The establishment of scientific proof for the identification of a marker for alcoholism has a number of in-built difficulties. Because alcoholism is a multi-factorial psychiatric disorder, with both psychosocial and biochemical/genetic factors leading to its manifestation in any one individual, the presence of biochemical/genetic factors alone may not lead to the manifestation of the disorder. There are numerous difficulties associated with identification of a trait abnormality in a disorder that requires suitable socio-cultural permissiveness with distinct behavioural characteristics to manifest a disorder that may not require that predisposing trait abnormality in order to develop. Numerous studies have been performed in the past to potentially identify a biochemical or genetic trait abnormality in alcoholism, and not all of them have addressed significant methodological flaws in this type of research. This review addresses some of the difficulties inherent in this research, and aims for a comprehensive review of the highlights of the search for a clinically useful trait abnormality. Some series of investigations hold promise that a trait marker for a particular subset of alcoholics may be developed, e.g. severe alcoholism and the dopamine D2 receptor gene; the level of reaction to alcoholism in family history-positive alcoholics; β-endorphin abnormalities in specific family groups of alcoholics; reduced P3 wave event-related potentials as markers and predictors of development of substance abuse in predisposed youths; reduced growth hormone response to apomorphine as a predictor of relapse to alcoholism in early abstinence; abnormal adenylyl cyclase activity in certain defined subgroups of alcoholics; and abnormal platelet monoamine oxidase levels in subjects with a behavioural predisposition to addictive disorders. The review concludes that while there has not yet been an identification of a comprehensive trait marker for alcoholism, there is hope for identification subgroups of alcoholics with consistent biological markers within that subgroup that may well prove fruitful over time. It will then be up to a future generation of clinicians to take that information and develop prevention programmes that can incorporate this information to help the predisposed individual avoid alcohol problems.
to differentiate a potential genetic marker for the development of alcoholism from a potential marker for the development of alcohol-related disease or tissue damage, a separate matter which is not encompassed within this review. To preserve the clinical focus of this review, we will also confine it to the discussion of human studies, rather than the much broader literature of basic research.

Investigations of non-alcoholic family members of genetically ‘loaded’ families can help identify predisposing factors. However, the extent of family history may have significant impact on whether a trait can be defined. Trait studies conducted in families with highly significant family histories may simply identify those traits in particular families that may not be applicable to traits of alcoholism in general. Trait studies conducted in families with more modest genetic loading run the risk of non-detection of a potential trait, because of dilution of that identifying factor. There is also no guarantee that any individual with a significant family history is actually going to develop the disorder, and thus spot studies on ‘those at risk’ may just present interesting associations that bear no relation to actual risk. Indeed, some individuals with significant family histories may be regarded as being at less risk of development of the disorder by virtue of becoming ‘teetotallers’ in response to this family experience of alcoholism.

Ideally an alcoholic trait marker would be identified by the following types of studies: identification in a population of alcoholics, preferably abstinent; reliability within the population of alcoholics; an absence of any significant confounder that renders the marker redundant; identification in a group of ‘at risk’ i.e. in those non-alcoholics with strong family histories of alcoholism; persistence of that trait in this population over time; and prediction of development of alcoholism or substance abuse by presence of the trait. Such an approach will avoid the possible confusion that may arise from a failure to distinguish between consumption or state markers, such as carbohydrate-deficient transferrin (Stibler and Borg, 1986), 5-hydroxytryptophol excretion (Beck et al., 1982), or haemoglobin modification (Lindross, 1989), from genuine markers of susceptibility to alcoholism.

By identifying not just the positive findings of various scientific groups, but by delineating the potential limitations of each line of investigation, we hope to clarify the current confusing scene and to help identify the most positive potential lines of investigation for the future. It is perhaps unlikely that the holy grail of a single genetic/biochemical marker for alcoholism, defining those predisposed to alcoholism before the development of the disorder, and also delineating those who are currently alcoholic, or who have suffered from it in the past, will ever be found. It is far more likely that various markers defining trait biochemical abnormalities or personality characteristics predisposing to alcoholism may be found, but these are unlikely in themselves to produce the complete disorder. It is far more likely that a number of trait markers will be identified that individually or in tandem may help predict genuine risk in those with a significant family history, or even those who may be at risk of alcoholism even without a significant family history. It will then be up to a future generation of clinicians to take that information and develop prevention programmes that can incorporate this information to help the predisposed individual avoid alcohol problems. The following is an account of important studies on trait markers for alcoholism, which are also outlined in Table 1.

ENZYMATIC MARKERS

Platelet monoamine oxidase

Platelet monoamine oxidase (MAO) activity has been investigated in psychiatric disorders for over thirty years. Originally MAO activity was investigated in schizophrenia (Wyatt et al., 1973), and in affective disorders (Belmaker et al., 1980) because of the theory of monoamine abnormalities in schizophrenia and affective disorders being reflected in platelet monoamine abnormalities, due to the similarity of the developmental origins of nerves and platelets, and the similarity of function of the serotonergic system in the neuronal and platelet membranes (Kleinman et al., 1979). Although these associations produced a significant amount of research, there was no specific psychiatric disorder associated with low platelet MAO activity (Fowler et al., 1982). In the 1970s, Gottfries and colleagues found a lowered MAO activity in the post-mortem brains of alcoholic suicides, relative to non-alcoholic controls, but found a lesser difference between alcoholic suicides and non-alcoholic suicides, implying some degree
of interaction between affective disorder and alcoholism (Gottfrries et al., 1975). This research provoked an interest in using platelet MAO as a peripheral marker of central MAO activity in alcoholism, and the next few years saw a significant number of studies exploring the association not only between low platelet MAO activity and alcoholism (Takashashi et al., 1976; Brown, 1977; Major and Murphy, 1978; Sullivan et al., 1978, 1979; Major et al., 1981; Faraj et al., 1987, 1994; Hallman et al., 1990; Mukasa et al., 1990; Yates et al., 1990) but also between low MAO activity and type 2 alcoholism (Von Knorr ing et al., 1985; Pandey et al., 1988; Sullivan et al., 1990; Devor et al., 1993a; Anthenelli et al., 1995). Type 2 alcoholism is a proposed subset of alcoholism that is associated with a positive family history of alcoholism, early age of onset of alcoholism, male
predominance, and a history of sociopathic activity (see Von Knorning et al., 1985), a distinction that may or may not be clinically relevant (Schuckit and Irwin, 1989). Because the original proponent of type 2 versus type 1 alcoholism (Cloninger, 1987) also proposed that type 2 alcoholics had abnormalities of serotonergic, dopaminergic and noradrenergic activity, platelet MAO activity might theoretically reflect serotonergic abnormalities in type 2 alcoholism.

Unfortunately this finding of lowered MAO activity in alcoholism has not been consistently replicated (Giller and Hall, 1983; Sherif et al., 1992; Farren et al., 1998a) and neither has the finding of lowered MAO activity in type 2 alcoholics (Yates et al., 1990; Anthenelli et al., 1998; Farren et al., 1998a). Another group (Tabakoff et al., 1988) reported that platelet monoamine oxidase activity, although not depressed in alcoholics, was significantly more sensitive to inhibition by ethanol in vitro. Some of these original studies were marred by the inclusion of subjects with affective disorders in the subject pool (Von Knorning et al., 1985; Pandey et al., 1988) and some studies have not clearly separated out subjects with primary antisocial personality disorder or a predominance of sociopathic traits (von Knorning et al., 1985; Yates et al., 1990; Faraj et al., 1994). Probably the biggest problem has been the failure to address the potential confounder of cigarette smoking on the results. Smoking has been found to produce lowered platelet MAO activity (Fowler et al., 1996), and indeed a diminished MAO activity has been found in smokers relative to healthy controls (Oreland et al., 1981; Norman et al., 1982; Von Knorning and Oreland, 1985). Analysis of the data of the large Collaborative Study on the Genetics of Alcoholism (COGA) has suggested that smoking status or male gender might explain most of the lowered platelet MAO activity found in alcoholics, relative to healthy controls, in that data set (Anthenelli et al., 1998) and that alcohol dependence per se did not predict a lowered level. Most of the previous studies in alcoholics simply did not take account of their subjects’ smoking status, and in one study that did, no differences between alcoholics and controls was found (Farren et al., 1998a).

Despite this, it is difficult to ignore the literature suggesting an association between alcoholism and lowered platelet MAO activity. It is quite probable that some association does exist, but the nature of that association is not yet clear. There is some evidence for lowered platelet MAO activity being associated with some personality characteristics and types, such as sensation-seekers and type A personalities (Ward et al., 1987; Smith, 1994), that may be associated with alcoholism (Von Knorning et al., 1987). There is also an association between lowered platelet MAO activity and non-specific psychopathology (Fowler et al., 1982), and a higher incidence of psychopathology has been found in relatives of alcoholics with a low platelet MAO activity (Devor et al., 1993b). Alcoholism is a disorder of genetic as well as psychosocial origin, but the nature of that genetic abnormality is not yet clear. If part of that genetic transmission is certain personality traits or characteristics, such as novelty seeking, reward dependence and harm avoidance (Cloninger, 1987), and these traits prove to be associated with platelet MAO activity, then platelet MAO has a future as a potential marker for alcoholism or of predisposition to alcoholism. However, that association has not yet been fully defined and thus it is difficult to predict the future status of platelet MAO as a marker for alcoholism. Aside from methodological problems, the story of platelet MAO and alcoholism, varying from significantly positive to significantly negative associations, illustrates the difficulty in identifying a single marker for a multi-factorial disorder.

Other enzymatic markers

Studies seeking a trait marker in an ill-defined group of alcoholics may have been confounded by a failure to treat the data from the two subgroups mentioned above separately. This may, for example, account for the conflicting reports as to whether reduced levels of erythrocyte aldehyde dehydrogenase are a trait or state marker for alcoholism (Thomas et al., 1982; Agarwal et al., 1983) or whether there are specific differences in the number or types of alcohol and aldehyde dehydrogenase isoenzymes that characterize alcohol-susceptible individuals (see Tipton et al., 1983). Similarly, this might account for the failure of Ward et al. (1983) to reproduce the reported (Schuckit and Rayses, 1979) elevation of acetaldehyde levels in first-degree relatives of alcoholics, as compared to control subjects, although both studies might have suffered from various methodological problems (see Lindross, 1989).
Adenylyl cyclase (AC) is an adenosine receptor-coupled enzyme and an important receptor-G-protein-coupled effector that is present in lymphocytes and platelets. Adenylyl cyclase activity can be stimulated by various agents including agents working through G-proteins, and some G-proteins may themselves be abnormal in short-term and long-term abstinent alcoholics (Lichtenberg-Kraag et al., 1995). A pilot study some years ago reported that lymphocytes from a number of alcoholics had significantly reduced adenosine-stimulated cAMP levels, as well as basal cAMP levels, relative to matched non-alcoholic control groups (Diamond et al., 1987). Lymphocytes from alcoholics cultured over many generations in the absence of ethanol show increased adenosine receptor-dependent cAMP production and also increased sensitivity to ethanol-induced desensitization, suggesting that these abnormalities could be used as markers for alcoholism (Diamond et al., 1991). In a larger study, Tabakoff et al. (1988) found a significant lowering of levels of platelet adenylate cyclase, notably after stimulation with fluoride, in alcoholics who were abstinent for up to 4 years. Subsequent studies have replicated this finding (Lex et al., 1993; Saito et al., 1994; Parsian et al., 1996) and also showing lowered levels of AC activity in family history-positive men, relative to family history-negative men and controls (Saito et al., 1994), and in family history-positive non-alcoholic women, relative to family history-negative non-alcoholic women (Lex et al., 1993). There were no differences, however, between alcoholics divided according to Cloninger’s hypothesis in adenylyl cyclase activity (Parsian et al., 1996). A subsequent study did find that platelet adenylyl cyclase activity was lowered in alcoholics, and was most closely correlated to subtypes of alcoholics: those that developed negative mood in relation to drinking; who continued drinking despite health effects; and those who became violent while drinking (Ikeda et al., 1998). This fluoride-stimulated effect was attributed to a single major genetic locus with a small multi-factorial background (Devor et al., 1991).

ELECTROPHYSIOLOGICAL MARKERS

The potential for electroencephalographic (EEG) response abnormalities predicting development of alcoholism in those with significant family histories for alcoholism has been explored in a number of ways. The overall EEG responses to alcohol challenges have been examined, and a hypothesis of greater EEG response to alcohol predicting later development of alcoholism was developed (Pollock et al., 1983). This theory was based on the tension-reduction theory of alcoholism, suggesting that pre-alcoholics are tense, and alcohol reduces tension, and thus such individuals are at increased risk for development of alcoholism. This has been refuted in a follow-up study of subjects at risk of alcoholism, where a low EEG response to alcohol was found to predict subsequent development of alcoholism at 10-year follow-up (Volavka et al., 1996). This finding was consistent with the theory that predisposition to alcoholism may be mediated by a low innate sensitivity to the effects of alcohol (Schuckit and Smith, 1996). Inter-hemispheric EEG coherence has also been studied in alcoholism, and a significant correlation found in the frontal and parietal leads in those at high risk for development of alcoholism (Michael et al., 1993), although the significance of this finding is not yet clear.

Event-related potentials (ERPs), most notably the P3 wave, in response to visual and auditory evoked potentials, have been studied in risk groups for alcoholism as potential markers for risk by a number of different groups of investigators. An early study examined the P3 auditory wave after doses of alcohol or placebo in young subjects with and without family histories of alcoholism, and found longer latencies and smaller amplitudes in the children of alcoholics (Elmasian et al., 1982). Others failed to replicate this finding (Polich and Bloom, 1988; Schuckit et al., 1988a). Studies on adults with positive family histories of alcoholism, tested without ethanol ingestion, have reported both a significant diminution in P3 amplitude in some studies (Porjesz and Begleiter, 1990; Benegal et al., 1995) and no difference in P3 amplitude (Parsons et al., 1990; Bauer et al., 1994). However, P3 latency does not appear to be as closely linked with family history of alcoholism in the adult, but rather appears to be related to amount of alcohol consumption (Polich and Bloom, 1987).

Studies examining the adolescent and child populations appear to show more consistent results, and do not vary as much as the adult studies in terms of sample populations studied. Begleiter’s
group reported that 7–15-year-old sons of alcoholics showed a smaller P3 wave amplitude in response to a visual task and that these patterns resembled those of abstinent alcoholics (Begleiter et al., 1984). These results were confirmed using auditory evoked potentials (Begleiter et al., 1988). Others also found reduced amplitude in ERPs in sons of alcoholics (Whipple and Noble, 1986). Recent studies with girls as well as boys have confirmed the reduced P3 amplitude in male children of alcoholics (Hill and Steinhauer, 1993), and one study in female children with multi-generational alcoholism reported delays in P3 visual latency (Rodriguez-Holguin et al., 1998). Most importantly, the predictive potential of these abnormalities has been confirmed in a number of studies that have followed up those children with significant P3 amplitude reduction and found these abnormalities to be significant predictors of subsequent adolescent and young-adult substance abuse (Berman et al., 1993; Hill et al., 1995). The largest data set used to examine this question overall, the COGA study, recently found that visual P3 amplitude ERP means were significantly lower in all alcoholic sex- and age-matched groups, relative to healthy controls, and also found that affected males had lower amplitudes than affected females, and that affected individuals from alcoholics’ families also had lower P3 amplitudes than unaffected individuals, strongly suggesting this as a phenotypic marker (Porjesz et al., 1998).

The EEG itself has a significant genetic heritable component, and a series of studies in healthy subjects, in monzygotic twins reared together, in monzygotic twins reared apart, in dizygotic twins reared together, and in dizygotic twins reared apart, has helped to demonstrate this heritability (Stassen et al., 1988). Moderate to strong genetic influences on EEG coherence (a coupling between two EEG signals from different brain areas), which is taken as an index of the connectivity of the brain, have been found in a large number of pairs of 5-year-old twins (van Baal et al., 1998). These studies also indicated that long connections, such as sensory-to-frontal connections, are primarily genetic in origin, whereas cortico-cortical connections between adjacent brain areas are largely explained by environmental influences. A recent study in 15–17-year-old twins has also confirmed these findings, with all monzygotic correlations being larger than corresponding dizygotic correlations (McGuire et al., 1998). These studies lend credence to the theory of heritability of EEG abnormalities in alcoholism.

NEUROCHEMICAL MARKERS

Abnormalities in different neurochemical systems in alcoholism have been described, and significant attempts made to correlate results with various phenotype characteristics of alcoholics such as severity, duration of abstinence, age of onset and family history of alcoholism.

The opioid system

Long-term abstinent alcoholics were reported to have decreased serum β-endorphin levels, relative to healthy matched controls, even after 10 years of abstinence in one study (Del Arbol et al., 1995). These authors suggested that this might be a cause, rather than a result, of alcoholism. Gianoulakis et al. (1989) also found lowered β-endorphin levels in non-alcoholic subjects with strong family histories of alcoholism (high risk), relative to those with no family history of alcoholism (low risk), and also in alcoholics who had been abstinent for a least 6 months (Gianoulakis et al., 1989, 1996). This helped lead to the development of the ‘opioid deficiency hypothesis’ of alcoholism, suggesting that high-risk subjects have an inherited deficiency in the basal activity of the endogenous opioid system (Volpicelli et al., 1990). This and other studies found that the β-endorphin response to a moderate dose of alcohol was increased in a dose-dependent manner in the high-risk subjects but not in the low-risk subjects, supporting the hypothesis (Gianoulakis et al., 1989, 1996). More recently, evidence for a significantly diminished cortisol response to low-dose naloxone in family history-positive, relative to family history-negative, non-alcoholics has given support to the theory for a central opioid ‘tone’ deficiency (less synaptic opioid content or reduced opioid receptor density) in those predisposed to alcoholism, or at least a difference in opioid receptor binding affinity for naloxone (Wand et al., 1998). The finding of a diminished hypothalamic–pituitary response to naltrexone in non-alcoholics, relative to abstinent alcoholics, also suggests a central opioid tone deficiency in alcoholism (Farren et al., 1998b),
although Inder et al. (1995) measured the cortisol responses to 20 mg of the opiate antagonist naloxone in abstinent alcoholics (mean 25 days abstinent) and healthy controls and found a similar cortisol response in both groups. Whereas the benefit of the opioid antagonist naltrexone in the treatment of alcoholism has demonstrated the significance of the various opioid system abnormalities in alcoholism, a definitive ‘opioid deficient’ marker has yet to be delineated (O’Malley et al., 1992; Volpicelli et al., 1992). Why naltrexone is beneficial is not absolutely clear. Naltrexone may decrease craving for alcohol (Volpicelli et al., 1992) but not consistently (O’Malley et al., 1992; Volpicelli et al., 1997), and may reduce the ‘high’ that is associated with alcohol consumption (Volpicelli et al., 1995), and also reduce the stimulating effects and increase the sedating effects of alcohol (Swift et al., 1994). Alcohol consumption may itself stimulate the endogenous opioid system (Gianoulakis, 1993), and this also is blocked by naltrexone. Since naltrexone is at its most effective in preventing relapse to heavy drinking in those who are not abstinent (O’Malley et al., 1992; Volpicelli et al., 1992), naltrexone may exert its therapeutic effect by blocking the stimulation of the opioid system by ethanol in these subjects, possibly through the extinction mechanism (Sinclair, 1997). Naltrexone also stimulates the hypothalamic–pituitary–adrenal axis in alcoholics (Farren et al., 1999), and this stimulation may be central to its anti-alcohol consumption effects in alcoholism (O’Malley et al., in preparation). Since the metabolism of ethanol can result in the formation of adducts with the biogenic amines, which may interact with opioid receptors (see Naoi et al., 1998) and the formation of acetaldehyde enkephalin adducts has also been reported (Summers and Lightman, 1981), it is important that any studies investigating the behaviour of the opiate system as a possible trait marker are performed with subjects without any recent history of alcohol abuse.

The hypothalamic–pituitary–adrenal (HPA) axis

Adrenocorticotropic hormone (ACTH) is also produced from the anterior pituitary; it shares the same precursor as β-endorphin and is co-released with it under various conditions (Schuckit and Smith, 1996). High-dose ethanol challenges have been found to produce lower levels of ACTH in non-alcoholics at high risk of alcoholism, relative to those at low risk (Schuckit et al., 1988b). Ovine corticotropin-releasing hormone (oCRH) has also been used to study ACTH release in non-alcoholic sons of alcoholics (family history-positive; FHP) and non-alcoholic sons of non-alcoholics (family history-negative; FHN), and the results showed that FHP men had lower peak ACTH reaction to oCRH than FHN men (Waltman et al., 1994). FHN men also had a blunted ACTH response to oCRH administered after an ethanol challenge. Inder et al. (1995) also found a blunted ACTH response to oCRH and to naloxone in recently detoxified alcoholics, relative to healthy controls, suggesting a hypo-responsiveness of the pituitary in alcoholics to CRH. Wand and Dobs (1993) found a diminished ACTH responsiveness to CRH in actively drinking alcoholics, and other investigators found a similar response in short-term abstinence from alcohol (Heuser et al., 1988; Loosen et al., 1991; Costa et al., 1996). However, a number of studies have found no abnormalities in basal ACTH levels in abstinent alcoholics (Vescovi et al., 1992; Del Arbol et al., 1995), and Adinoff et al. (1990) reported evidence for only a subtle attenuation of the ACTH response to oCRH in alcoholics who had been abstinent for longer than 3 weeks.

The cortisol response to high-dose ethanol has also been found to be diminished in sons of alcoholics relative to healthy controls (Schuckit et al., 1987a), but not in all studies (Gianoulakis et al., 1996), and one report showed an increase in cortisol in FHP women relative to FHN women (Lex et al., 1991). Indeed serum cortisol has been found to be elevated in abstinent alcoholics relative to healthy controls (Farren et al., 1995a), but not consistently (Del Arbol et al., 1995; Heinz et al., 1995). The cortisol response to stimulation by such probes as CRF has been reported to be diminished in alcoholics (Heuser et al., 1988; Costa et al., 1996), but again not consistently (Loosen et al., 1991; Waltman et al., 1994). The dexamethasone-cortisol suppression test has also been shown to remain abnormal in alcoholics in early withdrawal (Swartz and Dunner, 1982), and after a number of weeks of abstinence from alcohol (Abou-Saleh et al., 1984; Baldin et al., 1992a) but not consistently (DelPorto et al., 1985). The multiple influences upon the HPA, the wide variety of abnormalities described coupled with the inconsistency of the results make it unlikely that there will be a marker developed on the
basis of simple perturbation of the HPA in alcoholism.

The dopamine system

Dopaminergic probes have also been used to explore trait characteristics in alcoholics, and a number of specific abnormalities were found. Bromocriptine, a specific dopamine D2 receptor agonist, produced a blunted growth hormone (GH) response in a small number of recently abstinent alcoholics, relative to controls (Farren et al., 1995c). The less specific dopaminergic agonist apomorphine also produced a significantly reduced GH response in a group of alcoholics, abstinent for a 2-month period in one study (Balldin et al., 1992b), abstinent for an average period of 7 years in another (Balldin et al., 1993), and also appeared to produce a reduced GH response in abstinent alcoholics who were destined to relapse within a 3-month period (Dettling et al., 1995). Interestingly, a study performed during the alcohol withdrawal period showed a non-significant increase in GH response to apomorphine in the early phase of withdrawal, followed by a fall over a 2-month period (Balldin et al., 1985), whereas another study confirmed an increased dopamine receptor responsiveness in the first week of abstinence using the GH response to dopamine (Anunziato et al., 1983). There thus appears to be a significant change in receptor sensitivity from an increase in the period of withdrawal and early abstinence to a diminution in sensitivity with chronic abstinence. This reduced response may be both a trait marker in family history-positive alcoholics (Wiesbeck et al., 1995), or even also a predictor of relapse in one study of 49 alcoholics followed over 6 months (Schmidt et al., 1996).

There has been a considerable interest in the genetic associations of the dopaminergic system, most specifically the dopamine D2 receptor gene and alcoholism, over the last decade. There have been a considerable number of studies that have shown an association as well as a correlation with severity of alcoholism (Blum et al., 1991; Noble et al., 1991; Parsian et al., 1991; Lawford et al., 1997; Noble, 1998), a correlation with alcohol withdrawal severity and early relapse (Finckh et al., 1996), and an association with early onset of alcoholism (Kono et al., 1997). There have also been a number of negative studies (Gelernter et al., 1991; Geijer et al., 1994; Cook et al., 1996), and the most recent very large data-based COGA study was also negative (Edenberg et al., 1998). Some of the explanations for these disparate findings include heterogeneity among the samples studied, especially the control samples (Gelernter et al., 1993), and the difficulty in applying the rules of single-gene disorders to polygenic disorders (Comings, 1998). Studies on the dopamine D4 receptor (Chang et al., 1997), and the dopamine D3 receptor (Gorwood et al., 1995) have also proven negative to date, although a recent study in an American Indian population found evidence for genetic linkage at chromosome 11, in close proximity to the DRD4 receptor and the tyrosine hydroxylase gene (Long et al., 1998). Since other dopaminergic abnormalities, including a significant interaction between D1 and D2 receptors, have been described in abuse of other substances such as cocaine (Self et al., 1995), and since the dopamine D1 receptor gene is being implicated in addictive behaviours in general (Comings et al., 1997), the dopamine system may yet yield a biological marker for addictive disorders, if not specifically for alcoholism. Exciting evidence is now emerging from the COGA data for a linkage of an alcoholism-related severity phenotype to a site on chromosome 16 (Foroud et al., 1998), but it is too early to know if this will be replicable or translatable into a useful clinical marker.

The noradrenergic system

Noradrenergic receptor function is best studied using a dynamic test, such as a yohimbine challenge. In the study by Krystal et al. (1996) a significant elevation was found in the cortisol and prolactin response of alcoholics to intravenous yohimbine, whereas there was a significant diminution in the alcoholics’ pulse response relative to healthy controls. These findings were taken by the authors as evidence for a diminished postsynaptic noradrenergic response in the setting of a normal presynaptic response as evidenced by a normal baseline MHPG (3-methoxy-4-hydroxyphenylglycol) and MHPG response to yohimbine. Down-regulation of post synaptic α2 adrenoceptor function would be associated with greater sensitivity to α-receptor antagonists and decreased sensitivity to α2-receptor agonists, such as clonidine. This is consistent with alcoholic patients’ showing persistent blunting of the growth hormone response to clonidine during withdrawal.
suggested that the increased anxiety and anger in alcoholics (George et al., 1980). This down-regulation of the noradrenergic postsynaptic receptors may reflect adaptation to greater noradrenaline release during withdrawal (Linnola et al., 1987), rather than trait noradrenergic abnormality. Krystal et al. (1996) also noted a trend for greater elevations in cortisol after the yohimbine infusions in alcoholic patients without a family history of alcoholism. This finding, indicating some difference in response according to genetic predisposition, was not matched by findings of any differential baseline MHPG in a study of abstinent alcoholics divided according to family history of alcoholism (Farren and Tipton, 1999).

The serotonin system

Administration of the serotonin precursor 5-hydroxytryptophan produced a relatively reduced cortisol response, and a reduction in prolactin response after administration of the 5-HT2/5-HT1c receptor agonist MK-212 occurred in a group of recently drinking alcoholics (Lee and Meltzer, 1991), suggesting a subsensitivity of 5-HT2/5-HT1c receptors and possibly of 5-HT1a receptors as well. D-Fenfluramine-induced prolactin responses, primarily mediated through the 5-HT2 receptor, were also diminished in a group of abstinent alcoholics, relative to healthy controls (Farren et al., 1995b), as were prolactin responses to the less receptor-specific agent D,l-fenfluramine in actively drinking alcoholics (Balldin et al., 1994). m-CPP (m-chlorophenylpiperazine), another serotonergic probe with broad serotonin receptor activity including the 5-HT2c, 5-HT3, 5-HT2A, 5-HT1A, 5-HT7 and 5-HT6 receptors, has been reported to produce prolactin responses that were reduced in alcoholics, relative to controls (Buydens-Branchez et al., 1997), but blunted cortisol responses in abstinent alcoholics in another study (Krystal et al., 1996). A significant number of the alcoholics also reported feeling ‘high’ on the m-CPP, as has also been reported by other investigators (Benkelfat et al., 1991; Krystal et al., 1994). Recently, a diminished response to m-CPP in ACTH levels was found in alcoholics relative to controls, and there were also differences in behavioral measures between type 1 and 2 alcoholics (George et al., 1997). The authors suggested that the increased anxiety and anger in the type 1 relative to the type 2 alcoholics was evidence for a difference at the level of 5-HT2c receptor function. This finding together with the finding of a lower CSF concentration of the 5-HT metabolite 5-hydroxyindol-3-ylacetic acid in early-onset, relative to late-onset, alcoholics (Fils-Aime et al., 1996) suggest that there may be measurable serotonergic differences between types of alcoholics that could reach marker status with replication.

Total plasma tryptophan levels (Branchez et al., 1981), and the tryptophan/large neutral amino acid ratio that governs the availability of tryptophan for transport into the brain have also been reported to be lower in the early-onset subtypes of alcoholics compared with normal controls (Buydens-Branchez et al., 1989). In contrast, serum total tryptophan has been found to be elevated in a group of abstinent (mean 16 weeks) alcoholics, relative to healthy controls (Farren and Dinan, 1996), while Beck et al. (1983) reported higher cerebrospinal tryptophan levels in alcoholics after several weeks of abstinence, compared with healthy controls. The association of a high serum tryptophan with diminished central serotonin function in alcoholics has also suggested an abnormality in the conversion of tryptophan to 5-HT, at the level of tryptophan hydroxylase (Farren and Dinan, 1996).

These findings correlate nicely with research on the gene governing tryptophan hydroxylase, the rate-limiting step for the conversion of tryptophan to serotonin, which has been implicated in the transmission of vulnerability to alcoholism in a group of Finnish alcoholics (Nielsen et al., 1998). Indeed, the serotonin transporter gene was also significantly linked with severe alcohol dependence and withdrawal symptomatology in a recent German study (Sander et al., 1997). m-CPP, used in various studies mentioned above, binds to the 5-HT transporter at high concentrations (Hamik and Peroutka, 1989).

Other biological markers for alcoholism

Although not exactly a biochemical marker, the level of reaction (LR) to alcohol has been used to examine trait characteristics in potential alcoholics. The LR to alcohol primarily measured subjective feelings of intoxication and a motor performance task in response to an ethanol load. Schuckit and Smith (1996) evaluated a group of 453 sons of alcoholics and non-alcoholics for LR to ethanol, and followed them up for a period of up to 8 years...
to assess the development of alcoholism. There was a significant relative increase in alcohol dependence or abuse in those subjects with a positive family history of alcoholism. The LR to ethanol at the age of 20 years was a clear predictor of alcoholism in a manner that was independent of drinking pattern at the early age. This relationship was most robust for those subjects with clearly high LR scores, and considered to be a mediator of alcoholism risk.

Prolactin has also been studied as a marker of some abnormalities in alcoholism. Schuckit et al. (1987b) found that the sons of alcoholics had significantly lower prolactin levels in response to an alcohol challenge in comparison with the sons of non-alcoholics. Subjects were challenged on three occasions with placebo, 0.75 ml/kg and 1.1 ml/kg of ethanol, and the difference in response was found at the high dose level. A similar study by Lex et al. (1991) showed a lowered prolactin response to ethanol in a group of family history-positive women. In a follow-up on his original findings using the COGA data set, however, Schuckit et al. (1996) did not find a robust lowered prolactin response to ethanol in the sons of alcoholics as in the original studies.

The premise that personality characteristics predispose to later development of alcoholism rather than actual alcoholism being defined by a biological trait marker has been supported by the finding of significantly elevated free, and total, testosterone in a sample of 61 males taken from a criminal population. Not surprisingly, there was also an association between total testosterone and sex hormone binding hormone and antisocial personality disorder, as well as type 2 alcoholism (Staleheim et al., 1998). Also, higher basal testosterone and androstenedione levels have been proposed as markers of elevated alcohol drinking in females, although the evidence has come only from one study, which has yet to be replicated (Sarkola et al., 1998).

Overall, hormonal markers, either at baseline levels or when measured in response to a specific stimulating probe, have provided evidence for various neurochemical abnormalities in either drinking or abstinent alcoholics, but have not shown sufficient specificity or consistency to be regarded as trait makers for alcoholism. Hormonal responses to more specific probes may well prove to be trait markers for alcoholism in the future, but none of the probes yet tested looks sufficiently promising.

PROTECTIVE MARKERS FOR DEVELOPMENT OF ALCOHOLISM

The most consistently replicated findings for genetic markers in alcoholism are those involved in the protective effects of certain polymorphisms of the alcohol-metabolizing enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in Asian populations. Those Asians that inherit the mutant inactive ALDH enzyme have flushing reactions, following drinking, produced by high blood acetaldehyde levels (Thomasson et al., 1993). Various studies have found this to be specific to Orientals with Mongoloid origin, and not to be present in Caucasian, Native American or African populations (Goedde et al., 1985; Gilder et al., 1993). The relative risk for development of alcoholism in Japanese patients can be accurately estimated on the basis of the genotype frequencies of ADH-2 and ALDH-2 alleles, with a mutant inactive ALDH-2 and an atypical ADH-2 being protective (Higuchi et al., 1995).

GENERAL CONCLUSIONS AND COMMENTS

The difficulties in finding a trait marker for alcoholism include not just defining who exactly is alcoholic, but who is predisposed to being alcoholic by way of having a positive family history. The standard definition of a positive family history, namely of having a single first-degree relative with a history of alcoholism or having two second-degree relatives with a history of alcoholism, has not always been confirmed by the use of a collateral history in most studies, leaving that definition open to question on the basis of reliability as well as validity. In one group of studies examining the opioid system, the family history of the positive subjects was traced over generations, and included many positive relatives in a substantially homogeneous population group (Gianoulakis et al., 1989, 1996). This could not be considered to be the same genetic population as that from other studies, defining their family history group by broader criteria (Schuckit et al., 1988b; Waltman et al., 1994; Schuckit and Smith, 1996; Wand et al., 1998).
Similarly, studies on biochemical abnormalities in alcoholics in various stages of alcohol withdrawal may be confounded by the withdrawal effects of alcohol (Heuser et al., 1988; Wand and Dobs, 1993; Balldin et al., 1994; Costa et al., 1996) and the effects of long-term alcohol consumption (Farren et al., 1995a,b,c, 1998b).

There is, as yet, no convincing trait marker for susceptibility to alcoholism. Some series of investigations hold promise that a trait marker for a particular subset of alcoholics may be developed, e.g. severe alcoholism and the dopamine D2 gene; the level of reaction to alcoholism in family history-positive alcoholics; β-endorphin abnormalities in specific family groups of alcoholics; reduced P3 wave ERPs as markers and predictors of development of substance abuse in predisposed youths; reduced GH response to apomorphine as a predictor of relapse to alcoholism in early abstinence; abnormal adenyl cyclase activity in certain defined subgroups of alcoholics; and abnormal platelet MAO levels in subjects with a behavioural predisposition to addictive disorders. Identification of subgroups of alcoholics with consistent biological markers within that subgroup may well prove more fruitful than aiming for identification of a trait marker for alcoholism in general. It may well be that genotypic research will prove more fruitful in marker identification than phenotypic research (Foroud et al., 1998). Indeed, the arguments about whether only a small and readily identifiable minority of alcoholics (type 2), no more than 25% according to Crabb (1990), have a strong genetic aetiology, with the remainder (type 1-mileu-limited) being mainly influenced by environmental factors, requires resolution if the results of studies with undifferentiated alcoholic subjects are to be meaningfully interpreted.

Acknowledgements — The authors would like to acknowledge the use of the various databases: Index Medicus, PsychInfo, Biosis and Current Contents.

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