Biological vulnerability to alcoholism in children of alcoholics
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Neurochemical markers of alcoholism vulnerability in humans

ABSTRACT
This review considers possible neurochemical trait markers that may be related to alcoholism vulnerability. Potential trait markers for alcoholism of neurochemical origin have been studied in alcoholics and in the children of alcoholics. Indices of changes within five neurotransmitter systems, GABA, serotonin, dopamine, norepinephrine and β-endorphin have been implicated as important factors in alcoholism, and have been studied as trait markers for this condition. This review investigates whether possible neurochemical markers meet three criteria, to be: (1) heritable; (2) present in alcoholics but not in unrelated non-alcoholics; (3) state independent, as determined from studies of the children of alcoholics. Two neurochemical markers seemed to fulfill the three trait-marker criteria studied: (a) the measurement of increased baseline activity of the serotonin transporter in platelets; and (b) the measurement of increased responsiveness of the pituitary β-endorphin system to challenges. Regarding neurochemical vulnerability for alcoholism, arguments are presented to underline the necessity of studying trait marker properties in individuals at risk for alcoholism.

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INTRODUCTION

Alcoholism is a multifactorial disease, in which polygenic influences and environmental influences interact (Goldman, 1995). This has been demonstrated by adoption studies (Goodwin et al., 1973; Bohman et al., 1981; Cloninger et al., 1985; Cadoret et al., 1985) and by twin studies (Heath et al., 1997; Kendler et al., 1994; 1997; Reed et al., 1996; Romanov et al., 1991, Treu et al., 1996). The question arises as to what is inherited in alcoholism? The research on trait markers may contribute to knowledge about genetic risk factors for alcoholism. The search for trait markers has focused on behavioural, psychophysiological, or neurochemical trait markers for alcoholism (Begleiter and Porjesz, 1988; Farren and Tipton, 1999; Reich et al., 1999; Schuckitt, 1999; Van der Stelt, 1999). This review investigates whether the possible neurochemical trait markers that were previously reported represent trait effects, rather than state effects following the onset of alcoholism. Thus, in addition to the use of trait marker studies in alcoholics, neurochemical trait marker studies in the children of alcoholics (COAs) are reviewed.

A trait marker is a pathophysiological marker that has the potential to increase our understanding of the aetiology of the clinical condition in question. For example, how an inherited pathophysiological abnormality may lead to alcoholism within an individual. Unlike a diagnostic marker, a trait marker need not necessarily have high sensitivity and specificity for the clinical condition itself (Begleiter and Porjesz, 1988; Nurnberger, 1992). Trait markers for alcoholism are characteristics with two main features, to be reliable and valid. To be reliable, the trait marker needs to be identifiable,
easily measured, and have a good within-subject and between-subject reliability (Farren and Tipton, 1999; Froehlich et al., 2000). The features concerning the validity of trait markers are studied in this review. Firstly, they are heritable. Twin studies or adoption studies demonstrate that variations in the trait marker are largely based on genetic factors as opposed to environmental factors. Secondly, they are more prevalent in alcoholics than in unrelated non-alcoholics. Thirdly, they are state independent. This means that, rather than reflecting the current state of alcoholism, they are present throughout an individual’s lifetime (something that can be assessed in high-risk groups). The marker should also be co-segregated with alcoholism within families, as demonstrated by sib pair analyses, or other genetic analyses of pedigree data (Begleiter & Porjesz, 1988). This will prevent false positive conclusions based on population stratification. Although the state independence of the trait marker can be investigated in long-term abstinent alcoholics, this procedure does not discriminate accurately between state markers and trait markers. The effects of long-term daily alcohol use may have affected the activity of neurochemical trait markers in alcoholics. The trait marker’s mode of genetic transmission can be assessed either by a genetic analysis of pedigree data (using a genome-wide screen) or by a linkage analysis of candidate genes within families. The issue of transmission is not addressed in the present review.

This review will focus on neurochemical risk markers. These are indices of changes in neurotransmitter systems that are studied as trait markers in alcoholism, in accordance with the three above-mentioned criteria. The state effect of alcohol on changes in neurotransmission has
been widely studied within neurotransmitter systems. It has, for example, been the main approach adopted in studies of the NMDA receptor of the glutamatergic system. However, this review focuses on indices of changes within five neurotransmitter systems that may have a trait effect on alcoholism. This includes the following neurotransmitters: (1) γ-aminobutyric acid (GABA) (Frye and Breese, 1982; Koob et al., 1986); (2) 5-hydroxytryptamine (serotonin; importantly, the impaired functioning of CNS serotonin may result in diminished impulse control, a neurobiological feature of type II alcoholism) (Cloninger, 1987; Linnoila et al., 1983); (3) dopamine (Gessa et al., 1985; Imperato and Di Chiara, 1986; Wise and Bozarth, 1987); (4) norepinephrine (Cloninger, 1994); (5) β-endorphin. This last topic is the subject of two contradictory hypotheses. The endorphin compensation or deficiency hypothesis states that alcohol use compensates for a predisposed or acquired deficiency of endorphinergic receptor activity (Blum, 1983; Erickson, 1990; Volpicelli et al., 1985). Whereas the opioid surfeit hypothesis states that alcohol use is increased by a surfeit of opioiidergic receptor activity, rather than a deficit (Reid and Hunter, 1984; Hubbell et al., 1986; Reid et al., 1991). Both hypotheses are equally applicable to other neurotransmitter systems. G-proteins and adenylyl cyclase (or any other secondary messenger systems) have not been included in this review as they are not neurotransmitters.

The requisite theoretical background was provided by animal studies carried out in the 1950s, which reported that changes in neurotransmission in rat brains led to addiction-related behaviour. Olds and Milner (1954) found that intracranial stimulation of the hypothalamus and
associated structures can act to reinforce operant conditioning. Brain stimulation itself activated systems that were normally activated by a reinforcing stimulus, like feeding or drinking (Deutsch and Howarth, 1963). Brain self-stimulation (using electrodes placed in the vicinity of dopaminergic neurones) was accompanied by locomotor activation and positive reinforcement (Routenberg, 1978). Similarly, it has been hypothesized that euphoria and drug-seeking behaviour (whether induced by alcohol or other commonly abused substances) arise from activation of the mesolimbic dopaminergic reward pathways of the brain (Gessa et al., 1985; Imperato and Di Chiara, 1986; Wise and Rompre, 1989). Endogenous opioids are also reported to be involved in reward processes during brain self-stimulation (Schaefer, 1988). The intracerebroventricular self-administration of β-endorphin in rats has demonstrated that this substance acts as an endogenous positive reinforcer of behaviour. Thus it might intrinsically control behaviour like the self-administration of addictive drugs (Van Ree et al., 1979). In addition, the opioid antagonist naltrexone blocks the release of dopamine at the level of the nucleus accumbens, which follows alcohol administration (Benjamin et al., 1993). However, the results of animal and human studies suggest that the effects of alcohol consumption are not restricted to dopamine and endogenous opioids. Alcohol consumption may also affect several other brain neurotransmitters, including norepinephrine (Ahlenius et al., 1973; Brown and Amit, 1982), serotonin (McBride et al., 1988; Murphy et al., 1988) and GABA (Hwang et al., 1990; McBride et al., 1990).
Alcoholism-related behaviour patterns are defined in terms of the subtypes of alcoholics, like types 1 or 2 (with a clinical-theoretical basis) (Cloninger, 1987) or types A or B (with an empirical basis) (Babor et al., 1992). These types represent a specific clustering of phenotypes with a distinct aetiology of alcoholism. There may be areas in which different typologies overlap one another. For example, type 2 and type B are both related to the early onset of alcoholism, the presence of externalising psychopathology in childhood, multiple drug abuse and aggressive behaviour (Cloninger, 1987; Babor et al., 1992). Phenotypic components could be assessed by measuring neurophysiological parameters, such as the P3 event-related potential (Van der Stelt, 1999). It has been shown, for example, that alcoholics generate a smaller P3 than non-alcoholics (Cohen et al., 1995; Pfefferbaum et al., 1991; Porjesz et al., 1998). The same applies to the children of alcoholics, relative to control children (Begleiter et al., 1984; Hill et al., 1995; Porjesz et al., 1998; Van der Stelt et al., 1998). The P3 phenotypic event-related potential has also been associated with an increase in personality-dimension externalising behaviour (Bauer and Hesselbrock, 1999; Carlson et al., 1999; Van der Stelt et al., 1998).

The methods used to investigate human neurobiological vulnerability to alcoholism include baseline studies, challenge studies and a variety of post-mortem studies. Baseline studies identify individual variations in the trait marker, in the absence of any interference from alcohol. The aim is to investigate the state independence of the trait marker in question. Challenge studies often attempt to identify individual variations of the trait marker after alcohol intake, while some studies use challenge
drugs other than alcohol (acting on one or more of the five neurotransmitter systems under review). This is because any individual variation in sensitivity to the effects of alcohol (or other challenge drugs) might be associated with a variation in the risk of alcoholism (Schuckit et al., 1983; Schuckit and Smith, 1996). Generally, post-mortem studies simply reveal that alcohol has a toxic effect on neurotransmitter systems. The reported results do not usually draw a distinction between the trait and the state effects of alcohol. Baseline and challenge studies are not only performed in alcoholics, but also in high-risk individuals, such as the children of alcoholics (COA). The children of alcoholics represent a unique, high-risk group in studies of the state independence of the trait marker. This is because they have a heightened risk of alcoholism, and because the youngest groups do not generally consume alcohol on a regular basis (Eskay and Linnoila, 1991; Hill et al., 1991; Sher et al., 1991). This review includes neurochemical-trait-marker studies that investigated changes in the levels of neurotransmitters (or their metabolites) in cerebrospinal fluid (CSF) or plasma, and changes in the number, activity and affinity of receptors in the brain. Also included in this review are studies performed on the brain either directly or indirectly (by means of peripheral assessments). There is still some doubt concerning the degree of correspondence between the latter measurements and neurotransmitter activity in the central regions. However, there are problems associated with making some of the above-mentioned measurements in neurobiological studies on humans. For example, it would be ethically indefensible to subject one particular group of high-risk individuals - the young children of alcoholic patents - to invasive or
extensive examinations. This is because such examinations include investigations of the CSF, alcohol challenge tests, and various brain-imaging techniques that use radioactive substances. Neurobiological research in high-risk groups, on the other hand, can be carried out with the aid of peripheral blood samples, using platelets for example (Ratsma et al., 1999).

The aim of this review is to evaluate three trait-marker criteria of possible neurochemical trait markers by reporting challenge, baseline and post-mortem studies in alcoholics and their children.

**GAMMA-AMINOBUTYRIC ACID**

*Pharmacological properties*

γ-Aminobutyric Acid (GABA) acts on the GABA_A receptor, which is a transmitter-gated chloride channel. GABA_A receptors are composed of three sub-units: alpha, beta, and gamma. Fourteen sub-units have been identified to date, six alpha variants, three beta variants, three gamma variants and two delta variants. Alcohol and benzodiazepines act on the gamma sub-unit of the GABA_A receptor (Suzdak et al., 1986), while GABA acts on the alpha sub-unit. Changes in the sub-unit expression of GABA_A receptors are reflected in the pharmacological properties of the receptors (Levitan et al., 1988). The GABA_B receptor, is a G-protein-coupled receptor, without a benzodiazepine binding site.
Research findings

In the post-mortem frontal cortex, expression of mRNA for the GABA<sub>A</sub> receptor's α<sub>1</sub> and β<sub>3</sub> sub-units was higher in alcoholics than in controls (Lewohl et al., 1997) (Mitsuyama et al., 1998). These findings are consistent with the results of two post-mortem studies, in which noncirrhotic alcoholics were found to have more GABA receptors in their cerebral cortex and superior frontal gyrus than the control subjects (Dodd et al., 1992) (Tran et al., 1981). Brain imaging studies have also been performed, using single-photon emission-computed tomography (SPECT) (Abi-Dargham et al., 1998; Lingford-Hughes et al., 1998). These studies found that, in alcoholics and type II alcoholics, the benzodiazepine receptor distribution volume in the anterior cingulate, the prefrontal, frontal, parietal and temporal cortices and in the cerebellum was reduced relative to controls.

Some challenge studies have used the technique of positron emission tomography (PET). This revealed the existence of a blunted glucose utilization response to lorazepam in alcoholics (in the basal ganglia and thalamus) and in the children of alcoholics (in the cerebellum), relative to controls. These findings indicate abnormalities in brain GABA-benzodiazepine receptor reactivity (Volkow et al., 1993; 1995). This might be consistent with previous SPECT studies in alcoholics, which revealed a reduction in benzodiazepine receptor volume. In alcoholics in the early phase of abstinence and those who had been abstinent for 1-2 years, a challenge with gamma-hydroxybutyric acid (a GABA agonist) showed a blunted growth-hormone (GH) response (relative to controls). This also
represents a loss of GABA neurotransmission (Vescovi and Coiro, 1995; 1997).

The findings of blunted, post-challenge GABA responses in alcoholics and COA are not consistent with reported post-challenge plasma GABA levels in COA. GABA plasma levels of COA increased much more in response to alcohol than was the case in controls (Moss et al., 1990). Neutral results were also reported, where there was no difference between the GABA plasma levels in COA and in controls, following a diazepam challenge (Cowley et al., 1996).

A twin study (Berrettini et al., 1982) showed that the inter-individual difference in baseline plasma level of GABA is heritable. Abstinent, adult male alcoholics were found to have a lower baseline plasma GABA level than non alcoholics (Coffman and Petty, 1985; Petty et al., 1993). Plasma GABA levels were also studied in the children of alcoholics. Two studies of plasma GABA in male COA yielded conflicting results. One study showed that COA had lower baseline plasma levels of GABA than controls (Moss et al., 1990) while the other found no such difference (Cowley et al., 1996). The young adult COA in the latter study were highly selected for having no history of alcohol or drug abuse at ages 18-25. This may mean that they were at reduced risk of an early onset of alcoholism, which in turn may have biased the sample.

In conclusion, baseline studies have indicated that the measurement of GABA level in plasma seems to fulfil two criteria as a trait marker for alcoholism. Firstly, the inter-individual difference is heritable and secondly, alcoholics have reduced levels of GABA in their plasma. Challenge studies
have shown that the measurement of decreased GABA receptor responsiveness also appears to fulfil two trait marker criteria. Firstly, it has been reported in alcoholics and secondly, it has also been reported in COA (Table 1 and Table 2).

Table 1. Trait marker criteria for neurochemical baseline activities and responses to challenges

<table>
<thead>
<tr>
<th>Neurochemical markers</th>
<th>Heritable</th>
<th>Presence in alcoholics (compared with controls)</th>
<th>State independent (more common in high-risk groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GABA-ergic activity</strong></td>
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</tr>
<tr>
<td>Baseline (plasma GABA)</td>
<td>yes</td>
<td>decreased</td>
<td>inconsistent</td>
</tr>
<tr>
<td>Challenge (receptor reactivity)</td>
<td>-</td>
<td>decreased</td>
<td>decreased</td>
</tr>
<tr>
<td><strong>Serotonergic activity</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (5HT transporter activity)</td>
<td>yes</td>
<td>increased (long abstinence)</td>
<td>increased</td>
</tr>
<tr>
<td>Challenge (receptor reactivity)</td>
<td>-</td>
<td>decreased</td>
<td>increased (one study)</td>
</tr>
<tr>
<td><strong>Dopaminergic activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (plasma or CSF HVA)</td>
<td>-</td>
<td>inconsistent</td>
<td>inconsistent</td>
</tr>
<tr>
<td>Challenge (receptor activity)</td>
<td>-</td>
<td>decreased (long abstinence)</td>
<td></td>
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<tr>
<td><strong>Noradrenergic activity</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Baseline (CSF norepinephrine)</td>
<td>-</td>
<td>decreased</td>
<td></td>
</tr>
<tr>
<td>Challenge (receptor reactivity)</td>
<td>-</td>
<td>decreased</td>
<td></td>
</tr>
<tr>
<td><strong>β-Endorphinergic activity</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline (plasma or CSF β-endorphin)</td>
<td>-</td>
<td>decreased</td>
<td>decreased (receptor density)</td>
</tr>
<tr>
<td>Challenge (pituitary reactivity)</td>
<td>yes</td>
<td>increased</td>
<td>increased</td>
</tr>
<tr>
<td><strong>GABA Post-mortem studies</strong></td>
<td><strong>GABA Challenge studies</strong></td>
<td><strong>GABA Baseline studies</strong></td>
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<tr>
<td><strong>Alcoholics vs. controls:</strong></td>
<td><strong>Alcoholics vs. controls:</strong></td>
<td><strong>Alcoholics vs. controls:</strong></td>
<td></td>
</tr>
<tr>
<td>increased expression GABA&lt;sub&gt;α&lt;/sub&gt; receptors in frontal cortex (Lewohl et al., 1997; Mitsuyama et al., 1998)</td>
<td>decreased glucose utilization response to agonist lorazepam in basal ganglia, thalamus (Volkow et al., 1993)</td>
<td>decreased benzodiazepine receptor density in the cortex (Lingford-Hughes et al., 1998)</td>
<td></td>
</tr>
<tr>
<td>Alcoholics vs. controls: increased number of GABA receptors frontal and cerebral cortex studies (Dodd et al., 1992; Tran et al., 1981)</td>
<td>decreased growth hormone response to agonist GHB (Vescovi and Coiro, 1995; 1997)</td>
<td>Alcoholics type II vs. controls: decreased benzodiazepine receptor density in the cortex and cerebellum (Abi-Dargham et al., 1998)</td>
<td></td>
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<tr>
<td>COA vs. controls: decreased glucose utilization response to agonist lorazepam in cerebellum (Volkow et al., 1995)</td>
<td>COA vs. controls: neutral plasma GABA levels after alcohol (Moss et al., 1990)</td>
<td>Alcoholics vs. controls: decreased GABA plasma levels (Coffman and Petty, 1985; Petty et al., 1993)</td>
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<tr>
<td>COA vs. controls: neutral plasma GABA levels after agonist diazepam (Cowley et al., 1996)</td>
<td>COA vs. controls: neutral plasma GABA levels after agonist diazepam (Cowley et al., 1996)</td>
<td>COA vs. controls: decreased plasma GABA levels (Moss et al., 1990)</td>
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<tr>
<td>COA vs. controls: neutral plasma GABA levels after agonist diazepam (Cowley et al., 1996)</td>
<td>COA vs. controls: neutral plasma GABA levels (Cowley et al., 1996)</td>
<td>COA vs. controls: neutral plasma GABA levels (Cowley et al., 1996)</td>
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</table>
5-HYDROXYTRYPTAMINE

**Pharmacological properties**

5-Hydroxytryptamine (5-HT, serotonin) acts on 15 known subtypes of 5-HT receptors, which fall into four structural and functional 'families' of receptors. Three types (5-HT₁, 5-HT₂, and 5-HT₄) are coupled to G-proteins, while the 5-HT₃ receptor acts as a 5-HT gated ion channel. Although the genes of three other types (5-HT₅, 5-HT₆, and 5-HT₇) have been cloned, their physiological functions are as yet unknown. The 5-HT transporter is located in the serotonergic axon terminals, where it terminates 5-HT action in the synapse, and in platelet membranes, where it takes up 5-HT from the blood (Blakely et al., 1991). The synthesis of serotonin is influenced by the amount of the precursor tryptophan in the brain. In order to gain entry to the brain, tryptophan has to compete with other amino-acids. The extent to which tryptophan in the blood circulation is available to the brain may be decreased in alcoholics with a positive family history (see review by Badawy, 1999). The concentration of 5-Hydroxyindoleacetic acid (5-HIAA) (the major metabolite of serotonin) in cerebrospinal fluid (CSF) reflects central serotonergic processes (Banki and Molnar, 1981).

**Research findings**

Post-mortem studies of brain tissue from alcoholics have revealed reduced 5-HT transporter binding in the hippocampus (Gross-Isseroff and Biegon, 1988; Chen et al., 1991). Similarly, binding was found to be reduced in the 5-HT₁₅ receptors of individuals who consumed alcohol relative to those of subjects who did not (Dillon et al., 1991).
Following a challenge with a mixed serotonin partial agonist, m-chlorophenylpiperazine (mCPP), the ACTH response and the activation in the basal ganglia circuits (involving orbital and prefrontal cortices) was found to be lower in alcoholics than in non alcoholics (Krystal et al., 1994; George et al., 1997; Hommer et al., 1997). These findings were reflected by a decrease in the brain's utilisation of glucose, as shown by PET. This suggests that 5-HT2C receptors in alcoholics exhibit reduced sensitivity. Other studies reported challenges in which growth hormone, prolactin, and cortisol exhibited blunted responses to fenfluramine (releasing serotonin as an indirect agonist), mCPP, and sumatriptan (a 5-HT1D receptor agonist). The subjects in question were active alcoholics and abstaining alcoholics who had not used alcohol for 1-2 years (Balldin et al., 1994; Krystal et al., 1996; Vescovi and Coiro, 1997). Conversely, an elevated cortisol response was reported after a challenge with fenfluramine in a high-risk group of ADHD (Attention-Deficit Hyperactivity Disorder) boys, versus ADHD boys with a low risk of alcoholism. The assessed degree of risk was based on the presence or absence of parental alcoholism (Schulz et al., 1998).

The results of a study using single photon emission computed tomography (SPECT) showed a reduction in brain-stem serotonin transporters in alcoholics (after 3-5 weeks of abstinence) relative to controls (Heinz et al., 1998). A decrease in alcoholism-related serotonin transport was associated with a decrease in the 5-HT content of platelets in alcoholics (Banki, 1978). It also corresponded to a decrease (relative to controls) in the uptake of $^3$H-5HT by platelets in alcoholics undergoing a two-week period.
of detoxification (Kent et al., 1985). A twin study found that 5-HT uptake in blood platelets is moderated by genetic factors (Meltzer and Arora, 1988).

Alcoholics who had abstained from alcohol for periods of 1 month to 22 years (and their children) were found to have a higher $^3$H-5HT platelet uptake than controls (Ernouf et al., 1993). This is contrary to the findings in active alcoholics and in those who had only recently started practising abstinence. In addition, male COA were found to have a higher Vmax (capacity) of platelet serotonin uptake than controls (Rausch et al., 1991). As a result, it was suggested that an increase (rather than a decrease) in platelet serotonin transport was a trait marker for alcoholism (Ernouf et al., 1993). It is important to note that early-onset alcoholics exhibit higher platelet-serotonin-transporter activity than late-onset alcoholics (Javors et al., 2000).

With regard to 5-HIAA, the levels of this metabolite in CSF taken from abstinent alcoholics of both sexes were lower than in controls (Ballenger et al., 1979; Banki, 1981; Borg et al., 1985). Alcoholic impulsive offenders had lower CSF 5-HIAA levels, than alcoholic non-impulsive offenders, while the controls had intermediate levels (Virkkunen et al., 1994). Early-onset alcoholics had lower 5-HIAA levels than late-onset alcoholics (Fils-Aime et al., 1996). This was consistent with studies demonstrating low precursor serotonin availability in early-onset alcoholism (Buydens-Branchey et al., 1989). CSF 5-HIAA concentrations might be a state-related phenomenon, since essential dietary fatty acids could cause changes in CSF 5-HIAA concentrations (Hibbeln et al., 1998). However, an animal study of CSF 5-HIAA levels in monkeys revealed a high degree of
intra-individual stability (Raleigh et al., 1992). The above-mentioned CSF 5-HIAA studies, which were only performed in alcoholics, were consistent with the hypothesis that decreased CSF 5-HIAA levels represent a central serotonergic deficit in a subgroup of early-onset male alcoholics (Linnoila et al., 1983).

In conclusion, the inter-individual difference in baseline serotonin transporter activity in platelets seems to be heritable. There are also indications that it is elevated in COA and in alcoholics who have been abstinent for a protracted period of time. The measurement of increased baseline serotonin transporter activity in platelets would therefore appear to fulfil three of the criteria for trait markers for alcoholism. Challenge studies of serotonin receptor responsiveness have shown (albeit inconsistently) a decreased 5-HT response in alcoholics. There is also one report of an elevated response in COA, thus one criterion may have been met (Table 1 and Table 3).
<table>
<thead>
<tr>
<th>Serotonin post-mortem studies</th>
<th>Serotonin challenge studies</th>
<th>Serotonin baseline studies</th>
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<tbody>
<tr>
<td><strong>Alcoholics vs. controls:</strong></td>
<td><strong>Alcoholics vs. controls:</strong></td>
<td><strong>Alcoholics vs. controls:</strong></td>
</tr>
<tr>
<td><strong>decreased</strong> binding 5-HT transporter (Gross-Isseroff and Biegon, 1988; Chen et al., 1991)</td>
<td><strong>decreased</strong> glucose utilization in basal ganglia circuits and ACTH response to partial 5-HT agonist mCPP (Hommer et al., 1997)</td>
<td><strong>decreased</strong> brainstem serotonin transporters (SPECT) (Heintz et al., 1998)</td>
</tr>
<tr>
<td><strong>Alcohol consumers vs. controls:</strong></td>
<td><strong>Alcoholics vs. controls:</strong></td>
<td><strong>Alcoholics vs. controls:</strong></td>
</tr>
<tr>
<td><strong>decreased</strong> binding 5-HT$_{1A}$ receptor (Dillon et al., 1991)</td>
<td><strong>decreased</strong> ACTH response to mCPP (Krystal et al., 1994; George et al., 1997).</td>
<td><strong>decreased</strong> platelet serotonin uptake and content (Banki, 1978; Kent et al., 1985)</td>
</tr>
<tr>
<td>Alcoholics vs. controls:</td>
<td>Alcoholics vs. controls:</td>
<td>Alcoholics vs. controls:</td>
</tr>
<tr>
<td><strong>decreased</strong> prolactine and cortisol response to serotonin (Balldin et al., 1994; Krystal et al., 1996)</td>
<td>increased platelet serotonin uptake (Ernof et al., 1993) (long-term abstinence)</td>
<td>COA vs. controls:</td>
</tr>
<tr>
<td>Alcoholics vs. controls:</td>
<td>Alcoholics vs. controls:</td>
<td>increased platelet serotonin uptake (Ernof et al., 1993; Rausch et al., 1991)</td>
</tr>
<tr>
<td><strong>decreased</strong> growth hormone response to 5-HT1D receptor agonist sumatriptan (Vescovi and Coiro, 1997)</td>
<td>COA with ADHD vs. controls with ADHD:</td>
<td>Early-onset vs. Late-onset alcoholics:</td>
</tr>
<tr>
<td>COA with ADHD vs. controls with ADHD:</td>
<td><strong>increased</strong> cortisol response to 5-HT agonist fenfluramine (Schultz et al., 1998)</td>
<td>increased platelet serotonin uptake (Javors et al., 2000)</td>
</tr>
<tr>
<td>Alcoholics vs. Controls:</td>
<td>Alcoholics vs. Controls:</td>
<td>Alcoholics vs. Controls:</td>
</tr>
<tr>
<td>decreased CSF 5-HIAA level (Ballenger et al., 1979; Banki, 1981; Borg et al., 1985).</td>
<td>decreased CSF 5-HIAA level (Fils-Aime et al., 1996)</td>
<td>decreased CSF 5-HIAA levels (Virkkunen et al., 1994)</td>
</tr>
<tr>
<td>Early-onset vs. Late-onset alcoholics:</td>
<td>Impulsive vs. Non-impulsive alcoholics:</td>
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</table>
DOPAMINE

Pharmacological properties

Dopamine acts on at least five dopamine receptors, which are divided into two major groups: (1) the D1-like receptors (D1 and D5) and (2) the D2-like receptors (D2, D3, and D4). All dopamine receptors are G-protein-coupled receptors, the D1 receptor stimulates adenylyl cyclase activity while the D2 receptor inhibits adenylyl cyclase activity. Homovanillinic acid (HVA) is the major metabolite of dopamine.

Research findings

In one challenge study, male alcoholics who had been abstinent for a period of 7 years (+6 years) exhibited reduced postsynaptic-dopamine-receptor sensitivity relative to controls. This was expressed as a reduced maximum growth hormone (GH) response to the agonist apomorphine (Balldin et al., 1992). This report was consistent with the reduction in GH-response (relative to controls) seen in alcoholics who had been abstaining from alcohol for periods ranging from 8 days to 6 months (Dettling et al., 1995). However, an earlier study showed that, after a withdrawal state of 4-7 days, alcoholics exhibited an elevated GH response to dopamine infusion, whereas their GH responses to apomorphine were similar to those of controls (Annuziato et al., 1983; Balldin et al., 1985). The dopamine receptor involved in GH secretion was thought to be a D2-type receptor, while D1 receptors are believed to be involved in prolactin secretion (Fabbrini et al., 1988). The reduction in postsynaptic-dopamine-D2-receptor-sensitivity in alcoholics who had been abstaining from alcohol for
at least 8 days might be a trait marker for alcoholism. This may be consistent with the dopaminergic hypothesis of alcoholism (Eckardt et al., 1998; Ikemoto et al., 1999; Koob et al., 1998). It may also account for the fact that, prior to detoxification, active alcoholics exhibit a reduced prolactin response to the antagonist haldol. This response reverted to control values thirteen days after detoxification (Markanios et al., 2000).

Baseline studies of homovanillinic acid (HVA), found that alcoholics had lower levels of this metabolite in their plasma than did control subjects (Fulton et al., 1995). However, the HVA levels found in the CSF of alcoholics and in the plasma of male COA were similar to those found in controls (Petrakis et al., 1999; George et al., 1999; Howard et al., 1996; Limson et al., 1991; Roy et al., 1990). Early-onset alcoholics were found to have lower levels of HVA in their CSF than late-onset alcoholics (Fils-Aime et al., 1996). These results were contradicted by other studies, however, which found increased HVA CSF levels in early-onset alcoholics relative to late-onset alcoholics (George et al., 1999; Petrakis et al., 1999).

In conclusion, these inconsistent baseline studies show that the changes in dopaminergic neurotransmission that are associated with alcoholism do not appear to fulfil either of the reviewed criteria for a trait marker for alcoholism. The challenge studies in alcoholics who had been abstaining for a protracted period of time showed a decreased dopamine receptor response that seems to fulfil one of the criteria for a trait marker, namely that it is present in alcoholics (Tables 1 and 4).
<table>
<thead>
<tr>
<th>Dopamine challenge studies</th>
<th>Dopamine baseline studies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcoholics vs. controls:</strong> decreased GH response to apomorphine (Balldin et al., 1992) (Dettling et al., 1995) (long-term abstinence)</td>
<td><strong>Alcoholics vs. controls:</strong> neutral HVA CSF levels (Petrakis et al., 1999; George et al., 1990; Limson et al., 1991; Roy et al., 1990)</td>
</tr>
<tr>
<td><strong>Alcoholics vs. controls:</strong> increased GH response to dopamine infusion (Annuziato et al., 1983) (short-term abstinence)</td>
<td><strong>Alcoholics vs. controls:</strong> decreased HVA plasma levels (Fulton et al., 1995)</td>
</tr>
<tr>
<td><strong>COA vs. controls:</strong> neutral GH response to apomorphine (Balldin et al., 1985) (short-term abstinence)</td>
<td><strong>Early-onset vs. late onset alcoholics:</strong> decreased HVA CSF levels (Fils-Aime et al., 1996)</td>
</tr>
<tr>
<td><strong>Alcoholics vs. controls:</strong> decreased prolactine response to haldo during alcohol intake, neutral response after detoxification (Markianos et al., 2000)</td>
<td><strong>Early-onset vs. late onset alcoholics:</strong> increased HVA CSF levels (George et al., 1999; Petrakis et al., 1999)</td>
</tr>
</tbody>
</table>
NOREPINEPHRINE

Pharmacological properties
Norepinephrine acts on various adrenergic receptors (two α receptors and two β receptors), all of which are G-protein coupled. The α₁ adrenergic receptor is linked to the mobilisation of intracellular calcium by phospholipase C, while the α₂ adrenergic receptor inhibits adenylyl cyclase. The β₁ and β₂ adrenergic receptors stimulate adenylyl cyclase. A major metabolite of norepinephrine is 3-methoxy-4-hydroxyphenylglycol (MHPG).

Research findings
Reduced numbers of melanin-containing noradrenergic neurones were found in the post-mortem locus ceruleus of alcoholics, which indicates a lower level of noradrenergic neurotransmission (Arango et al., 1994). This was inconsistent with the findings of Baker et al., (1994), however, which showed no differences post-mortem. In a baseline study, cerebrospinal fluid and plasma were continuously sampled in controls and in alcoholics who had been abstaining for 38-124 days (Geracioti et al., 1994). The results showed that CSF norepinephrine was lower in alcoholics than in controls, which was consistent with the findings of Arango et al., (1994). Although there were no differences in terms of the norepinephrine levels in plasma (Geracioti et al., 1994), alcoholics were found to have a lower basal level relative to controls (Ehrenreich et al., 1997).

The same study reported that, following a challenge with human CRF or a stress-test, the norepinephrine response was unaffected.
In addition, alcoholics were found to have a blunted growth hormone response to clonidine (an $\alpha_2$ adrenergic receptor agonist) in comparison to controls. The effect was found shortly after detoxification and also after six months of abstinence (Glue et al., 1989; Berggren et al., 2000). Similarly, in comparison to controls, alcoholics were found to have an enhanced cortisol response to yohimbine (an $\alpha_2$ adrenergic receptor antagonist). This reflects the down-regulation of postsynaptic noradrenergic receptors that is observed in alcoholics (Krystal et al., 1996).

In conclusion, both the basal studies of decreased norepinephrine levels in CSF (but not in plasma) and the challenge studies of receptor responsiveness showed that a decrease in noradrenergic neurotransmission seems to fulfil one criterion for a trait marker, that it should be present in alcoholics (Table 1 and Table 5).

Table 5. Norepinephrine studies

<table>
<thead>
<tr>
<th>Norepinephrine post-mortem studies</th>
<th>Norepinephrine challenge studies</th>
<th>Norepinephrine baseline studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholics vs. controls: decreased number of noradrenergic neurones in locus ceruleus (Arango et al., 1994)</td>
<td>Alcoholics vs. controls: neutral response of norepinephrine in plasma after CRF or a stress test (Geraciotti et al., 1994; Ehrenreich et al., 1997)</td>
<td>Alcoholics vs. controls: decreased levels of norepinephrine in CSF (Geraciotti et al., 1994)</td>
</tr>
<tr>
<td>Alcoholics vs. controls: neutral number of noradrenergic neurones in locus ceruleus (Baker et al., 1994)</td>
<td>Alcoholics vs. controls: increased cortisol response to antagonist yohimbine (Krystal et al., 1996)</td>
<td>Alcoholics vs. controls: neutral levels of norepinephrine in plasma (Geraciotti et al., 1994)</td>
</tr>
<tr>
<td>Alcoholics vs. controls: decreased growth hormone response to agonist clonidine (Glue et al., 1989; Berggren et al., 2000)</td>
<td>Alcoholics vs. controls: decreased levels of norepinephrine in plasma (Ehrenreich et al., 1997)</td>
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</tr>
</tbody>
</table>
BETA-ENDORPHIN

Pharmacological properties
Opioids act on three major classes of opiate receptors: mu, delta and kappa. These G-protein-coupled receptors, which inhibit adenylyl cyclase, are distributed throughout the central nervous system. The distribution of β-Endorphin, however, is somewhat restricted. It is primarily confined to neurones in the hypothalamus which send projections to the periaqueductal grey region and to noradrenergic nuclei in the brain stem.

Research findings
Baseline studies in humans have shown that, in individuals at risk of alcoholism, the basal activity level of the endogenous opioid system might be lower than in individuals with a low risk of alcoholism (Volpicelli et al., 1990). Such a finding would be consistent with the opioid-deficiency hypothesis. This was supported by the fact that the β-endorphin levels in the plasma and cerebrospinal fluid of alcoholics were lower than those found in controls (Aguirre et al., 1990; Genazzani et al., 1982). It was reported in one study that alcohol may have an equally strong effect on the central levels of β-endorphin as it does on the peripheral levels (Peterson et al., 1996). A lower dose of naloxone completely blocked any reduction in synaptic opioid content and/or opioid receptor density in high risk individuals. This indicates that endogenous hypothalamic opioid activity levels in these individuals are lower than those in low-risk subjects (Wand et al., 1998). Using a placebo, it was found that cortisol levels in high-risk subjects were higher than in low-risk subjects. This indicates that there is probably no
increase in opioid receptor affinity, as this would result in more inhibitory tone, and lower cortisol levels.

With regard to challenge studies, the opioid antagonist naloxone produced higher cortisol and β-endorphin responses in alcoholics than in controls. It also caused alcoholics to reduce their consumption of ethanol (Kemper et al., 1990; O'Malley et al., 1992; Volpicelli et al., 1992). The ingestion of a moderate dose of ethanol induced a dose-dependent increase in plasma β-endorphin levels in high-risk subjects but not in low-risk subjects. Although these elevated levels were not produced by the peripheral adrenal cortisol system, the more central pituitary β-endorphin system showed an increased sensitivity to ethanol. This might mediate ethanol’s reinforcing effects in high-risk subjects (Gianoulakis et al., 1989;1996). These baseline and challenge studies are consistent with the opioid-deficiency hypothesis. In a twin study, the inter-individual difference in β-endorphin response to alcohol was found to be heritable (Froehlich et al., 2000).

In conclusion, the measurement of decreased β-endorphin baseline activity appears to fulfil two of the reviewed criteria for a trait marker for alcoholism. These are lower β-endorphin levels in alcoholics and decreased opioid receptor density in COA. However, the measurement of increased β-endorphin responsiveness seems to fulfil three such criteria in that it is heritable, it is present in alcoholics and it is state independent (present in COA). (Table 1 and 6).
### Table 6. β-Endorphin studies

<table>
<thead>
<tr>
<th>β-Endorphin challenge studies</th>
<th>β-Endorphin baseline studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholics vs. controls:</td>
<td>Alcoholics vs. controls:</td>
</tr>
<tr>
<td><em>increased</em> cortisol response to naloxone (Kemper et al., 1990)</td>
<td><em>decreased</em> basal level of β-endorphin in plasma (Aguirre et al., 1990)</td>
</tr>
<tr>
<td>Alcoholics vs. controls:</td>
<td>Alcoholics vs. controls:</td>
</tr>
<tr>
<td><em>increased</em> β-endorphin response to naloxone (O'Malley et al., 1992)</td>
<td><em>decreased</em> basal level of β-endorphin in CSF (Genazzani et al., 1982)</td>
</tr>
<tr>
<td>Alcoholics vs. controls:</td>
<td>COA vs. controls:</td>
</tr>
<tr>
<td><em>decreased</em> alcohol consumption with naloxone (Volpicelli et al., 1992)</td>
<td><em>decreased</em> opioid receptor density (Wand et al., 1998)</td>
</tr>
<tr>
<td>COA vs. controls:</td>
<td></td>
</tr>
<tr>
<td><em>increased</em> β-endorphin response to alcohol (Gianoulakis et al., 1989; 1996)</td>
<td></td>
</tr>
</tbody>
</table>
CONCLUSIONS

The present report reviewed five neurotransmitters: GABA, serotonin, dopamine, norepinephrine and β-endorphin (see Table 1). Two neurochemical markers seemed to fulfil the three reviewed criteria of a trait marker for alcoholism. The first possible marker is the measurement of increased baseline activity of the serotonin transporter in platelets. This is heritable, and is present in both long-term abstinent alcoholics and in COA. The second marker is the measurement of increased responsiveness of the pituitary β-endorphin system to challenges. This is heritable, and is present in both alcoholics and COA. Both possible neurochemical markers have yet to be assessed in family co-segregation studies to determine whether the marker segregates with alcoholism within families, to prevent false positive conclusions.

It can be concluded from the GABA challenge studies in alcoholics and in COA that the measurement of decreased responsiveness in GABA neurotransmission fulfils two of the reviewed criteria for a trait marker, it is present in both alcoholics and in COA. Further studies on the question of heritability are needed. Another possible trait marker is the measurement of reduced baseline GABA levels in plasma. Its properties must be further studied in the children of alcoholics, to assess the state independence of these effects.

Studies on serotonin activity in alcoholics and COA have shown that increases in serotonin transporter activity can be masked by the recent consumption of alcohol, which may lead to a decrease in serotonin transporter activity in active and newly abstinent alcoholics. This may also
hold true for the use of challenge studies to determine the presence of a decreased postsynaptic serotonin receptor response in alcoholics versus an elevated response in COA. The serotonin post-mortem studies that have been reviewed also showed decreased binding to the serotonin transporter and receptor. This may be consistent with a masking effect produced by alcohol (Menninger et al., 1998).

Although the challenge and baseline studies of dopamine activity in alcoholics produced contradictory results, decreased receptor reactivity was found in alcoholics who had been abstaining for a protracted period of time. The measurement of dopamine levels in plasma poses certain problems, however the use of neuro-imaging techniques (which measure central dopaminergic functions) holds considerable promise for the future.

Indices of changes in the norepinephrine system all corresponded to a fall in norepinephrine baseline levels, and to a reduction in postsynaptic-α2-adrenergic-receptor reactivity. There may be reduced activity in the norepinephrine system, of which the assessment might represent a trait marker for alcoholism. However, further studies are required on the presence of these possible markers in COA, and on their heritability.

The reduced baseline level of β-endorphin in alcoholics, together with reduced opioid receptor density in COA and an elevated β-endorphin response to alcohol and naloxone in alcoholics and COA, might be consistent with the opioid-deficiency hypothesis. The finding that inter-individual differences in challenged β-endorphin levels are heritable suggests that the measurement of increased responsiveness of the β-endorphin system may be a trait marker for alcoholism.
In the course of producing this review, no familial co-segregation studies were found on possible neurochemical trait markers and alcoholism. Such studies would have been of use in addressing the issue of possible false-positive conclusions. However, many familial and population-based linkage studies have been carried out into candidate, neurochemistry-related, alcoholism genes. The Collaborative Study of the Genetics of Alcoholism (COGA) has performed linkage analyses in a large number of families with severe alcoholism. The COGA is a multicentre study for the detection and characterization of genes that influence susceptibility to alcohol dependence and related phenotypes. Data from COGA have been used to study the linkage of vulnerability for alcoholism on regions of chromosomes 1, 2 and 7 (Reich et al., 1998). Neurogenetic candidate genes on chromosomes 4 and 11 have been reported in a genome-wide scan for genetic linkage to alcohol dependence in a South-western American Indian Tribe (Long et al., 1998). The regions in question are those near the β1 GABA receptor gene on chromosome 4, and near the tyrosine hydroxylase and dopamine D4 receptor genes on chromosome 11.

Genome-wide screenings must be followed by investigations in alcoholics and COA, to determine whether the candidate gene has a real causal relationship with the development of alcoholism over time. As pointed out in the review by Rutter (1994), it is essential that the component of a multidimensional phenotype of alcoholism be accurately defined and that it be strongly homogeneous in genetic terms. This indicates that further research is needed on the etiological heterogeneity, mode of transmission
and incomplete penetrance of the genes involved in alcoholism (Devor et al. 1994).

In conclusion, this review presents three main items. Firstly, two possible neurochemical markers fulfil three criteria of a trait marker for alcoholism. The markers in question are the measurements of increased baseline activity of the serotonin transporter in platelets and increased responsiveness of the pituitary β-endorphin system to challenges. Secondly, a description of changes in GABA, dopamine and norepinephrine neurotransmitter systems is presented. As yet, however, these do not fulfil all three reviewed criteria for a trait marker for alcoholism. Thirdly, we would like to emphasize that the use of high-risk groups is essential (a view supported by the many inconsistent studies in COA). Such an approach facilitates investigations into the state independence of a trait marker. This is due to the marked influence of alcohol on neurotransmitter systems, which results in state dependent changes after alcohol use. It also makes it possible to determine (preferably by the use of longitudinal data) whether an index of a change within a neurotransmitter system has a causal relationship with the development of alcoholism. If so, this would mean that it could serve as a trait marker. Trait markers for alcoholism which do not yet fulfil all of the criteria may do so in the future, once they have been more extensively studied.
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