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Platelet Adenylyl Cyclase Activity as a Trait Marker of Alcohol Dependence

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Background: There is compelling evidence that genetic factors play a major role in the development of alcohol dependence. Platelet adenylyl cyclase (AC) activity has been proposed as a biochemical marker for differentiating alcohol-dependent and nondependent subjects, but the sensitivity and specificity of this marker have not been ascertained. The objective of this study was to determine the sensitivity and specificity of platelet AC activity in identifying alcohol-dependent subjects and to ascertain the effect of medical/psychiatric variables, drinking and smoking history, and age and body weight on AC activity.

Methods: The cross-sectional study was conducted from 1995 to 1998. Participants were 210 Australian White men who were community volunteers and alcohol treatment inpatients in Sydney, Australia. There were 41 nondrinkers, 140 drinkers, and 29 men who were entering alcohol treatment. The main outcome measure was platelet AC activity. Classification variables were plasma ethanol, γ-glutamyltransferase, aspartate aminotransferase, serum carbohydrate-deficient transferrin (CDT), and urinary 5-hydroxytryptophol/5-hydroxyindoleacetic acid (5-HTOL/5-HIAA) levels, and World Health Organization/International Society for Biomedical Research on Alcoholism Interview Schedule variables, which included alcohol use and dependence criteria.

Results: Among subjects who reported abstinence for at least 4 days, both cesium fluoride (CsF)- and forskolin-stimulated platelet AC activities were significantly lower in those with a lifetime history of alcohol dependence compared with those with no such history ($p < 0.005$ and $p < 0.05$, respectively). The sensitivity and specificity of CsF-stimulated AC activity to discriminate individuals with a lifetime history of alcohol dependence were 75% and 79%, respectively. Similar values for sensitivity and specificity for CsF-stimulated AC activity were calculated when discriminating current alcohol dependence in the subjects in our sample. Irrespective of the history of alcohol dependence, persons who had consumed alcohol recently (within the last 3–4 days) showed significantly higher mean basal, CsF-stimulated, and forskolin-stimulated AC activity ($p < 0.001$), as did those who had elevated 5-HTOL/5-HIAA ratios or CDT levels, indicative of recent (heavy) drinking. The "normalization" of platelet AC activity to baseline levels after an individual stops drinking may be related to the generation of new platelets during the abstinence period. Conduct disorder and antisocial personality disorder were not associated with low AC activity, but low forskolin-stimulated AC activity was associated with major depression.

Conclusions: We found that CsF- and forskolin-stimulated platelet AC activity discriminates between subjects with and without alcohol dependence in a population of subjects who had not consumed significant quantities of ethanol recently. Recent alcohol consumption is a confounding variable that can alter the measured levels of AC activity. Forskolin-stimulated platelet AC activity also may be influenced by a history of major depression.

Key Words: Platelet, Adenylyl Cyclase, Alcohol Dependence, Abstinence, Markers.

ALTHOUGH THE DIAGNOSIS of alcohol dependence is widely accepted to reflect a heterogeneous disorder, genetic influences have been shown to play a significant role in the development of alcoholism. A number of markers for the predisposition or vulnerability to

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developing alcohol dependence have been proposed and investigated, including monoamine oxidase activity (Anthenelli et al., 1995; von Knorring et al., 1991), event-related potential P300 amplitude (Keenan et al., 1997), electroencephalographic activity (Bauer and Hesselbrock, 1993), aldehyde dehydrogenase polymorphism (Nakamura et al., 1996), and low level of response to an ethanol challenge (Schuckit et al., 1996). The enzyme adenylyl cyclase (AC) is a biochemical marker that also has been studied for its potential relationship to alcohol dependence. AC is responsible for the formation of the second messenger adenosine 3′:5′-cyclic monophosphate (cAMP), which in turn acts on a number of target molecules, particularly cAMP-dependent protein kinase, to control such diverse phenomena as metabolism, gene transcription, and memory. AC activity, measured in either platelets (Lex et al., 1993; Saito et al., 1994; Tabakoff et al., 1988) or lymphocytes (Diamond et al., 1987; Nagy et al., 1988; Waltman et al., 1993) of alcohol-dependent subjects, has been shown to be less responsive to agents known to increase enzymatic activity. More recently, low platelet AC activity has been proposed as a trait marker for predisposition to alcohol dependence (Devor et al., 1991; Tabakoff and Hoffman, 1998).

The finding of lower platelet AC activity in alcohol-dependent subjects comes with two caveats that need further exploration. First, to demonstrate differences between alcohol-dependent and nondependent subjects, alcohol-dependent subjects appear to need a period of abstinence from alcohol. Studies that demonstrate low platelet AC activity in alcohol-dependent subjects all have included subjects who were abstinent for extended periods of time. Tabakoff et al. (1988) assessed alcohol-dependent men who had been abstinent for an average of 23 days and found significantly lower platelet AC activity when measured in the presence of agents that stimulate AC through the G_s protein, that is, cesium fluoride (CsF) and the guanine nucleotide analog 5′-guanylyl-imidodiphosphate, or Gpp(NH)p. Some of the subjects with the lowest CsF-stimulated AC activity in this study were abstinent for 12 to 48 months. Lex et al. (1993) reported significantly lower AC activity when AC was stimulated by either a guanine nucleotide or CsF in alcohol-dependent women who had an average of 3.9 years of sobriety. In another study of platelet AC activity in alcohol-dependent subjects, Saito et al. (1994) listed a minimum of at least 1 year of abstinence in their criteria for study entry. In a study that investigated platelet AC activity in subjects who were recovering from heavy drinking, we recently demonstrated that low platelet AC activity (CsF-stimulated) seen in alcohol-dependent subjects was not present immediately after cessation of heavy drinking but became evident after abstinence of 1 to 2 weeks (Menninger et al., 1998).

Second, family history for alcohol dependence appears to be important in determining low platelet AC activity. We reported that low CsF-stimulated platelet AC activity was significantly associated with a history of alcohol dependence in first-degree relatives among both sober alcohol-dependent subjects and control subjects (Menninger et al., 1998). Our finding corroborated the results of others who reported lower AC activity associated with familial alcoholism. Lex et al. (1993) noted significantly lower basal, Gpp(NH)p-stimulated, and CsF-stimulated platelet AC activity among female non–alcohol-dependent controls who were family history positive (FHP) for alcohol dependence, compared with those who were family history negative (FHN). In a separate study of alcohol-dependent men, the FHP alcohol-dependent men had significantly lower Gpp(NH)p-stimulated platelet AC activity than either the control group or the FHN alcohol-dependent group (Saito et al., 1994). In a recent study of platelet AC in young children of alcoholics, Ratsma et al. (1999) reported reduced platelet AC activity when assayed in the presence of fluoride ion in these children, compared with controls, which further supported AC activity as a trait marker for predisposition to alcoholism. However, in none of the studies to date has platelet AC activity been investigated in a large group of subjects to determine the diagnostic utility of the AC measure for distinguishing alcohol-dependent subjects.

In 1988, the WHO/ISBRA Collaborative Project on State and Trait Markers of Alcoholism was established. Investigators from the International Society for Biomedical Research on Alcoholism (ISBRA) and the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and representatives of the World Health Organization (WHO) collaboratively agreed to pursue a research project focused on (1) the characterization of alcohol dependence in different countries and (2) the substantiation of biological state and trait markers for alcohol dependence. In this paper we present data about platelet AC activity in 210 Australian men that were collected as part of the WHO/ISBRA Study on State and Trait Markers of Alcoholism. The extensive characterization of this population-based sample of subjects allowed us to examine variables such as level of alcohol consumption, length of abstinence, history of alcohol dependence, other medical and psychiatric conditions, and changes in state markers of alcohol consumption in relation to platelet AC activity.

METHODS

Subjects

Altogether, 285 Australian men were interviewed by research staff who had been trained to use the WHO/ISBRA Interview Schedule. Subjects included 32 men admitted to the Detoxification Unit of Royal Prince Alfred Hospital for alcohol treatment and 253 men who responded to research solicitations. Platelet AC activities were missing in 34 of the subjects, due to either loss of samples from thawing during shipment from Australia or inadequate amount of platelet protein in the sample. They were therefore excluded, together with 41 non-White subjects (because the 32 non-White nondrinkers had significantly different mean platelet AC activities compared with their White counterparts). The studied sample thus comprised 210 White men.

Subjects were segregated into four categories on the basis of the information elicited in the complete interview: nondrinkers, light/moderate drinkers, heavy drinkers, and patients who were receiving treatment for alcohol dependence. Subjects were considered nondrinkers if they stated that they had never had a 1-year period during which they consumed at least nine drinks. Subjects were classified as light/moderate or heavy drinkers if their reported weekly alcohol consumption during the past 3 months was below or above, respectively, a cutoff of 210 g of ethanol (i.e., less or greater than 15.4 drinks per week). Alcohol-dependent subjects met DSM-IV criteria for alcohol dependence (American Psychiatric Association, 1994).

Exclusion criteria included major medical and psychiatric disorders, intravenous drug abuse, and recent (past 10 days) treatment with aldehyde dehydrogenase inhibitors (e.g., disulfiram).

Interview

The WHO/ISBRA Interview Schedule is a structured interview schedule composed of the following major sections: (1) sociodemographic background information; (2) lifetime and past 30 day occurrence of medical conditions; (3) frequency and quantity of beverage-specific alcohol consumption during the past 30 days and during the period when the subject was drinking most heavily; (4) various symptoms experienced with drinking, and information on alcohol-related treatment history; (5) frequency, quantity, and duration of smoking during the past 30 days and the period of heaviest smoking; (6) history of prescription and illicit drug use; (7) history of depression, antisocial behaviors, and treatment for mental or emotional problems; and (8) family history of alcohol and drug problems, major depression, and antisocial behaviors in the biological first- and second-degree relatives. The Interview Schedule was adapted from the Alcohol Use Disorders and Associated Disabilities Interview Schedule developed by the NIAAA (Grant et al., 1995) and was translated into Finnish, Japanese, French, and Portuguese versions for the purpose of this study.

The WHO/ISBRA Interview Schedule enables diagnoses to be extracted according to DSM-IV and International Classification of Diseases (ICD)-10 criteria for the following conditions: alcohol abuse, alcohol dependence, abuse of and dependence on other psychoactive substances (including sedatives and tranquilizers; heroin, methadone, and other opiates; stimulants and cocaine; cannabis; inhalants; hallucinogens; and anabolic steroids), major depression, antisocial personality disorder, and conduct disorder. The test-retest kappa values, the best measure of agreement for categorical data, of the major data elements that appear in the WHO/ISBRA Interview Schedule range from a low of 0.55 for items such as DSM-IV diagnosis of marijuana dependence to values of 1.0 for family history of alcohol dependence in the biological mother. The kappa values for various alcohol dependence criteria in the respondents ranged from 0.70 to 0.90 and 0.60 to 0.80 for DSM-IV and ICD-10 diagnosis of alcohol abuse and dependence (Grant et al., 1995; Tabakoff, 1996).

Blood and Urine Samples

Blood and urine samples were collected at the time of the interview. Blood was collected via standard venipuncture technique into vacutainers that contained ethylenediaminetetraacetic acid (EDTA) for preparation of plasma and platelets. Within 2 hr of collection, the platelets were prepared by centrifuging the blood samples at $700 \times g$ for 10 min at room temperature (21 ± 3°C). The platelet-rich plasma layer was transferred to a fresh centrifuge tube and again centrifuged for 10 min at $700 \times g$ at room temperature. The upper platelet-rich layer was then transferred to a fresh centrifuge tube and centrifuged at $2800 \times g$ for 15 min at room temperature. The platelet pellet was recovered and stored at −70°C until being shipped in dry ice to the Coordinating Center in Helsinki, Finland, and from there to Denver, Colorado, for analysis. The supernatant from the platelet pellets was recovered as the plasma fraction, frozen, and shipped in dry ice to the Helsinki Coordinating Center and assayed for plasma ethanol, γ-glutamyltransferase (GGT), and aspartate aminotransferase (AST) activities at the laboratories of ALKO and KTL, Helsinki, Finland. The urine samples were frozen and shipped via the Helsinki Coordinating Center to the Alcohol Diagnostic Laboratory at St. Gorans Hospital, Stockholm, Sweden, for assay of 5-hydroxytryptophol/5-hydroxyindoleacetic acid (5-HTOL/5-HIAA) ratios. A separate vacutainer for preparation of serum was drawn, and the serum was frozen, shipped, and assayed for carbohydrate-deficient transferrin (CDT) levels in the Alcohol Diagnostic Laboratory at St. Gorans Hospital, Stockholm, Sweden.

Preparation of Platelet Membranes

Before assay of platelet AC activity, the platelet pellet was thawed and washed at 4°C. For washing, the platelet pellet was suspended in 1.5 ml of 50 mM Tris-HCl (pH 7.5) that contained 20 mM EDTA and then centrifuged at $17{,}000 \times g$ for 10 min. This procedure was repeated, and the platelet pellet was suspended in 1.5 ml of 5 mM Tris-HCl (pH 7.5) that contained 5 mM EDTA and was centrifuged at $17{,}000 \times g$ for 10 min. The platelet pellet then was suspended in 1.5 ml of 5 mM Tris-HCl (pH 7.5) that contained 1 mM EDTA by using a hand-held Teflon homogenizer. The homogenate was diluted as necessary with 5 mM Tris-HCl (pH 7.5) that contained 1 mM EDTA to attain a protein concentration of approximately 200 to 1000 μg/ml and was used immediately for the assays of platelet AC activity. Protein determinations were performed by using the Pierce bicinchoninic acid protein microtiter method (Smith et al., 1985).

Adenylyl Cyclase Assay

Approximately 10 to 50 μg of the prepared platelet membrane protein (50 μl) was added to 200 μl of assay buffer that consisted of 25 mM Tris-maleate (pH 7.5), 10 mM theophylline, 5 mM $MgCl_2$, 0.25 mM adenosine triphosphate (ATP), and [α-^{32}P]ATP (1.2 to 2.0×10^6 cpm/assay). AC activity was measured in duplicate under four separate conditions: basal activity, and with the addition of 10 μM Gpp(NH)p, 10 mM CsF, or 10 μM forskolin. After equilibration of the assay mixture at 30°C for 5 min, the reaction was initiated by adding the platelet membranes, and the reaction mixture was incubated at 30°C for 10 min. The reaction was terminated by the addition of 750 μl of ice-cold solution that contained 4 mM ATP, 1.4 mM cAMP, and 10,000 cpm [^{3}H]cAMP (25–40 Ci/mmol) to each assay tube. [^{3}H]cAMP, together with [^{32}P] cAMP generated by AC, was isolated by sequential chromatography on Dowex and alumina columns as described by Salomon et al. (1974) and quantified by liquid scintillation counting.

All reported values were corrected for recovery of [^{3}H]cAMP, and AC activity was expressed as picomoles of cAMP generated per milligram of protein per minute. An aliquot of human erythroleukemia (HEL) cell membranes with known and stable levels of AC activity was assayed with each group of samples. The HEL cell membrane preparation was used as a reference standard to reduce between-assay variability. The value of the AC activity obtained with HEL cell membranes within each day's assay was divided by the HEL cell membrane activity averaged over the entire project period. The resulting factor was applied to each individual's platelet AC activity result obtained on a particular day to standardize all AC activity values obtained throughout the study period. Intraassay coefficients of variation for the AC activity assay performed in our laboratory have been reported to be 14.0% for basal AC activity, 4.3% for CsF-stimulated AC activity, and 5.0% for forskolin-stimulated AC activity. Without the use of the HEL cell membrane standardization factor, interassay coefficients of variability for the respective AC activities in our laboratory have been reported at 23.4%, 9.2%, and 13.5% (Menninger and Tabakoff, 1997). With the use of the HEL cell membrane standardization factor, interassay coefficients of variability were found to be 15.4%, 10.8%, and 11.9% for basal, CsF-stimulated, and forskolin-stimulated AC activity, respectively.

GGT and AST Assays

Plasma GGT and AST activities were determined by reflectance spectrophotometry by using a Vitros 250 Analyser (Ortho Clinical Diagnos-

Table 1. Demographic Characteristics Based on Postinterview Classification of Subjects

	Nondrinkers	Light/moderate drinkers	Heavy drinkers	Drinkers without alcohol dependence	Drinkers with lifetime alcohol dependence*
No. of subjects	41	46	123	56	113
Age (years)	43.6 ± 13.4	37.3 ± 11.5	39.1 ± 13.7	39.4 ± 13.0	38.3 ± 13.2
Marital status*					
Married	27 (66%)	24 (52%)	46 (37%)	31 (55%)	39 (34%)
Separated	1 (3%)	6 (13%)	31 (25%)	9 (16%)	28 (25%)
Never married	13 (32%)	16 (35%)	46 (37%)	16 (29%)	46 (41%)
Education					
High school or less	14 (34%)	13 (26%)	42 (34%)	16 (29%)	38 (34%)
Undergraduate	18 (44%)	31 (67%)	71 (58%)	33 (59%)	69 (61%)
Postgraduate	9 (22%)	3 (7%)	10 (8%)	7 (13%)	6 (5%)
Religion					
Protestant	16 (40%)	20 (44%)	55 (46%)	28 (50%)	47 (42%)
Catholic	9 (22%)	16 (35%)	35 (58%)	14 (25%)	37 (33%)
Jewish	1 (3%)	1 (2%)	2 (2%)	2 (4%)	1 (1%)
None	1 (3%)	6 (13%)	25 (21%)	9 (16%)	22 (20%)
Other	13 (32%)	3 (7%)	4 (3%)	3 (5%)	4 (4%)
Area where now live					
Inner city	5 (12%)	8 (17%)	33 (27%)	6 (11%)	35 (31%)
Suburban city	30 (73%)	29 (63%)	80 (66%)	41 (73%)	68 (61%)
Other	6 (15%)	9 (20%)	9 (17%)	9 (16%)	9 (8%)
Weight (kg)	78.3 ± 14.8	80.6 ± 10.2	80.4 ± 14.3	80.1 ± 11.7	80.6 ± 14.1
Tobacco use					
Current smoker	3 (8%)	8 (17%)	65 (53%)	13 (23%)	60 (53%)
Lifetime smoker	9 (23%)	25 (54%)	93 (76%)	29 (52%)	89 (79%)
Alcohol dependence					
Current*	—	6 (13%)	81 (66%)	—	87 (77%)
Lifetime	—	19 (41%)	94 (76%)	—	113 (100%)
Never	41 (100%)	27 (59%)	29 (24%)	56 (100%)	—

* Alcohol dependence refers to DSM-IV alcohol dependence; married includes living with someone as if married and domestic partner relationships; separated includes separated, divorced, and widowed: current refers to in the past year. Values are means ± SD or frequency and percentage.

tics, Raritan, NJ). Values were considered elevated if >50 Units/liter for AST and >80 Units/liter for GGT.

CDT Assays

Serum CDT determinations were carried out in duplicate by using the commercially available CDTect™ test (Pharmacia Diagnostics, Uppsala, Sweden, and Axis Biochemicals, Oslo, Norway) based on the separation of transferrin isoforms on an anion exchange chromatography microcolumn followed by a double antibody radioimmunoassay. CDT levels >20 Units/liter were considered elevated.

Plasma Ethanol Determinations

Ethanol determinations in plasma samples were carried out by headspace gas chromatography essentially as described by Roine et al. (1989). Analytical conditions were as follows: column 60/80 Carbopack B/5% Carbowax (20 m, 6 ft × 2 mm inner diameter; Supelco, Bellefonte, PA); oven temperature 75°C; detector temperature 140°C; carrier gas (N_2) at 40 ml/min. Ethanol levels >100 μmol/liter were taken as positive evidence for the presence of ethanol in plasma.

Urinary 5-HTOL and 5-HIAA Determination

The level of the serotonin metabolites 5-HTOL and 5-HIAA were determined by using gas chromatographic-mass spectrometric and high-performance liquid chromatographic methods (Beck et al., 1982; Helander et al., 1991; Voltaire et al., 1992). The reference limit applied for a high urinary 5-HTOL/5-HIAA ratio was >15 pmol/nmol (Helander et al., 1994).

Statistical Analysis

Values are given as mean ± SD unless noted otherwise. We used Student's *t* tests and analysis of variance to compare mean AC activities across two or more groups, as defined by type of drinker, lifetime alcohol dependence, recent sobriety, and family history for alcohol dependence. Linear contrasts were used to examine linear trends in AC activities related to duration of sobriety. We conducted post hoc tests for multiple pairwise comparisons by using Tukey's Honestly Significant Difference statistic. The 0.05 level of significance was used for each overall comparison. SPSS for Windows version 8.0 (SPSS Inc., Chicago, IL) was used for data analysis. MedCalc version 4.3 (MedCalc Software, Mariakerke, Belgium) was used for receiver operating characteristic (ROC) curve analysis.

Ethical Considerations and Informed Consent

The study was approved by the Ethics Review Committee of the Royal Prince Alfred Hospital, Sydney, Australia. Informed consent was obtained in writing from all subjects before participation in the study.

RESULTS

Demographic Characteristics of Subjects

Table 1 includes demographic characteristics on the 210 subjects. After analyzing the completed interviews, we categorized subjects into nondrinkers, light/moderate drinkers, and heavy drinkers, whereas drinkers also were grouped according to those who did and did not meet criteria for lifetime alcohol dependence. There were no differences in age or body weight among the groups. The heavy drinkers were more likely to be separated, divorced, or widowed, to be less well educated, to live in an inner city, and to have smoked tobacco (ever or recently). All of the groups consisted primarily of Protestant and Catholic subjects.

Table 2. Recent and Heaviest Ethanol Consumption Based on Postinterview Classification of Subjects

	Light/moderate drinkers	Heavy drinkers	Drinkers without alcohol dependence	Drinkers with lifetime alcohol dependence*
No. of subjects	46	123	56	113
Sobriety (days since last drank)	5.4 ± 6.0	1.8 ± 2.6[a]	3.4 ± 5.1	2.4 ± 3.5
Recent drinking (last month)				
Avg. daily ethanol consumption (g)**	14.1 ± 8.3	118 ± 92[a]	50 ± 56	110 ± 99[b]
% Ethanol as beer	69 ± 32	59 ± 36	59 ± 34	63 ± 36
% Ethanol as wine	24 ± 29	29 ± 35	32 ± 33	25 ± 34
% Ethanol as liquor	7 ± 17	12 ± 22	8 ± 19	12 ± 22
Heaviest drinking period				
Age at onset (years)	17.8 ± 2.2	17.9 ± 4.5	17.8 ± 2.2	17.9 ± 4.4
Duration (months)	54 ± 56	62 ± 77	60 ± 72	59 ± 71
Avg. daily ethanol consumption (g)	60 ± 61	195 ± 192[a]	78 ± 82	198 ± 198[b]

* Alcohol dependence refers to DSM-IV alcohol dependence. **A conversion factor of 13.6 g of ethanol per standard drink can be applied to this data. Values are means ± SD. Tests of significance (Student's *t* test): [a]$p < 0.001$ compared with light drinkers; [b]$p < 0.001$ compared with nondependent subjects (independent *t* tests for equality of means assuming unequal variances).

Table 3. Platelet Adenylyl Cyclase Activities

Subject groups	Platelet AC activity (*n*) (pmol cAMP/mg protein/min)			
	Basal	Gpp(NH)p (10 μM)	CsF (10 mM)	Forskolin (10 μM)
Nondrinkers	15.2 ± 8.4 (40)	73.1 ± 40.1 (41)	127.3 ± 44.0 (39)	367.8 ± 156.1 (39)
Light/moderate drinkers	16.4 ± 5.7 (44)	84.7 ± 20.9 (45)	126.4 ± 23.3 (46)	353.5 ± 84.3 (46)
Heavy drinkers	17.4 ± 6.5 (123)	82.6 ± 27.1 (121)	129.9 ± 28.1 (123)	387.6 ± 105.7 (122)
Lifetime alcohol dependence	17.1 ± 6.5(113)	80.3 ± 26.1 (111)	128.2 ± 29.2(113)	375.5 ± 112.2 (112)
No lifetime alcohol dependence	16.5 ± 7.2 (94)	82.2 ± 32.4 (96)	129.2 ± 32.5 (95)	377.2 ± 115.0 (95)

The values in this table were derived without consideration of the individual's recent alcohol drinking history. Values are means ± SD. No significant differences between groups were noted by ANOVA.

Alcohol Dependence and Recent Alcohol Consumption

After analyzing the complete interviews, we categorized 27% of the alcohol drinkers as light/moderate drinkers and 73% as heavy drinkers. Nineteen (41%) of the light/moderate drinkers met lifetime criteria for DSM-IV alcohol dependence, compared with 94 (76%) of the heavy drinkers, for a total of 113 alcohol-dependent subjects.

Recent and heaviest alcohol consumption of the subject groups is listed in Table 2. The light/moderate drinkers had a significantly higher mean duration of recent sobriety than the heavy drinkers. The light/moderate drinkers drank a greater proportion of beer and a smaller proportion of hard liquor in the last month compared with the heavy drinkers, although this difference was not significant. They also drank significantly less ethanol during their heaviest period of drinking than the heavy drinkers. The proportion of alcohol beverage type (beer, wine, or liquor) consumed was similar between drinkers with a lifetime history of alcohol dependence and nondependent drinkers, although the alcohol-dependent group drank more ethanol both in the last month and during their heaviest period of drinking.

Comorbid Drug Dependence

Few of the alcohol-dependent subjects had additional lifetime dependence on drugs other than alcohol and nicotine. Nine met criteria for cannabis dependence; four for heroin dependence; two each for dependence on sedatives, tranquilizers, and other opiates; and one each for dependence on cocaine, methadone, inhalants, and hallucinogens. None of the non–alcohol-dependent drinkers or nondrinkers had a history of any abuse of or dependence on these drugs.

Platelet AC Activity

On initial examination, the platelet AC activities were not statistically different among any of the three drinker-level groups (Table 3). There were also no significant differences in any of the platelet AC activities between subjects with a lifetime history of alcohol dependence and those without.

Platelet AC Activity: Effects of Abstinence

The group of alcohol drinkers was subdivided into current and abstaining drinkers by self-report (by using cutoffs ranging from 2 to 7 days of abstinence, Table 4). Significant differences in basal, CsF-stimulated, and forskolin-stimulated platelet AC activities were noted between abstaining and current drinkers. After 4 days of abstinence, both mean CsF- and forskolin-stimulated AC activities remained constant and lower in the abstinent drinkers. For the remainder of this article, the term *abstinent* will refer to individuals who had not consumed ethanol for at least 4 days.

Alcohol drinkers also were divided into two groups defined by normal and elevated levels of several state biochemical markers for alcohol consumption: plasma ethanol, urinary 5-HTOL/5-HIAA ratio, serum CDT, and plasma GGT and AST (Table 5). Classifying the alcohol-

Table 4. Platelet Adenylyl Cyclase Activities as a Function of Self-Reported Abstinence in Alcohol Drinkers

Subject groups	Platelet AC activity (*n*) (pmol cAMP/mg protein/min)			
	Basal	Gpp(NH)p (10 μM)	CsF (10 mM)	Forskolin (10 μM)
Abstinent at least 2 days	15.6 ± 6.2 (70)[c]	81.4 ± 27.0 (68)	124.4 ± 26.0 (71)[c]	344.4 ± 93.2 (70)[d]
Drank in the last 2 days	18.6 ± 6.1 (89)	86.6 ± 24.3 (89)	134.6 ± 26.2 (89)	410.6 ± 97.8 (89)
Abstinent at least 3 days	14.9 ± 6.4 (44)[c]	81.4 ± 26.3 (42)	123.2 ± 26.0 (45)[a]	336.3 ± 90.9 (44)[d]
Drank in the last 3 days	18.1 ± 6.1 (115)	85.4 ± 25.3 (115)	132.8 ± 26.3 (115)	398.8 ± 99.7 (115)
Abstinent at least 4 days	14.3 ± 5.1 (29)[c]	79.0 ± 20.5 (28)	116.8 ± 20.8 (30)[d]	319.2 ± 80.7 (30)[d]
Drank in the last 4 days	17.9 ± 6.4 (130)	85.4 ± 26.4 (129)	133.2 ± 26.8 (130)	396.0 ± 100.0 (129)
Abstinent at least 5 days	14.6 ± 5.0 (21)[a]	80.6 ± 18.9 (21)	118.1 ± 18.4 (22)[c]	323.3 ± 72.5 (22)[d]
Drank in the last 5 days	17.7 ± 6.4 (138)	84.9 ± 26.5 (136)	132.0 ± 27.1 (138)	390.8 ± 102.0 (137)
Abstinent at least 6 days	14.6 ± 4.9 (18)[a]	79.7 ± 19.0 (18)	118.4 ± 19.7 (19)[a]	322.2 ± 76.4 (19)[c]
Drank in the last 6 days	17.6 ± 6.4 (141)	84.9 ± 26.3 (139)	131.7 ± 26.9 (141)	389.5 ± 101.5 (140)
Abstinent at least 7 days	14.0 ± 4.4 (17)[c]	77.9 ± 17.9 (17)	116.8 ± 19.0 (18)[b]	317.2 ± 75.3 (18)[d]
Drank in the last 7 days	17.7 ± 6.4 (142)	85.1 ± 26.3 (140)	131.8 ± 26.9 (142)	389.7 ± 101.2 (141)

Values are means ± SD. Tests of significance: [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.005$, and [d]$p < 0.001$ compared with recent drinkers (independent *t* tests for equality of means assuming unequal variances).

Table 5. Platelet Adenylyl Cyclase Activities in Alcohol Drinkers in Relation to Biochemical Markers of Recent Drinking

Subject groups	Platelet AC activity (*n*) (pmol cAMP/mg protein/min)			
	Basal	Gpp(NH)p (10 μM)	CsF (10 mM)	Forskolin (10 μM)
No detectable ethanol	17.3 ± 6.5 (146)	83.2 ± 25.6 (146)	128.6 ± 26.8 (148)	377.0 ± 100.1 (148)
Positive ethanol level	16.3 ± 5.3 (21)	83.2 ± 25.8 (20)	131.3 ± 27.7 (21)	387.9 ± 111.9 (20)
Normal 5-HTOL/5-HIAA	16.8 ± 6.9 (113)	82.9 ± 27.7 (113)	126.5 ± 27.0 (115)	366.2 ± 102.0 (115)[a]
Elevated 5-HTOL/5-HIAA	17.9 ± 4.8 (54)	84.0 ± 20.3 (53)	134.1 ± 26.1 (54)	404.4 ± 95.4 (53)
Normal CDT level	16.7 ± 6.3 (112)	82.9 ± 26.1 (112)	125.5 ± 25.8 (114)[a]	365.8 ± 100.4 (114)[a]
Elevated CDT level	18.2 ± 6.2 (55)	83.8 ± 24.4 (54)	136.2 ± 27.8 (55)	404.6 ± 98.7 (54)
Normal GGT level	17.0 ± 6.4 (133)	82.8 ± 26.4 (134)	128.9 ± 27.6 (135)	378.4 ± 103.8 (135)
Elevated GGT level	17.7 ± 5.8 (34)	84.9 ± 21.8 (32)	129.1 ± 24.1 (34)	377.8 ± 91.6 (33)
Normal AST level	17.2 ± 6.3 (137)	82.9 ± 25.7 (138)	128.8 ± 26.3 (139)	379.0 ± 101.0 (139)
Elevated AST level	17.7 ± 5.7 (29)	86.7 ± 23.6 (27)	132.2 ± 27.4 (29)	382.4 ± 96.8 (28)

Elevated marker levels are defined as follows: 5-HTOL/5-HIAA > 15 pmol/nmol; CDT > 20 Units/liter; GGT > 80 Units/liter; and AST > 50 Units/liter. Values are means ± SD. Tests of significance: [a]$p < 0.05$, [b]$p < 0.01$,[c] $p < 0.005$, and [d]$p < 0.001$ compared with elevated marker levels (independent *t* tests for equality of means assuming unequal variances).

Table 6. Platelet Adenylyl Cyclase Activities in Self-Reported Abstinent* Drinkers: Relation to Lifetime and Current Alcohol Dependence

Subject groups	Platelet AC Activity (*n*) (pmol cAMP/mg protein/min)			
	Basal	Gpp(NH)p (10 μM)	CsF (10 mM)	Forskolin (10 μM)
Lifetime alcohol dependence	13.6 ± 5.6 (16)	72.5 ± 21.6 (15)	106.8 ± 18.8 (16)[b]	286.8 ± 75.6 (16)[a]
No lifetime alcohol dependence	15.1 ± 4.5(13)	86.6 ± 16.9(13)	128.2 ± 17.1(14)	356.3 ± 71.8(14)
Current alcohol dependence	12.2 ± 4.5(12)	68.5 ± 21.8(11)	103.6 ± 18.0(12)[b]	279.5 ± 77.0(12)[a]
No current alcohol dependence	15.8 ± 5.1 (17)	85.8 ± 17.0 (17)	125.6 ± 18.0 (18)	345.7 ± 73.7 (18)

* Abstinent at least 4 days by self-report. Values are means ± SD. Tests of significance: [a]$p < 0.05$ and [b]$p < 0.005$ compared with subjects without lifetime alcohol dependence (independent *t* tests for equality of means assuming unequal variances).

consuming subjects on the basis of a positive plasma ethanol level, or elevation of either of the hepatic enzymes, GGT or AST, yielded no significant differences in mean platelet AC activity. On the other hand, in the alcohol-consuming subjects, normal 5-HTOL/5-HIAA ratios were associated with significantly lower mean forskolin-stimulated platelet AC activity, and normal CDT levels were associated with significantly lower mean CsF- and forskolin-stimulated platelet AC activities.

Platelet AC Activity: Effects of Alcohol Dependence

Among subjects who were currently abstinent (>4 days without alcohol), those with a history of alcohol dependence had significantly lower mean CsF- and forskolin-stimulated platelet AC activities ($p < 0.005$ and < 0.05, respectively). Similar significant findings were noted for those subjects with current (i.e., past year) alcohol dependence (Table 6). Such differences were not seen among those subjects who had been drinking within 4 days of the assessment, whether the comparison was for lifetime or current alcohol dependence. Figure 1 further illustrates the relationship between current drinking, lifetime history of alcohol dependence, and platelet AC activity. Irrespective of whether basal, CsF-stimulated, or forskolin-stimulated AC activity was being measured, the lowest values were noted in the group of subjects who had been abstinent for at least 4 days *and* who had a lifetime history of alcohol dependence. When Gpp(NH)p was used as a means of stimulating platelet AC activity, the results were not definitive because nondrinkers also displayed low enzymatic activity under these assay conditions.

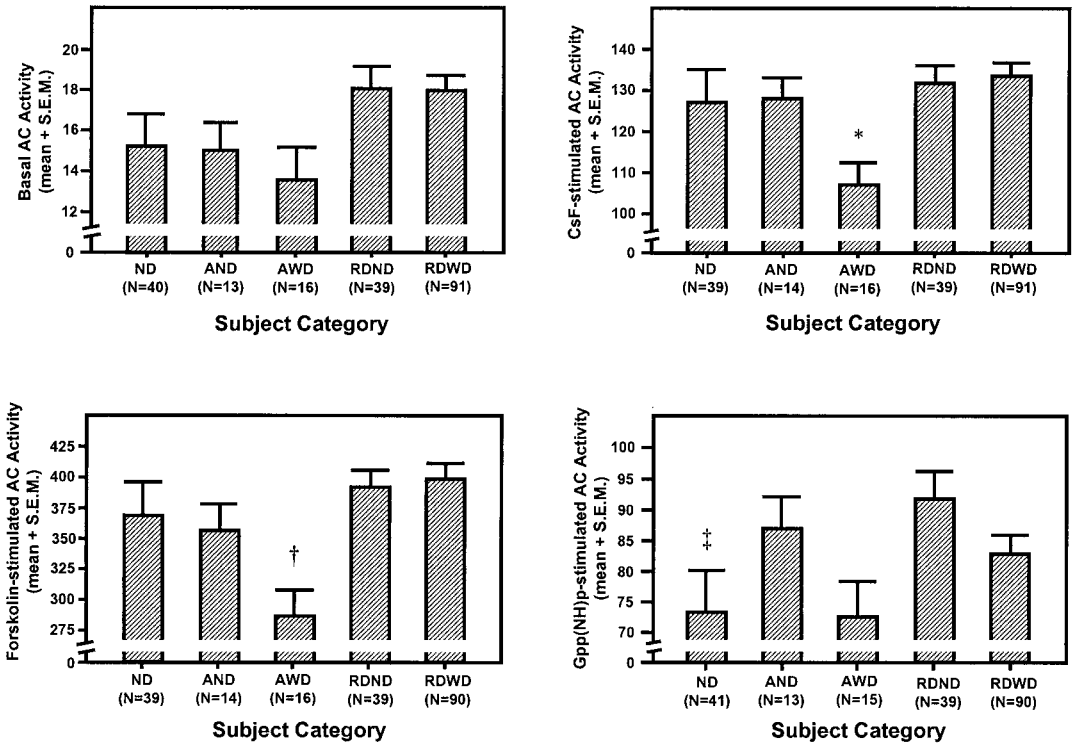

Fig. 1. Effect of abstinence and lifetime DSM-IV alcohol dependence on platelet adenylyl cyclase activity. One-way ANOVA of platelet AC activity and subject category was performed (ND, nondrinker; AND, abstinent drinker with no alcohol dependence; AWD, abstinent drinker with alcohol dependence; RDND, recent drinker with no alcohol dependence; RDWD, recent drinker with alcohol dependence). Values are presented as mean ± SEM. Platelet AC activity is expressed in picomoles of cAMP per milligram of protein per minute. Post hoc tests of significance: *CsF-stimulated AC activity: AWD vs. RDND, $p < 0.05$, and AWD vs. RDWD, $p < 0.01$. †Forskolin-stimulated AC activity: AWD vs. RDND, $p < 0.05$, and AWD vs. RDWD, $p < 0.005$. ‡Gpp(NH)p-stimulated AC activity: ND vs. RDND, $p < 0.05$.

Because prior studies did not attempt to examine the diagnostic utility of platelet AC activity, we determined the sensitivity and specificity of CsF-stimulated AC activity for discriminating lifetime alcohol dependence versus no history of alcohol dependence in abstinent drinkers. An ROC analysis showed an area under the curve (AUC) of 0.79 with a 95% confidence interval (CI) of 0.61 to 0.92. The optimal cutoff level for CsF-stimulated AC activity was 109 pmol cAMP/mg protein/min, which yielded a sensitivity of 75% and a specificity of 79% (Fig. 2). We found similar results for CsF-stimulated AC activity in distinguishing abstinent subjects with current alcohol dependence from those without alcohol dependence (AUC = 0.81; 95% CI = 0.62, 0.93; sensitivity 67% and specificity 83% at a cutoff of 105 pmol cAMP/mg protein/min).

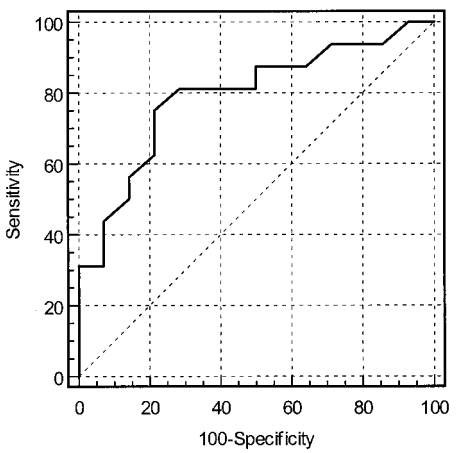

Fig. 2. ROC curve analysis of CSF-stimulated platelet AC activity for determining lifetime alcohol dependence in alcohol drinkers abstinent at least 4 days; 12 subjects with lifetime DSM-IV alcohol dependence and 14 subjects with no history of DSM-IV alcohol dependence.

Platelet AC Activity: Effects of Quantity of Alcohol Consumed, Tobacco Smoking, Age, Body Weight, and Alcohol-Related Behaviors

We also considered a number of other factors that may distinguish alcohol drinkers who had or had not consumed ethanol over the past 4 days (Table 7). The abstinent alcohol drinkers had consumed significantly less ethanol in the past month and during their heaviest drinking period compared with the recent drinkers. However, ethanol quantity, consumed either in the past month or during the heaviest drinking period, did not significantly correlate with any of the platelet AC activities (data not shown). As expected, after we adjusted for quantity of ethanol consumed in the past month, the previously noted differences in basal, CsF-stimulated, and forskolin-stimulated AC activity between abstinent and recent drinkers remained statistically significant (data not shown).

There was no difference in almost any of the platelet AC activity measures between current smokers and nonsmokers. Only basal platelet AC activity was significantly differ-

Table 7. Recent and Heaviest Ethanol Consumption and Tobacco Use of Abstinent* and Recent Male Drinkers

	Abstinent drinkers (n = 30)	Recent drinkers (N = 130)
Recent drinking		
Sobriety (days since last drank)	9.1 ± 6.0	1.2 ± 0.9
Average daily ethanol consumption last month (g)	57.4 ± 91.7[a]	100.0 ± 88.9
% Ethanol last month as beer	63 ± 38	61 ± 35
% Ethanol last month as wine	27 ± 33	27 ± 33
% Ethanol last month as liquor	11 ± 20	11 ± 22
Heaviest drinking period		
Age at onset (years)	19.7 ± 7.2	17.4 ± 2.4
Duration (months)	44.4 ± 40.4	64.2 ± 77.3
Average daily ethanol consumption (g)	90.1 ± 110.4[b]	179.1 ± 189.0
Tobacco use		
Lifetime smokers	17 (57%)	97 (75%)
Current smokers	7 (23%)	63 (48%)
Total packs of cigarettes ever smoked	4500 ± 5353	6760 ± 7576

*Abstinent at least 4 days by self-report. Values are reported as means ± SD or as frequency and percentage. Tests of significance: [a]$p < 0.05$ and [b]$p < 0.005$ compared with recent drinkers (independent *t tests* for equality of means assuming unequal variances).

ent between abstinent subjects who ever smoked and nonsmokers ($p < 0.05$, data not shown), and this difference remained significant even after adjustment for lifetime alcohol dependence.

Forskolin-stimulated AC activity weakly correlated with age in the group of abstinent subjects ($r = 0.39$, $p < 0.05$, data not shown), although this correlation disappeared in the overall group of subjects. Forskolin- and CsF-stimulated AC activity remained significantly different between recent and abstinent drinkers even after we adjusted for age and body weight. No significant association was seen between any measure of AC activity and alcohol-related behaviors such as violent behavior during or after drinking (Ikeda et al., 1998).

Platelet AC Activity: Effects of Family History for Alcohol Dependence

Abstinent alcohol drinkers who were FHP, defined separately as (1) subjects with one or more first- or second-degree alcohol-dependent relatives and, more strictly, as (2) subjects with both a first- and a second-degree alcohol-dependent relative, showed a decline in CsF-and forskolin-stimulated platelet AC activity with increasing family loading for alcohol dependence, but the observed differences did not reach significance (Table 8).

Platelet AC Activity: Effects of Antisocial Personality Disorder

Neither antisocial personality disorder nor prior conduct disorder, as defined by DSM-IV, were associated with significant differences in any mean platelet AC activity, whether the comparisons were among all subjects, all drinkers, or only abstinent drinkers.

Platelet AC Activity: Effects of Major Depression

A history of major depression, as defined by DSM-IV, was associated with significantly lower levels of forskolin-stimulated platelet AC activity in the group that consisted of nondrinkers and abstinent drinkers, compared with individuals without any history of depression (256.2 ± 76.0 vs. 382.2 ± 134.2, $p < 0.01$). A ROC analysis of low forskolin-stimulated AC activity for discriminating major depression showed an AUC of 0.79 with a 95% CI of 0.70 to 0.86. The optimal cutoff level of 286 pmol cAMP/mg protein/min yielded a sensitivity of 83% and a specificity of 76%. The basal, Gpp(NH)p-stimulated, and CsF-stimulated AC activities were not different in subjects with major depression compared with those who had no history of major depression. The results obtained with depressed subjects should be viewed with caution because the number of depressed subjects in this study was small ($n = 6$).

Platelet AC Activity: Effects of Medications and Medical Conditions

Examination of the effect of various self-reported medical conditions, both in the past 30 days and ever, on mean platelet AC activity in abstinent drinkers and nondrinkers revealed significant effects of hepatomegaly and hyperlipidemia on AC activity. Basal, CsF-stimulated, and forskolin-stimulated AC activities were significantly lower in the six subjects with a history of liver enlargement compared with 63 subjects without any history of hepatomegaly (data not shown). However, when we accounted for lifetime history of alcohol dependence, no independent effects of hepatomegaly were seen on AC activity. Within the group of abstinent drinkers and nondrinkers, 13 subjects with hyperlipidemia had significantly lower mean platelet Gpp(NH)p- and CsF-stimulated AC activity compared with the 56 subjects without elevated lipids (60.7 ± 21.8 vs. 79.0 ± 35.0, $p < 0.05$, and 103.2 ± 22.7 vs. 127.6 ± 37.3, $p = 0.005$, respectively), although no significant difference in forskolin-stimulated activity was found. Even after we accounted for lifetime history of alcohol dependence, there still remained a significant effect of hyperlipidemia on CsF- and Gpp(NH)p-stimulated AC activity. There were no significant differences in mean platelet AC activity for subjects with hypertension ($n = 11$), hepatitis ($n = 3$), or gastritis ($n = 3$), and no subjects reported a history of cirrhosis or diabetes. The recent use of various medication types, over both the past week and the past month, revealed no significant effects on mean platelet AC activity (data not shown), nor did the reported recent use of various illicit drugs.

CONCLUSIONS

In this study we report findings on platelet AC activity in a recruited population of alcohol drinkers and nondrinkers.

Table 8. Platelet Adenylyl Cyclase Activities in Abstinent Drinkers*: Relation to Family History for Alcohol Dependence

Subject groups	Platelet AC Activity (*n*) (pmol cAMP/mg protein/min)			
	Basal	Gpp(NH)p (10 μM)	CsF (10 mM)	Forskolin (10 μM)
FHN†	14.0 ± 5.1 (17)	78.1 ± 21.9 (17)	119.6 ± 21.5 (18)	331.9 ± 88.3 (18)
FHP†† in a 1st or 2nd degree relative	14.2 ± 5.4 (9)	82.3 ± 16.6 (8)	115.1 ± 20.4 (9)	305.6 ± 71.0 (9)
FHP†† in both a 1st and 2nd degree relative	12.0 ± 5.4 (4)	76.3 ± 9.0 (3)	108.2 ± 22.5 (4)	258.0 ± 68.2 (4)

* Abstinent at least 4 days by self-report; †FHN (family history negative) refers to absence of DSM-IV alcohol abuse and dependence in 1st and 2nd degree relatives; ††FHP (family history positive) refers to presence of DSM-IV alcohol dependence (with symptom clustering) in 1st and/or 2nd degree relatives. Values are means ± SD. No significant differences between groups were noted by ANOVA.

Because the assessed population consisted of White Australian men, the generalization of our results to women or to other racial/ethnic groups should be undertaken with significant caution. In the abstinent drinkers, CsF- and forskolin-stimulated platelet AC activities were significantly lower in individuals diagnosed with either current or lifetime alcohol dependence compared with those who had no history of alcohol dependence. The results obtained with CsF mirror the results of prior studies that examined AC activity in abstinent alcohol-dependent subjects, using both platelets (Lex et al., 1993; Parsian et al., 1996; Tabakoff et al., 1988) and lymphocytes (Waltman et al., 1993). Our results obtained with forskolin add yet another dimension to results that demonstrate lower platelet AC activity in alcohol-dependent individuals. Forskolin is a diterpene alkaloid, which binds directly to AC protein and activates the enzyme (Seamon and Daly, 1981). The results of assays with forskolin indicate that the AC enzyme protein may be the determinant of the lower platelet AC activity.

Two other studies of subjects with a lifetime history of alcohol dependence (Saito et al., 1994; Tabakoff et al., 1988) showed that individuals with a history of alcohol dependence, but who had been abstinent for 1 to 4 years, displayed lower CsF-stimulated platelet AC activity compared with individuals who had never been alcohol dependent. Prior work also has focused attention on the fact that individuals with a family history of alcohol dependence display lower platelet AC activity than FHN subjects (Lex et al., 1993; Saito et al., 1994). In the current study, a trend was again evident for lower CsF- and forskolin-stimulated AC activity in our subjects as the loading for alcohol dependence in their families increased. Additionally, because CsF-stimulated platelet AC activity has been demonstrated to be a heritable trait (Devor et al., 1991), one can consider that low platelet AC activity may represent a biological "trait" marker for alcohol dependence.

However, recent consumption of alcohol influences the observed levels of platelet AC activity. The effect of recent alcohol consumption on platelet AC activity is evident through comparisons of basal-, CsF-, and forskolin-stimulated AC activity in individuals currently drinking and those reporting abstinence for 2 or more days. The highest levels of AC activity are evident in the self-reported current drinkers, whereas significantly lower levels are evident in the abstaining subjects. The mean values for basal-, CsF-stimulated, and forskolin-stimulated activity stabilized at their lowest levels in individuals who reported 4 or more days of abstinence. Similarly, a recent study that serially monitored platelet AC activity in individuals who recently had ended heavy alcohol consumption (measurements made at 1–3 days, 7 days, and 14 days of monitored abstinence) found that CsF- and forskolin-stimulated platelet AC activity diminished significantly within 1 week of abstinence, and the levels of AC activity remained constant at 7-day and 14-day assessments (Menninger et al., 1998).

In the current study, a number of biological markers of recent alcohol use were also available to assess abstinence and relate to platelet AC activity. Plasma 5-HTOL/5-HIAA ratios have been reported to remain elevated for 5 to 20 hr after clearance of ethanol from an alcohol-consuming individual (Sillanaukee, 1996). Individuals with elevated 5-HTOL/5-HIAA ratios in the current study had significantly higher forskolin-stimulated platelet AC activity than individuals with normal 5-HTOL/5-HIAA ratios. Serum CDT levels are also a measure of recent alcohol consumption with sensitivity and specificity values equal to or better than those for the conventional laboratory markers (Anton and Moak, 1994; Stibler, 1991). CDT levels have been reported elevated in most individuals who consume ethanol at levels at or exceeding 60 to 80 g per day for extended periods (Stibler et al., 1986). With abstinence, serum CDT levels normalize with a half-life of about 15 days (Stibler et al., 1991). Individuals with elevated CDT levels in our study also displayed higher levels of CsF- and forskolin-stimulated platelet AC activity compared with individuals who had normal CDT levels.

Ethanol metabolism and elimination have been reported to range from 58 to 148 mg/kg/hr (Kopun and Propping, 1977), or 4 to 11 g/hr in a 70 kg man, with more rapid metabolism and elimination of ethanol noted in heavy drinkers (Jones, 1993) and chronic alcoholics (Nuutinen et al., 1985). Thus, the quantity of alcohol present in three standard drinks (one standard drink is considered equivalent to 13.6 g absolute ethanol; Sobell and Sobel, 1992) can be metabolized in 4 to 6 hr in an average male drinker. The presence of plasma ethanol in certain individuals in our study did not differentiate these individuals with regard to their platelet AC activity. If one assumes that regular ethanol consumption elevates certain components of platelet AC activity, then one can hypothesize that normalization of the platelet AC activity follows a time course that is significantly slower than the time course of ethanol elimination in human males.

Elevated plasma GGT and AST levels also did not dif-

ferentiate platelet AC activity. The elevated GGT and AST levels in alcohol-consuming individuals in many cases reflect liver damage (Sillanaukee, 1996) rather than alcohol consumption per se. Thus, one can assume that elevated platelet AC activity values may not be a consequence of changes in liver function, which would be reflected by the elevations in plasma GGT and AST.

Overall, by using either self-report of recent alcohol intake or the results of the biological markers (5-HTOL/5-HIAA ratios or CDT levels) for measuring recent alcohol intake, the results indicate that recent (within 2–4 days) alcohol intake elevates platelet AC activity when the enzyme is activated by CsF or forskolin. Given that platelet half-life in humans is approximately 4 to 5 days (Gewirtz and Poncz, 1991), one may conjecture that the lowering (normalization) of platelet AC activity after the individual stops drinking alcohol may be related to generation of new platelets during the abstinence period. Interestingly, platelet AC activity measured in the presence of Gpp(NH)p was not statistically higher in currently alcohol-consuming subjects compared with abstinent subjects. This observation may reflect a lower sensitivity of this assay condition to measure ethanol-induced differences in platelet AC activity, or it may reflect a mechanism of ethanol's actions that leaves unperturbed the path by which Gpp(NH)p activates AC. Although both CsF and Gpp(NH)p act through G proteins to modulate AC activity, their actions are different. Gpp(NH)p is a nonhydrolyzable analog of GTP that displaces GDP and binds to the GTP binding site of G proteins and activates these proteins. On the other hand, the fluoride ion interacts with G proteins (e.g., $G_S\alpha$) that have GDP bound at the guanine nucleotide binding site and activates this complex by stabilizing the $G_S\alpha$ transition state responsible for the activation of AC (Skiba and Hamm, 1998).

A final important observation about the effect of recent alcohol consumption on platelet AC activity was that the AC activity did not correlate with self-report on the quantity of alcohol consumed in the last 30 days. This observation may reflect the difficulty in obtaining accurate information on prior alcohol consumption without using various memory aids (Babor and Del Boca, 1992), or it may reflect the fact that platelet AC activity is a better reflector of more recent drinking (the preceding 2 to 4 day period) than of drinking that occurred within the past 30 days. However, our interview instrument was not designed to accurately quantify the most recent daily alcohol consumption, and future studies would benefit from using memory-enhancing methods such as the timeline follow-back technique (Sobell and Sobel, 1992) to assess quantitative relations between recent ethanol intake and platelet AC activity.

Because our subject sample was recruited primarily on the basis of their alcohol consumption habits rather than on the a priori determination of a diagnosis of alcohol dependence, we further assessed the utility of platelet AC activity as a biological marker for distinguishing alcohol-dependent subjects in the abstaining (4 or more days) subject population. A ROC analysis provided a cutoff value for CsF-stimulated platelet AC activity that allowed for a sensitivity and specificity of approximately 75%. Although a marker with this level of sensitivity and specificity can be considered of moderate utility in identifying alcohol-dependent subjects in a general population, the current analysis represents one of the first attempts to ascertain the sensitivity and specificity of this putative biological "trait" marker for alcohol dependence.

Other enzymological differences between alcohol-dependent and nondependent subjects have been proposed to represent "trait" markers for alcohol dependence, but evidence for their heritability, sensitivity, and specificity is lacking (Li, 1995). For example, a link between esterase D polymorphism and alcohol dependence (Tanna et al., 1988) has not been replicated in subsequent studies (Neiswanger et al., 1995; Wesner et al., 1991). More recently, however, genetic analyses have produced a report about a link between an alcoholism-related severity phenotype and microsatellite markers (e.g., D16S675) on chromosome 16 (Foroud et al., 1998). Interestingly, two AC genes are known to be located on chromosome 16: The AC type VII gene is located between 16q12 and 16q13 (Hellevuo et al., 1995), and the AC type IX gene is located at 16p13.3 (Hacker et al., 1998). The gene for AC type IX is located in the same region of chromosome 16 as the D16S675 marker identified by Foroud et al. (1998) (i.e., 16p13.3). It would be of interest to further examine the gene for AC IX and to identify the possible structural or regulatory differences that may contribute to a predisposition for low platelet AC activity.

We again found a relationship between low forskolin-stimulated platelet AC activity and a diagnosis of depression in our subjects. Prior work in nondependent individuals demonstrated significantly lower forskolin-stimulated AC activity in subjects with unipolar depression (Menninger and Tabakoff, 1997). Although the current study contained only a limited number of subjects who could be assigned a diagnosis of major depression, the mean value for forskolin-stimulated platelet AC activity in these subjects was significantly lower than in subjects with no history of major depression. The distinguishing feature of the platelet AC activity between the subjects with a history of major depression versus the alcohol-dependent subjects was that the alcohol-dependent subjects were low in both CsF and the forskolin-stimulated platelet AC activity, whereas the depressed subjects displayed lower forskolin-stimulated AC activity in conjunction with normal CsF-stimulated activity. Further work in this area may allow for development of a diagnostic tool for distinguishing alcohol-dependent subjects with and without comorbid unipolar depression.

The current study also allowed us to examine effects of a number of medical conditions and licit and illicit drug use on platelet AC activity in our subjects. In general, most

such variables had little or no effect on platelet AC activity. The current study did reveal an altered AC activity in subjects with hepatomegaly, though this effect appeared to be explained by alcohol dependence. Hyperlipidemia, however, appears as an independent factor that results in lower CsF-stimulated AC activity (in abstinent and nondrinker subjects). This observation appears consistent with prior reports of reduced AC activity associated with hypercholesterolemia. Schmidt et al. (1993) and Gerasimova (1977) noted reduced AC activity in smooth muscle and liver tissue, as well as in thrombocytes of hypercholesterolemic rabbits. The effect of hyperlipidemia on platelet AC activity is, however, distinguishable from the effect of alcohol dependence: alcoholism was associated with both low CsF-stimulated and low forskolin-stimulated AC values, whereas hyperlipidemia was associated with lower CsF-stimulated but not forskolin-stimulated AC activity. Prior studies have reported little association between smoking and platelet AC activity (Menninger et al., 1998; Tabakoff et al., 1988). Similarly, in the current study, although smoking increased basal AC activity in abstinent drinkers, there was no significant difference in stimulated AC activity levels between smokers and nonsmokers.

In summary, CsF-stimulated platelet AC activity may act as a marker for lifetime incidence of alcohol dependence, as long as the subject is abstinent for 4 days or more before testing. Additional factors that may contribute to low platelet AC activity are depression, hyperlipidemia, and family history of alcohol dependence. Measurements of both forskolin- and CsF-stimulated platelet AC activity may allow researchers to distinguish individuals with a lifetime history of alcohol dependence from those individuals with affective illness and/or hyperlipidemia.

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