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Effects of Chronic Alcohol Use and Age on Human Lymphocyte Protein Kinase C Activity

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DEPETRILLO, P. B. *Effects of chronic alcohol use and age on human lymphocyte protein kinase C activity.* PHARMACOL BIOCHEM BEHAV **48**(4) 999–1004, 1994.—The effects of alcohol exposure on human peripheral circulating lymphocyte protein kinase C (PKC) activity were characterized in lymphocytes harvested from two sample groups. The first group (control) consisted of 30 nonalcoholic male subjects and the second group consisted of nine male subjects with chronic alcoholism. Alcoholic subjects were admitted for detoxification to a substance abuse unit located in a nonprofit community hospital. In this group of subjects, blood was sampled on admission for detoxification (pre-A), and after 5 days (post-A). Subjects received chlordiazepoxide for treatment of alcohol withdrawal symptoms. PKC activities measured in the control, pre-A, and post-A groups expressed as pmol/μg/min ± SEM were 5.09 ± 0.50, 1.81 ± 0.43, and 3.95 ± 0.44. Control PKC was significantly higher than pre-A PKC ($p \leq 0.05$) and post-A PKC was significantly higher than pre-A PKC ($p \leq 0.05$). Total lymphocyte PKC activity was also found to be inversely related to age, expressed by the relationship log(PKC) = 0.870 − 0.005(Age), with $R = 0.433$.

Protein kinase C Alcohol Ethyl Lymphocyte Age factors

CELLULAR mechanisms implicated in the acquisition of alcohol tolerance and expression of withdrawal syndromes are not well understood. However, dysregulation of key intracellular transduction systems involving cyclic adenosine 3′5′-monophosphate (11), alterations in β-adrenergic receptor ligand affinity (16), levels of stimulatory G-proteins (19), and changes in adenylate cyclase activity (25), have all been associated with components of alcohol dependence including the alcohol withdrawal syndrome. A more complete understanding of in vivo alterations in key intracellular signaling systems during alcohol abuse and withdrawal will not only provide insight into biochemical correlates of alcoholism, but may characterize future therapeutic targets for the treatment of alcohol habituation, tolerance, and withdrawal.

The phosphatidylinositol cascade has emerged as a cellular signaling system of major importance, mediating transduction of a wide variety of substances such as norepinephrine (18), acetylcholine (6), and serotonin (1), all of which are critical to central nervous system function. The intracellular expression of activation of the phosphatidylinositol cascade is characterized by a series of events which focus on control of intracellular Ca^{++} homeostasis and control of protein kinase C (PKC) activity. Protein kinase C (PKC) is the descriptive term for a heterogeneous family of enzymes activated by receptor-mediated hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2). This results in the production of inositol 1,4,5-triphosphate (1,4,5 IP_3) and 1,2-*sn*-diacylglycerol (DAG), accompanied by an increase in cytosolic Ca^{++} (21).

Regulation of PKC activity is a dynamic process, with PKC activity in tissues decreasing after chronic exposure to agents that activate the enzymes (34). Anaesthetics, including alcohols of varying chain length, appear to acutely inhibit PKC activity in purified preparations, possibly by interacting with the regulatory subunit of the enzyme (28). In contrast, in vitro exposure of circulating lymphocytes to ethanol results in an increase in PKC activity (10). Evidence from animal studies suggests that in vivo chronic exposure to ethanol decreases phorbol ester binding, a measure of available PKC sites, as well as decreasing PKC activity itself in rat brain (4). Phorbol ester binding may not reflect total PKC activity, but appears

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to correlate with increases in membrane-bound activity (20). In light of these findings, this study was undertaken to examine whether chronic alcohol exposure was associated with changes in total PKC activity measured in freshly isolated human circulating lymphocytes.

METHOD

Subjects

Human subjects participating in this study gave written informed consent for the protocol which had the approval of the institutional review board. Eighteen nonalcoholic male subjects comprising the control group (mean age 41.2 years ± 5.4, range 19–75) were recruited for the study by means of advertisements placed in local media. Ten alcoholic male subjects were recruited from a population of chronic alcoholics admitted to an in-patient detoxification and short-term rehabilitation program at Roger Williams Hospital (Providence). This program enrolls approximately 650 in-patients/year, with primary diagnoses of alcohol dependence, sedative-hypnotic, opiate, and cocaine dependence. Consequently, a substantial percentage of patients with a primary diagnosis of alcohol dependence were found to be abusing a variety of substances, including benzodiazepines and cocaine, by history and confirmatory urine drug screen. Over an 8 month period, 10 consecutive alcoholic males meeting criteria for study participation on initial history were entered into the study. Nine alcoholic subjects completed the study (mean age 54.2 years ± 5.9, range 26–79) with one subject excluded from the study because of positive urine toxicologic analysis for benzodiazepines. Inclusion criteria for alcoholic subjects included a diagnosis of alcohol dependence by DSM-III-R criteria (2), an average alcohol intake of >80 g/day for at least two weeks prior to admission for detoxification, a blood alcohol level estimated by breathalyzer of at least 4.34 mM (20 mg%) on admission, and no other prescribed or nonprescribed drug use within 4 weeks of hospitalization with the exception of caffeine, nicotine, or acetaminophen. Subjects with severe coexisting medical or psychiatric disorders were excluded from participation. Nonalcoholic volunteer subjects were healthy males without evidence of substance abuse by structured history, physical, and screening laboratory examinations.

Alcohol use was quantitated by a structured interview estimating the average frequency of intake over the preceding year. Parameters estimating alcohol consumption were: beer (one bottle or can), wine (one glass), and liquor (whiskey, gin, vodka, etc). Drinks were converted to grams of alcohol per day as follows: one beer (360 ml) = 13.2 g ethanol, one glass of wine (120 ml) = 10.8 g ethanol, one drink of liquor (45 ml) = 15.1 g ethanol (13). Drug use history was validated by urine toxicologic analysis on admission (Abbott ADx, North Chicago, IL) (15). Blood alcohol concentration (BAC) was estimated using a hand-held intoximeter (Alco-Sensor III, Intoximeters, Inc., St. Louis, MO).

All subjects admitted for alcohol detoxification underwent complete history, physical, and laboratory examinations. Hourly estimations of blood alcohol concentration by breathalyzer were obtained. After two consecutive BAC readings of 0 mg%, 40 ml of blood were drawn by venipuncture into four sterile tubes containing 1.67 ml of Acid-Citrate-Dextrose (ACD) (5 g/l citric acid, 25 g/l sodium citrate, 20 g/l *d*-glucose) which were gently mixed by inversion several times and transported to the laboratory within 20 min for separation of lymphocytes. Blood was drawn again 5 days after admission in a nonfasting state approximately 2 h after breakfast. A second sampling time of 5 days was chosen to maximize the length of time between the first and second blood samples while minimizing dropout from the study because of discharge, based on a known mean length of stay of 6.2 days in this population. Blood was sampled in control subjects 2 h after breakfast in a nonfasting state. Alcoholic subjects, all of whom were cigarette smokers, were allowed to smoke ad lib during the hospitalization.

Symptoms of withdrawal in alcoholic subjects were treated according to a standard protocol (32). The severity of alcohol withdrawal was quantitated every 1 to 2 h by specially trained nursing staff employing the Clinical Institute Withdrawal Assessment Scale for Alcohol (CIWA-A) instrument (26). Chlordiazepoxide was administered for withdrawal symptoms consistent with a CIWA-A score ≥ 15 and was discontinued when the score remained < 10 over a 24-h period.

Isolation of Lymphocytes and Cell Extract Preparation

ACD anticoagulated blood was diluted 1 : 1 with ice cold calcium deficient Eagle's medium containing 5 mM HEPES pH 7.42 and the mononuclear cells isolated by the method of Boyum (7). The suspension of mononuclear cells was then passed at a rate of 2 ml/min through a eight layers of 200 μm pore nylon mesh packed in a sterile 10 ml syringe to adsorb macrophages and platelets. The resulting eluate was >99% free from white blood cell and platelet contamination and was >99% viable by trypan blue dye exclusion. The volume of the solution was adjusted to a final concentration of 1×10^7 cells/ml.

For preparation of cell homogenate, 200 μl of cell suspension containing 2×10^6 cells was added to 200 μl of ice-cold homogenization buffer (HB) containing 20 mM Tris, pH 7.5, 0.5 mM EDTA, 0.5 mM EGTA, 0.5% Triton X-100, 25 μg/ml of aprotinin, and leupeptin. Cells were disrupted by sonication (Fisher Model II) at 35% power employing three 20-s bursts, followed by centrifugation at 14,000 × *g* × 2 min at 4°C.

Purification and Determination of PKC Activity

For purification of cell extract, 2 ml disposable microcolumns (Bio-Rad, Richmond, CA) were packed with 0.25 g of DEAE (DE52) cellulose (Whatman, Hillsboro, OR) suspended in 1 ml of wash buffer (WB) containing 20 mM Tris pH 7.5, 0.5 mM EDTA, 0.5 mM EGTA. Prior to use, the columns were washed with 2 ml of WB. Cell extract was added and washed with 2.5 ml of WB. Subsequently, PKC activity was eluted with 2 ml of buffer containing 20 mM Tris pH 7.5, 0.5 mM EDTA, 0.5 mM EGTA, 10m M β-mercaptoethanol and 0.2 M NaCl. About 20% of the input protein was recovered in the eluate.

PKC activity was determined using a commercially available kit (GIBCO BRL, Gaithersburg, MD) with a fragment of myelin-basic-protein employed as a phosphate-acceptor peptide (33). PKC activity measured after an incubation time of 5 min is reported as the amount of γ-^{32}P incorporated into substrate per μg protein per minute (pmol/μg/min). For the assay, 10 μl of eluate was used according to the protocol supplied by the manufacturer. The protein concentration of the eluate was chosen so as to be within the linear limits of the

assay. Protein concentrations were measured by the method of Bradford (8) using bovine serum albumin standards.

Statistical Analysis

Experimental data were analyzed using parametric ANCOVA procedures and descriptive statistics, with age as a fixed covariate. For all analyses, a graph of the residual error plotted against the fitted PKC values showed increasing variance with larger values of PKC. Therefore, the log transform of PKC values were entered into the model. Violation of independence assumptions in repeated-measures ANCOVA was corrected by means of the Greenhouse-Geisser procedure, and the resulting F-value adjusted accordingly. A similar procedure was used to test for significant differences in isolated lymphocyte cell counts between the three groups.

All means comparisons statistics were adjusted by the Bonferroni/Dunn correction for multiple mean comparisons. A p-value of ≤ 0.05 was accepted as significant for all analyses. All values are expressed as mean ± standard error (SEM).

RESULTS

In the group of chronic alcoholics, the mean PKC activity on admission was 1.81 pmol/μg protein/min ± 0.43. On repeat sampling 5 days after admission, PKC activity was 3.95 pmol/μg/min ± 0.44. PKC activity contrasted between these two groups is significantly different ($F = 7.718$, $p = 0.02$), as shown in Fig. 1. Parameters derived from ANCOVA comparing the control group with pre-A showed no effect of age ($F = 1.987$, $p = 0.17$) and no effects of age × group ($F = 1.686$, $p = 0.19$). Control PKC was significantly different from pre-A PKC ($F = 24.236$, $p = 0.0001$). Comparing mean PKC activity between control and post-A, there was no group effect on PKC activity ($F = 0.186$, $p = 0.67$), and no effect of group × age ($F = 0.430$, $p = 0.52$). However, age was a significant factor in explaining variability of PKC ($F = 8.56$, $p = 0.006$) ($R = 0.433$ for the regression, which is shown in Fig. 2). Parameters derived from the ANCOVA were $\beta_0 = 0.870 \pm 0.087$, $p = 0.0001$; $\beta_1 = -0.005 \pm 0.002$, $p = 0.006$. The relationship can be expressed by log(PKC) = 0.870 − 0.005(Age).

One subject in the alcohol group was excluded from analysis because of urine toxicologic evidence of benzodiazepine use. Baseline parameters associated with the alcoholic subjects are shown in Table 1. At the time of the first blood sampling, mean ± SEM systolic and diastolic blood pressures (mm Hg) measured in the right arm in supine position were 134.9 ± 4.9 and 87.3 ± 3.8, respectively. Mean heart rate was 94.0 ± 5.0 beats per minute. At sampling time, CIWA-A withdrawal scores were < 15 in all subjects. The average dose of chlordiazepoxide administered for treatment of alcohol withdrawal symptoms was 155.5 mg ± 67.9, given in divided doses within 72 h of admission. There were no significant differences in the number of circulating lymphocytes isolated from 40 ml of

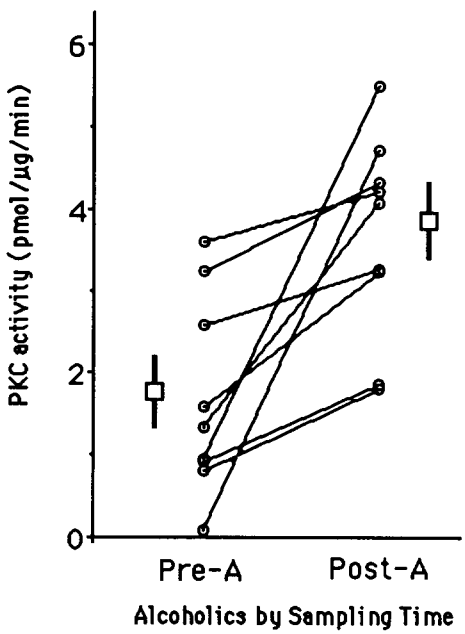

FIG. 1. Individual values for PKC activities are represented by open circles for $n = 9$ alcoholic subjects sampled on admission (pre-A) and after 5 days (post-A). The lines connect values obtained from an individual subject. To the right or left of the individual points for each group is a box with error bars depicting the mean value of PKC activity ± SEM. Mean PKC activities in alcoholic subjects were 1.81 ± 0.43 and 3.95 ± 0.44 for the pre-A and post-A groups, respectively, significantly different at $p \leq 0.05$.

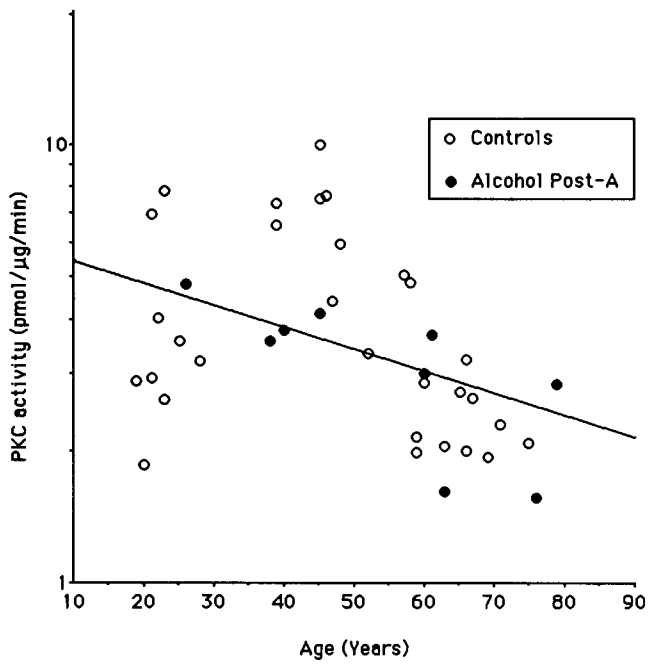

FIG. 2. Plot of age of individual subjects against PKC activity, represented by open circles for control subjects ($n = 30$) and filled circles for alcoholic subjects ($n = 9$), sampled 5 days after cessation of drinking, post-A. The Y-axis representing PKC activity in pmol/μg protein/min is a log scale. There was no difference in PKC activity between these two groups, but a significant effect of age on PKC activity was found. The linear regression model derived from the ANCOVA is log(PKC) = 0.870 − 0.005(Age), $R = 0.433$, $F = 8.559$, and $p = 0.0058$.

TABLE 1
BASELINE CHARACTERISTICS OF ALCOHOLIC SUBJECTS (n = 9)

	Mean	SEM	Range
Age, years	54.2	5.9	26-79
Alcohol use, g/day	244	32	119-362
Duration of use, years	28.8	5.9	3-50
Admission alcohol level, mM (mg%)	30.4 (140.1)	5.9 (27.8)	11.9-61.9 (55-285)

whole blood between the the control group and the pre-A and post-A groups. (control 2.750 ± 0.163, pre-A 2.856 ± 0.365, post-A 2.567 ± 0.302, all values × 10^6; F = 0.225, NS).

DISCUSSION

Compared to lymphocyte PKC activity sampled at 5 days, PKC activity was lower in lymphocytes of chronic alcoholics sampled just after drinking ceased. When compared to healthy, nonalcoholic volunteers, lymphocyte PKC activity was decreased in alcoholics when sampled immediately after alcohol cessation, but was not significantly different when sampled after a short period of abstinence. Although efforts were made to sample alcoholic subjects at a time when blood alcohol levels were zero, it is possible that some of the effects seen on PKC activity at the time of the first sampling were due to the acute effects of the drug. However, because acute alcohol exposure appears to increase, rather than decrease PKC activity in circulating lymphocytes, it is unlikely that the effects of acute alcohol exposure can explain decreases in PKC activity seen in the pre-A group when compared to the post-A group. The time course of the change in PKC activity after alcohol cessation is unknown, because only two time points were obtained in this preliminary study.

An age-related alteration in total lymphocyte PKC activity is seen both in the control and alcoholic groups. Total PKC activity appears to decrease with age. A similar age-related decrease in PKC activity has been found in rat hippocampal slices (12). Decreases in circulating lymphocyte PKC activity may parallel decreases in responses to humoral factors which rely on PKC-mediated signal transduction. Such age-related decreases in response have been found to occur both in murine (23) as well as human (5) models. The finding suggests that age-related factors are important in the interpretation of PKC-activity data derived from human tissues.

There are a number of potentially confounding variables in this study including nutritional status, ingestion of other drugs, and chronic sympathetic adrenergic stimulation secondary to alcohol withdrawal. Although nutritional status was not formally evaluated, none of the subjects appeared clinically malnourished. This is supported by the finding of a mean serum albumin value in alcoholic subjects of 42.3 g/l ± 0.6 (± SEM; range 40.0-46.0 g/l), as well as hemoglobin and hematocrit values within normal range for males, suggesting that nutritional status was comparable to that of the general population (17).

Drug administration for pharmacotherapy of alcohol withdrawal symptoms might have contributed to the increase in PKC activity seen at the time of the second blood sampling in the alcoholic subjects. However, at the time of first blood sampling, the subjects were verified by urine toxicology screening to be free of other drugs with the exceptions noted above. This confounder would not alter the conclusion that alcohol intake contributed significantly to differences in total lymphocyte PKC activity between control subjects and alcoholic subjects at the time of the first blood sampling.

The possible effects of elevated circulating catecholamine levels as a result of early alcohol withdrawal are not likely to explain differences in circulating lymphocyte PKC activity. The peripheral manifestations of the alcohol withdrawal syndrome are largely due to increases in levels of circulating catecholamines (27). There is no data regarding the effects of catecholamine exposure on total lymphocyte PKC activity. However, agents that alter total PKC levels by potent direct activation of the enzyme, such as phorbol ester, require at least 6 h of exposure to result in measurable decreases in enzyme levels and activity (30). Blood samples from alcoholic patients were obtained prior to the development of severe alcohol withdrawal symptoms. Blood sampling occurred at CIWA-A scores < 15 with minimally elevated systolic and diastolic blood pressures and heart rates. These data argue against rising serum catecholamine levels as an explanation for the lower PKC activity seen in lymphocytes sampled from the Admission group.

Changes in the composition of circulating pools of lymphocytes are also unlikely to account for changes in PKC activity because of the relatively long half life of circulating lymphocytes as compared to the time course of alcohol withdrawal. It has been shown that the only change in surface-marker expression in circulating pools of lymphocytes sampled from chronic alcoholics was a decrease in the percentage of cells expressing alpha-1 acid glycoprotein as well as a marked deficiency in the ability of these lymphocytes to participate in a functional test of immune competence (29).

Chronic nicotine exposure appears to increase PKC activity in bovine adrenal medullary cells (31). Because all subjects in the alcohol group consumed cigarettes, it is possible that nicotine exposure may have altered PKC activity in circulating lymphocytes as well. Because the alcoholic subjects served as their own controls, it is unlikely this would alter the conclusion that alcohol exposure was the main determinant of differences in PKC activity. If nicotine exposure increased PKC activity in circulating lymphocytes, this would have tended to bias the results in favor of the null hypothesis when comparing PKC activity measured in the Control group and the pre-A group.

These results support the hypothesis that chronic alcohol exposure modulates PKC activity in vivo. The results also suggest an age-related decrease in human circulating lymphocyte PKC activity. Because activation of PKC is an

integral step in the activation of T-lymphocytes (22) and B-lymphocytes (3,24) in response to antigen receptor cross-linking, the effect of chronic ethanol consumption on circulating lymphocyte PKC activity might help to explain the immune system dysfunction seen with such exposure (9,14). If alcohol-related in vivo modulation of PKC activity in humans is found to occur in other tissues, as suggested by similar findings in animal models (10), such changes might prove to be of particular pathophysiologic importance in explaining the multiorgan system effects of chronic alcohol intake.

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REFERENCES

1. Aghajanian, G. K.; Sprouse, J. S.; Sheldon, P.; Rasmussen, K. Electrophysiology of the central serotonin system: Receptor subtypes and transducer mechanisms. Ann. NY Acad. Sci. 600:93–103; 1990.
2. American Psychiatric Association: Diagnostic and statistical manual of mental disorders: DSM-III-R. 3rd ed., revised. Washington, DC: American psychiatric Association; 1987.
3. Bandekar, J. R.; Castagna, R.; Sultzer, B. M. Roles of protein kinase C and G proteins in activation of murine resting B lymphocytes by endotoxin-associated protein. Infect. Immunol. 60(1): 231–236; 1992.
4. Battaini, F.; Del Vesco, R.; Govoni, S.; Trabucchi, M. Chronic alcohol intake modifies phorbol ester binding in selected brain areas. Alcohol 6:169–172; 1989.
5. Beckman, I.; Dimopoulos, K.; Xu, X. N.; Ahern, M.; Bradley, J. A. Age-related changes in the activation requirements of human CD4+ T-cell subsets. Cell. Immunol. 132(1):17–25; 1991.
6. Berstein, G.; Blank, J. L.; Smrcka, A. V.; Higashijima, T.; Sternweis, P. C.; Exton, J. H.; Ross, E. M. Reconstitution of agonist-stimulated phosphatidylinositol 4,5-bisphosphate hydrolysis using purified m1 muscarinic receptor, Gq/11, and phospholipase C-beta 1. J. Biol. Chem. 267(12):8081–8088; 1992.
7. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. Suppl. 97(21):77–89; 1968.
8. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254; 1977.
9. Chang, M. P.; Norman, D. C. Immunotoxicity of alcohol in young and old mice. II. Impaired T cell proliferation and T cell-dependent antibody responses of young and old mice fed ethanol-containing liquid diet. Mech. Aging Dev. 57(2):175–186; 1991.
10. DePetrillo, P. B.; Liou, C. S. Ethanol exposure increases total PKC activity in human lymphocytes. Alcohol.: Clin. Exp. Res. 17(2):351–354; 1993.
11. Diamond, I.; Wrubel, B.; Estrin, W.; Gordon, A. Basal and adenosine receptor-stimulated levels of cAMP are reduced in lymphocytes from alcoholic patients. Proc. Natl. Acad. Sci. USA 84: 1413–1416; 1987.
12. Fordyce, D. E.; Wehner, J. M. Effects of aging on spatial learning and hippocampal protein kinase C in mice. Neurobiol. Aging 14(4):309–317; 1993.
13. Giovanucci, E.; Colditz, G. A.; Stampfer, M. J. The assessment of alcohol consumption by a simple self-administered questionnaire. Am. J. Epidemiol. 133:810–817; 1991.
14. Jerrells, T. R.; Smith, W.; Eckardt, M. J. Murine model of ethanol-induced immunosuppression. Alcohol.: Clin. Exp. Res. 14(4): 546–550; 1990.
15. Jolley, M. E.; Stroupe, S. D.; Schwenzer, K. S.; Wang, C. J.; Lu-Steffes, M.; Hill, H. D.; Popelka, S. R.; Holen, J. T.; Kelso, D. M. Fluorescence polarization immunoassay. III. An automated system for therapeutic drug determination. Clin. Chem. 27:1575–1579; 1981.
16. Kuriyama, K.; Muramatsu, M.; Aiso, M.; Ueno, E. Alteration in β-adrenergic receptor binding in brain, lung, and heart during morphine and alcohol dependence and withdrawal. Neuropharmacology 20:659–666; 1981.
17. Micozzi, M. S.; Albanes, D.; Stevens, R. G. Relation of body size and composition to clinical and hematologic indices in US men and women. Am. J. Clin. Nutr. 50(6):1276–1281; 1989.
18. Minneman, K. P.; Atkinson B. Interaction of subtype-selective antagonists with alpha 1-adrenergic receptor-mediated second messenger responses in rat brain. Mol. Pharmacol. 40(4):523–530; 1991.
19. Mochly Rosen, D.; Chang, F. H.; Cheever, L.; Kim, M.; Diamond, I.; Gordon, A. S. Chronic ethanol causes heterologous desensitization of receptors by reducing as messenger RNA. Nature 333:848–850; 1988.
20. Nordstedt, C. Synergism between phorbol ester and the Ca^{2+} ionophore A23187 on protein kinase C translocation, [^{3}H]PDBu binding and adenosine A2-receptor activation in Jurkat cells. Eur. J. Pharmacol. 188(6):349–357; 1990.
21. Ohno, S.; Akita, Y.; Hata, A.; Osada, S.-I.; Kubo, K.; Konno. Y.; Akimoto, K.; Mizuno, K.; Saido, T.; Kuroki, T.; Suzuki, K. Structural and functional diversities of a family of signal transducing protein kinases, protein kinase C family; Two distinct classes of PKC, conventional cPKC and novel nPKC. Adv. Enzyme Regul. 31:287–303; 1991.
22. Pardi, R.; Inverardi, L.; Rugarli, C.; Bender, J. R. Antigen-receptor complex stimulation triggers protein kinase C-dependent CD11a/CD18-cytoskeleton association in T lymphocytes. J. Cell. Biol. 116(5):1211–1220; 1992.
23. Patel, H. R.; Miller, R. A. Age-associated changes in mitogen-induced protein phosphorylation in murine T lymphocytes. Eur. J. Immunol. 22(1):253–256; 1992.
24. Rooney, J. W.; Dubois, P. M.; Sibley, C. H. Cross-linking of surface IgM activates NF-kappa B in B lymphocyte. Eur. J. Immunol. 21(12):2993–2998; 1991.
25. Saffey, K.; Gillman, M. A.; Cantrill, R. C. Chronic in vivo ethanol administration alters the sensitivity of adenylate cyclase coupling in homogenates of rat brain. Neurosci. Lett. 84:317–322; 1988.
26. Shaw, J. M.; Kolesar, G. S.; Sellers, E. M.; Kaplan, H. L.; Sandor, P. Development of optimal treatment tactics for alcohol withdrawal. I. Assessment and effectiveness of supportive care. J. Clin. Psychopharmacol. 1(6):382–387; 1981.
27. Sjoquist, B.; Borg, S.; Kvande, H. Catecholamine derived compounds in urine and cerebrospinal fluid from alcoholics during and after long-standing intoxication. Subst. Alcohol. Actions Misuse 2:63–72; 1981.
28. Slater, S. J.; Cox, K. J.; Lombardi, J. V.; Ho, C.; Kelly, M. B.; Rubin, E.; Stubbs, C. D. Inhibition of protein kinase C by alcohols and anaesthetics. Nature 364(6432):82–84; 1992.
29. Stefanini, G. F.; Mazzetti, M.; Zunarelli, P.; Piccinini, G.; Amorati, P.; Capelli, S.; Cicognani, G.; Gasbarrini, G. In vivo effect of chronic ethanol abuse on membrane alpha 1-glycoprotein of lymphocytes and immune response to various stimulating agents. Alcohol.: Clin. Exp. Res. 13(3):444–448; 1989.
30. Tsutsumi, A.; Kubo, M.; Fujii, H.; Freire-Moar, J. Regula-

tion of protein kinase C isoform proteins in phorbol ester-stimulated Jurkat T lymphoma cells. J. Immunol. 150(5):1746–5; 1993.
31. Tuominen, R. K.; McMillian, M. K.; Ye, H.; Stachowiak, M. K.; Hudson, P. M.; Hong, J. S.; Long-term activation of protein kinase C by nicotine in bovine adrenal chromaffin cells. J. Neurochem. 58(5):1652–1658; 1992.
32. Wartenberg, A. A.; Nirenberg, T. D.; Liepman, M. R.; Silvia, L. Y.; Begin, A. M.; Monti, P. M. Detoxification of alcoholics: Improving care by symptoms-triggered sedation. Alcohol.: Clin. Exp. Res. 14(1):71–75; 1990.
33. Yasuda, I.; Kishimoto, A.; Tanaka, S.; Tominaga, M.; Sakurai, A.; Nishizuka, Y. A synthetic peptide substrate for selective assay of protein kinase C. Biochem. Biophys. Res. Commun. 166(3): 1220–1227; 1990.
34. Young, S.; Parker, P. J.; Ulrich, A.; Stabel, S. Down-regulation of protein kinase C is due to an increased rate of degradation. Biochem. J. 244:775–779; 1987.