT mice, which are relatively insensitive to the hypothermic effects of alcohol (Bachtell *et al*. 2002b).

However, as the authors point out, the c-Fos response in the Edinger-Westphal nucleus occurs at far lower concentrations of alcohol than the hypothermic response (Bachtell *et al*. 2002b).

Bachtell *et al*. (2002b) have also determined that alcohol-non-preferring DBA/2 J mice have fewer urocortin I-positive cells than alcohol-preferring C57BL/6 J mice. Suggestive of a role in alcohol consumption, Bachtell *et al*. (2003) have further demonstrated that alcohol consumption under limited access conditions by heterogenous B6D2 F2 mice positively correlates with urocortin I immunoreactivity and urocortin I-positive cells in the Edinger-Westphal nucleus but negatively correlates with urocortin I-immunoreactive processes in the lateral septal nucleus (Table 5). COLD mice also demonstrate higher ethanol consumption than HOT mice at concentrations above 5% (Cunningham *et al*. 1991). However, although alcohol-preferring HAP mice demonstrated greater levels of urocortin I immunoreactivity and urocortin I-positive cells in the Edinger-Westphal nucleus compared with alcohol-non-preferring LAP mice (Bachtell *et al*. 2003), HAP mice either had greater number of urocortin I-immunoreactive processes in the lateral septal nucleus (replicate 1) or no significant difference (replicate 2) compared with LAP mice. Thus far, the data would appear to support a role for the Edinger-Westphal nucleus/urocortin I in the differential ethanol consumption of alcohol-preferring/non-preferring strains of mice; however, the primary projection site(s) through which urocortin may produce this differential ethanol consumption is as yet unclear.

As urocortin I has greater affinity than CRF for the CRF_2 receptor and slightly greater affinity for the CRF_2 than the CRF_1 receptor (Vaughan *et al*. 1995), urocortin I was suggested to be an endogenous ligand for the CRF_2 receptor (Vaughan *et al*. 1995). However, neuropeptides more selective for the CRF_2 receptor have since been discovered – urocortin II (Reyes *et al*. 2001) and urocortin III (Lewis *et al*. 2001). Urocortin II has been shown to cause a decrease in food (Reyes *et al*. 2001; Inoue *et al*. 2003) and water intake (Inoue *et al*. 2003), a mild decrease in locomotor activity (Valdez *et al*. 2002a) and decreased anxiety-like behaviour (Valdez *et al*. 2002a). Urocortin III similarly caused a decrease in locomotor activity and decreased anxiety-like behaviour (Valdez *et al*. 2003). The effects of selective non-peptide CRF_2 receptor antagonists such as K41498 (Lawrence *et al*. 2002) on alcohol consumption have not yet been tested. Although the anxiolytic actions of urocortin II and III, presumably mediated via CRF_2 receptors, may facilitate a decrease in 'dysphoria'-induced alcohol consumption, it is unclear whether the appetite suppressant effects may be counterproductive.

Neuropeptide Y (NPY)

An increasing body of research has indicated an interaction between neuropeptide Y and alcohol consumption (Pandey *et al*. 2003a). The main impetus for the examination of this interaction was the finding that NPY-deficient mice show increased ethanol consumption, whereas NPY-overexpressing mice show decreased ethanol consumption (Thiele *et al*. 1998; Table 2). Although the NPY-deficient mice were less sensitive to the sedative/hypnotic effects of ethanol, whereas the NPY-overexpressing mice were more sensitive (Thiele *et al*. 1998), a second strain of NPY-deficient mice on a different genetic background did not demonstrate this decreased sensitivity to the hypnotic effects of ethanol, but did demonstrate greater ethanol consumption than wild-type mice when a 20% solution was offered (Thiele *et al*. 2000). Alcohol consumption by several other NPY-related knockout mice have now been analysed (Table 2). The regulatory effect of NPY on ethanol consumption appears to be mediated via the Y1 receptor, as Y1–/– mice show increased ethanol consumption relative to wild-type mice, but normal consumption of sucrose or quinine solutions (Thiele *et al*. 2002). In contrast, Y2–/– mice (the Y2 receptor believed to be an inhibitory autoreceptor on NPY-containing terminals; Naveilhan *et al*. 1999) were shown to have decreased ethanol consumption relative to wild-type controls (Pandey *et al*. 2003a). This effect was replicated with the selective Y2 receptor antagonist BIIE0246, which decreased responding for a sweetened ethanol solution (Thorsell *et al*. 2002). Evidence from other experimental models also indicate that dysregulation of NPY expression may have a role in heavy/excessive alcohol consumption. Badia-Elder *et al*. (2001) have demonstrated that intracerebroventricular (i.c.v.) delivery of NPY caused decreased ethanol consumption in alcohol-preferring P rats, but not in Wistar or alcohol-non-preferring NP rats and interestingly, low levels of NPY have been reported in the CeA of P and HAD rat strains compared with alcohol-non-preferring NP and LAD rat strains (Table 6; Hwang *et al*. 1999). However, no similar difference was found in alcohol-preferring AA rats compared with ANA rats (Caberlotto *et al*. 2001), although there was a significantly lower expression of NPY mRNA in the hippocampus; nor did i.c.v. administration of NPY affect ethanol consumption by AA rats under operant conditions (Slawecki *et al*. 2000), clearly indicating strain differences in the probable etiology of excessive alcohol consumption.

In humans, an association between alcoholism and a polymorphism in the signal peptide region of the NPY gene (resulting in a substitution of Pro7 for Leu7) has been suggested but conflicting data have been obtained (Pandey *et al*. 2003a; Zhu *et al*. 2003). To date, no study examining the effect of NPY (or non-peptide analogues) in the treatment for alcoholism has been published. However, given the antistress/anxiolytic-like effects of NPY in several animal models (Heilig and Thorsell 2002) as well as the reversal of anxiolytic effects of NPY by antisense inhibition of the Y1 receptor (Heilig 1995), it may be anticipated that non-peptide NPY analogues, particularly those selective for the Y1 receptor, or possibly Y2 antagonists (Thorsell *et al*. 2002),

Study	Rats strains	Significant effects
(Hwang *et al.* 1999)	Alcohol-preferring P vs. alcohol-non-preferring NP;	↑ NPY immunoreactivity in the PVN, Arc ↓ NPY immunoreactivity in the CeA
(Hwang *et al.* 1999)	Alcohol-preferring HAD vs. alcohol-non-preferring LAD	↓ NPY immunoreactivity in the PVN, Arc ↓ NPY immunoreactivity in the CeA
(Caberlotto *et al.* 2001)	Alcohol-preferring AA vs. alcohol-non-preferring ANA	↓ NPY mRNA expression in HC

Table 6 Alterations in NPY-like immunoreactivity and NPY mRNA in alcohol-preferring/non-preferring strains of rats. Arc, arcuate nucleus; HC, hippocampus; PVN, paraventricular nucleus of the hypothalamus.

may produce positive outcomes in the treatment of alcohol dependence.

Other neuropeptides

The data for other neuropeptides appears to be less complete. Substance P and neurokinin-like immunoreactivity were shown to be low in the frontal cortex of alcohol-preferring P rats compared with alcohol-non-preferring NP rats (Slawecki *et al*. 2001); interestingly, the NK_3 receptor agonist senktide was shown to decrease alcohol consumption in this same strain of alcohol-preferring rat (Ciccocioppo *et al*. 1995); however, further data by the same authors suggest that senktide may have rewarding properties itself (Ciccocioppo *et al*. 1998). The neurotensin (NTS1) receptor appears to have a significant role in the hypnotic effects of ethanol (Erwin *et al*. 2001). Differential CCK-1 and CCK-2 receptor expression and density were recently found between the alcohol-preferring Fawn-Hooded and alcohol-non-preferring Wistar Kyoto strains of rats (Lodge and Lawrence 2001). Finally nociceptin, which is an endogenous ligand for the opioid receptor-like 1 (ORL1) receptor, decreases alcohol consumption in rats under a range of experimental protocols (Ciccocioppo *et al*. 2002).

Central nucleus of the amygdala

Alluded to throughout this review is the significant role that the central nucleus of the amygdala (CeA; Koob *et al*. 1998; McBride 2002; see Fig. 1) may play in excessive alcohol consumption. We hypothesize that the reinforcing properties of ethanol, both positive and negative, are mediated via the CeA, although not exclusively. Several other strands of evidence not heretofore mentioned would support this view, notably the release of dopamine and serotonin within the CeA induced by systemic alcohol administration (Yoshimoto *et al*. 2000); the alteration in local cerebral glucose utilization within the CeA induced by alcohol self-administration (McBride 2002), the alterations in ethanol consumption induced by drugs that modulate GABAergic neurotransmission (Hyytiä and Koob 1995; Roberts *et al*. 1996) and protein kinase A (PKA) activity (Pandey *et al*. 2003a; Pandey *et al*. 2003b) when microinjected into the CeA; finally the decrease in ethanol consumption induced by bilateral lesions of the central but not the basolateral nuclei of the amygdala (Moller *et al*. 1997). Other neuroanatomical strands of evidence may be woven into this model; for example, the presence of β-endorphin-immunoreactive terminals in the medial portion of the CeA (Finley *et al*. 1981; Gray *et al*. 1984) and the presence of urocortin-immunoreactive terminals, which although occurring 'sparsely' throughout the cerebral cortex, basal ganglia and amygdala, occur at moderately dense levels in the medial central nucleus (and posterior cortical nucleus) of the amygdala (Bittencourt *et al*. 1999), suggestive that these two neuropeptides, implicated in the etiology of excessive alcohol consumption, may mediate some of their effects at this brain nucleus.

How the various neurotransmitters and peptides interact within the CeA in response to ethanol consumption, ethanol withdrawal and stress (and other dysphoric states) has yet to be ascertained, although several aspects can be outlined. Although not yet demonstrated directly, we would suggest that alcohol consumption facilitates the release of opioid peptides within the CeA, whereas alcohol withdrawal, as noted previously (Merlo Pich *et al*. 1995) causes the release of CRF in the CeA. Interestingly, CRF and enkephalin are both expressed in GABAergic neurons within the (lateral) CeA (Veening *et al*. 1984; Cassell *et al*. 1986; Fallon and Leslie 1986; Sakanaka *et al*. 1986; Veinante *et al*. 1997) but are apparently not coexpressed (Veinante *et al*. 1997). Why ethanol should differently regulate these two neuronal populations is as yet unclear. Low levels of NPY within the CeA (Hwang *et al*. 1999) and/or possibly the release of serotonin and dopamine by alcohol (Yoshimoto *et al*. 2000) may facilitate or inhibit one or other of these two groups of neurons. However, experiments examining the relevant colocalization of neuropeptides and receptors within this brain region have yet to be performed.

Liu and Weiss (2002), as noted previously, demonstrated that reinstatement of alcohol-seeking behavior by conditioned olfactory cues was significantly diminished by the opioid antagonist naltrexone, suggestive of role of opioidergic signalling in this behavior, whereas reinstatement of alcohol-seeking behavior by footshock stress was reversed by the CRF antagonist d-Phe-CRF. Whether alcohol-associated cues may lead to the release of opioid peptides within the CeA is a matter for speculation; however, that neurons within the CeA (in particular those expressing CRF) are responsive to stress are well documented. For example,

bilateral electrolytic lesions of the CeA prevented the turnover of dopamine in the prefrontal cortex in rats in response to both a novel stressful environment and footshock stress (Davis *et al*. 1994); bilateral lesions of the CeA using ibotenic acid have been shown to prevent the increase in anxiety-like behavior caused by one hour of restraint stress prior to the elevated plus-maze test (Moller *et al*. 1997). Restraint stress was shown to lead to CRF release directly within the CeA (Merlo Pich *et al*. 1995; Richter *et al*. 2000) and psychological stress (placement of non-stressed rats in the same cage as a group of rats previously stressed by footshock) was shown to cause an up-regulation of CRF mRNA and content in the CeA (Makino *et al*. 1999).

How alterations in neuropeptide and neurotransmitter signalling within the CeA should influence drug-seeking behavior is not completely clear; whether this involves, for example, modulation of the mesolimbic dopaminergic system, does not appear to have been determined. Although there is a projection from the CeA to the substantia nigra/ventral tegmental area (Fig. 1; Veening *et al*. 1984; Cassell *et al*. 1986; Wallace *et al*. 1989, 1992), Maeda and Mogenson (1981) demonstrated that electric stimulation of the CeA results in activation (or in one-third of cases, suppression) of dopaminergic and non-dopaminergic neurons within the ventral tegmental area primarily via multisynaptic pathways, although a relatively high number of non-dopaminergic neurons were activated by stimulation of the central nucleus via a monosynaptic pathway.

Combination pharmacotherapy & conclusions

Clearly, alcohol interacts with several neuropeptide systems and modulation of these could potentially provide useful therapies in the treatment of alcoholism. The clinical data appear to support a combination pharmacotherapy approach; for example naltrexone in combination with a CRF antagonist or NPY Y1 receptor agonist; there is also the possibility of combination with drugs that target other neuronal systems, such as serotonin (Pettinati 2001). In the clinical studies outlined previously, levels of anxiety/depression would appear to have had a contributory role in relapse even if they were not the primary etiologic cause (Hershon 1977; Marlatt and Gordon 1980). We suggest that an appropriate broad-band pharmacological intervention will facilitate the continuation of abstinence or the prevention of relapse while other factors (social support structure, coping skills, Brown *et al*. 1995) develop or improve. With regard however, to the role of neuropeptides in alcoholism and its treatment possibilities, perhaps the most significant problem is the relative lack of water-soluble, non-peptide analogues of the neuropeptides (both agonists and antagonists). The development of such compounds would of course have benefits not only for the treatment of alcoholism but for the treatment of other widespread problems such as anxiety and depression.

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