Ethanol Ingestion: Differences in Blood Acetaldehyde Concentrations in Relatives of Alcoholics and Controls

Abstract. *Blood acetaldehyde concentrations were significantly elevated after a moderate ethanol dose in 20 healthy young men with alcoholic parents or siblings compared to matched controls with no familial alcoholism.*

Alcoholism appears to be a multifactorial genetically influenced disorder (*1*). This conclusion is supported by studies showing (i) a 25 to 50 percent lifetime risk for alcoholism in the sons and brothers of severely alcoholic men; (ii) the presence of a number of genetic markers associated with alcoholism; (iii) the fact that alcohol preferences can be bred in strains of animals; and (iv) a 55 percent or higher concordance rate for alcoholism in identical twins with only a 28 percent rate for same-sex fraternal twins (*2*).

The most compelling information, however, comes from studies using either the half-sibling method in the United States or adoption samples in Scandinavia (*1*, *3*). This approach has demonstrated a fourfold or higher increase in alcoholism for the children of alcoholics over controls, even when the children had been separated from their biological parents near birth and raised without knowledge of the biological parents' drinking problems. Individuals adopted through the same agencies but without alcoholic biological parents showed relatively low rates of alcoholism, even if they were reared by an alcoholic parent figure or experienced a subsequent parental death or divorce.

While these studies demonstrate the importance of unknown social and environmental factors (for example, the concordance rate in identical twins is only 55 percent), the evidence for a genetic influence is strong enough to justify longitudinal investigations. The genetic information allows for adequate prospective testing of potentially important factors in groups of young individuals at theoretical high and low risk for alcoholism. This circumvents the problems of retrospective studies in which differences between alcoholics and controls may reflect the consequences of years of heavy drinking.

There are many factors that might, individually or in combination, underlie a genetic influence in higher risk individuals. These include, but are not limited to, a possible unique reaction to a single dose of alcohol (for instance, it might give greater pleasure to those at high risk or greater discomfort to those at low risk), differences in the metabolism of alcohol, and differential susceptibility to the consequences of the long-term exposure to alcohol (*4*). In the present study, we explore differences between children of alcoholics and controls with respect to metabolism of alcohol. This approach was suggested by reports of elevated levels of acetaldehyde in alcoholics given single doses of alcohol when compared to control subjects given comparable doses (*5*).

The subjects were chosen from 304 physically healthy male students and nonacademic staff at the University of Washington who were paid to respond to a questionnaire covering demography, drinking and drug patterns and problems, and family history of alcohol, drug, and psychiatric difficulties. Psychiatric diagnoses were established for subjects and families by the criteria of Woodruff *et al.* (*6*). Diagnosis of primary alcoholism is based on the occurrence of major life problems related to alcohol in individuals with no preexisting psychiatric disorders.

After the 3 percent of young men who fulfilled the criteria for alcoholism were screened out, the first 20 of the 25 subjects with an alcoholic parent or sibling were chosen as candidates for study. A matched control with no familial alcoholism, but similar in respect to age, sex, race, marital status, and drinking history, was selected for each subject. The subjects were all Caucasian males with a mean age of 23; 70 percent reported drinking on one to four occasions per week, with an average intake of two to four drinks.

At 8:00 a.m. after an overnight fast, a participant was seated in a quiet, temperature-controlled room where vital signs were monitored and blood could be drawn through an indwelling venous catheter. Reagent quality ethanol (0.5 ml per kilogram of body weight) combined with sugar-free 7-Up at room temperature was then drunk over 5 minutes. Each person was monitored continuously on the polygraph, and blood samples were taken before ethanol administration, 15 and 30 minutes after administration, and every subsequent half-hour during the following 3 hours. Blood acetaldehyde concentration was determined on previously frozen blood samples by a modification of the method of Caldwell *et al.* (*7*) in which 0.2 ml of blood was transferred into a 5-ml Reacti-Vial and incubated for 20 minutes at 50°C, after which 500 μl was injected by the head space technique into a Perkin-Elmer 3920 gas chromatograph equipped with a flame ionization detector. A 90 cm by 2 mm Porapak S column (80 to 100 mesh) was used and maintained at 110°C. The analyses were performed by a technician who was unaware of the source of the samples.

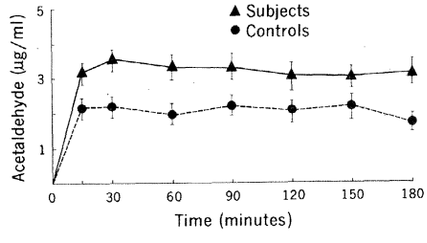

Fig. 1. Mean acetaldehyde concentrations in blood and standard errors of the mean for subjects and controls after a single dose of ethanol (0.5 ml/kg).

Mean acetaldehyde concentrations for the 20 subjects and their matched controls are shown in Fig. 1. The difference between the two groups was significant ($P < .004$; $F = 9.44$; d.f. 1, 38; two-way analysis of variance for repeated measures).

The pattern of heightened acetaldehyde concentrations for subjects is similar to data comparing abstinent alcoholics with control populations after exposure to moderate alcohol doses (*5*). Those data were generally believed to reflect possible somatic damage in alcoholics (such as impaired liver mitochondria), but our results indicate that young, healthy men with family histories of alcoholism who might be predisposed to alcoholism themselves also demonstrate increased acetaldehyde levels.

These results have potential clinical significance. Increased acetaldehyde concentrations could mediate the short-term effects of alcohol, resulting in an altered (perhaps heightened) state of intoxication. It is equally possible that those individuals predisposed to alcoholism are more vulnerable to organ damage from acetaldehyde—an example of a potential difference in response to long-term exposure to alcohol. This higher acetaldehyde plateau might facilitate the formation of condensation products with monoamine metabolites, with the resulting production of addicting morphine-like alkaloids (*8*). These possibilities, as well as others, can now be studied prospectively in healthy relatives of alcoholics and control groups.

It will be necessary to replicate the differences reported here for acetaldehyde

0036-8075/79/0105-0054$00.50/0

levels in larger and more diverse samples. In the future, participants should be monitored for 5 hours or until the acetaldehyde concentration returns to zero to better understand the differences in production and metabolism of this substance. The acetaldehyde levels we report are higher than those noted by others (*5*), perhaps because our blood samples were frozen, with resultant hemolysis—a bias which would apply equally to subjects and controls. Nonetheless, it is important to determine whether the same results are seen with different methods of analysis, especially those using fresh samples analyzed after perchlorate precipitation.

It is important to note that the young adult offspring of alcoholics—who, according to other studies, are at elevated risk for the development of alcoholism themselves (*1, 2*)—show significantly elevated levels of acetaldehyde when exposed to moderate doses of alcohol. This study demonstrates the potential importance of carrying out prospective investigations into the possible causes of alcoholism and the need to carefully test children of alcoholics.

MARC A. SCHUCKIT*
VIDAMANTAS RAYSES
Alcoholism and Drug Abuse Institute, University of Washington, Seattle 98105

References and Notes

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9. We thank D. Segal for help in preparing the manuscript.
* Present address: Psychiatry Service, Veterans Administration Hospital, San Diego, Calif. 92161, and Department of Psychiatry, School of Medicine, University of California, San Diego, La Jolla 92037. Address reprint requests to M.A.S.

14 July 1978; revised 29 August 1978

N-Acetyl Gramicidin: Single-Channel Properties and Implications for Channel Structure

Abstract. *Substitution of a methyl group for the* N*-terminal hydrogen of gramicidin greatly increased the rate of dissociation of conductive channels in lipid bilayer membranes. The finding of short lifetimes for conductive channels, comparable to those seen for the neuromuscular junction, lends support to the head-to-head dimer structure for the conductive channel.*

Gramicidin A, a linear pentadecapeptide, is a particularly useful model for ion permeation in cell membranes not only because it forms well-defined conductive channels through lipid bilayer and cell membranes but also because its chemical structure is well established (*1-3*). Kinetic as well as structural data indicate that the conductive channels consist of two gramicidin molecules (*3, 4*). Considerations of the chemical properties of gramicidin have yielded two possible models for the structure of the conductive dimer in the membrane. End-to-end dimerization of two gramicidin monomers, each folded in a single-stranded $\beta_{3,3}$ helix, was proposed to occur by hydrogen bonding between the formyl heads of the molecules (*5-7*). An alternative model, consisting of double-stranded β-helices of gramicidin, aligned in either parallel or antiparallel orientation, was later proposed as a possible structure for the conductive dimer (*8-10*). Both models exhibit a channel coincident with the helix axis. Initially the malonyl amino end to amino end covalent dimer (*6, 11*), then the *N*- and *O*-pyromellityl derivatives (*12*), and more recently the *N*- and *O*-succinyl derivatives (*13*), when examined for their activity in lipid bilayers, individually and more so collectively, lead to the conclusion that the head-to-head dimer is the likely structure for the gramicidin A channel.

In an effort to characterize more closely the relation between structure and function in conductive channels, we have examined the electrical properties of single channels formed by the *N*-acetylated analog of gramicidin A. This analog has previously been reported to increase the conductance of lipid bilayer membranes (*14*). As shown in Fig. 1, *N*-acetyl gramicidin A (*N*-acetyl-G) differs from gramicidin A (*N*-formyl-G) only by the presence of an acetyl, instead of a formyl residue at the amino terminus. This substitution of a methyl for a hydrogen has no effect on the association to form the double-stranded helical dimer in ethanol solution (*9*), yet the methyl residues are expected to interfere with the hydrogen bonding that results in head-to-head dimerization of single-stranded β helices. This should result in a decrease in the stability of the channel. In contrast, substitution of the *N*-formyl by *N*-acetyl residues is not expected to have much effect on channel properties if these consist of double helices.

Gramicidin A (N-formyl-G)

HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2CH_2OH$

N-acetyl-G

CH_3CO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2CH_2OH$

Fig. 1. Chemical structures for gramicidin A (*N*-formyl-G) and its *N*-acetyl analog.

Picomolar aqueous concentrations of *N*-acetyl gramicidin produced unitary changes in the membrane conductance. Figure 2A shows these single-channel events for 1*M* RbCl. For comparison, Fig. 2B shows single-channel events for gramicidin A observed under identical conditions. The conductance of the *N*-acetyl-G channel is about half as large as that of gramicidin A (compare channel heights for Fig. 2, A and B). However, the most striking difference is clearly in the duration of the channels which appears to be reduced by nearly two orders of magnitude for *N*-acetyl-G relative to gramicidin A (compare the time scales of 0.5 second and 20 seconds, respectively, for Fig. 2, A and B). The lifetime of *N*-acetyl-G channels was characterized by measuring the duration, ΔT, of a large number of channels. Figure 3 shows the distribution of ΔT's for a record containing 285 single-channel events measured in 1*M* RbCl. The solid line, which is an exponential drawn for a least-square fit to the data, agrees well with the experimental data, indicating that the channel lifetimes are distributed exponentially. Thus the probability of channel breakdown is independent of the time during which the channel has been open. The same type of random breakdown has been observed for gramicidin A channels (*3, 15*). The distribution of channel lifetimes in Fig. 3 has a time constant of 52 msec, which is expected and is found to be close to the calculated mean of the channel lifetimes, 65 msec.

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