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.

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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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Table 1. Comparison of mean channel lifetimes for *N*-acetyl-G and *N*-formyl-G. Lifetimes were measured in 1*M* chloride solutions, with 100 mV of applied potential.

Cation	τ_N (sec)	τ_G (sec)	τ_N/τ_G
Na	0.037	2.1	0.018
K	0.056	3.3	0.017
Rb	0.052	3.0	0.017
Cs	0.066	3.5	0.019

Mean lifetimes (τ_N's) of *N*-acetyl-G channels, estimated from exponential fits of the channel lifetime distributions, are summarized in Table 1 for 1*M* NaCl, KCl, RbCl, and CsCl solutions. Corresponding data (τ_G's) for *N*-formyl-G (that is, gramicidin A) are also shown for comparison. A small but significant increase of τ_N's is observed for ions in the Na < Rb ≃ K < Cs sequence, with a similar trend also evident for *N*-formyl-G. It is interesting, however, that the ratios on mean channel lifetimes for *N*-acetyl-G relative to *N*-formyl-G are independent of the cationic species present ($\tau_N/\tau_G \simeq 0.018$) in spite of the specific ion effects on mean channel lifetimes. Clearly the most striking difference between *N*-acetyl-G and *N*-formyl-G channels is in the channel lifetimes, the former being shorter lived by a factor of 56 relative to the latter. The mean lifetimes of *N*-acetyl-G channels are quite similar to those of neuromuscular junction channels [see, for example, Neher and Sakman (*16*)] indicating that *N*-acetyl-G channels my be useful as a realistic model for physiological channels.

The conductance of open *N*-acetyl-G channels was found to fluctuate somewhat. Examples of this phenomenon, not seen for gramicidin A, are evident in the single-channel records of Fig. 2A. Fluctuation of channel conductance appears to be a general characteristic of *N*-acetyl-G channels since it is observed in all of the alkali metal cations (data not shown). The single-channel conductance, Λ_N, of *N*-acetyl-G channels was characterized by measuring the maximal conductance at an applied potential of 100 mV for a large number of single channels in 1*M* solutions of the appropriate chloride salt. The results are summarized in Table 2. For comparison, single-channel conductances (A_G) of gramicidin A channels measured under identical conditions, are also shown. It is clear that *N*-acetylation reduces the channel conductance for all of the alkali metal cations. Furthermore, the factor A_N/A_G (Table 2, column 4) by which channel conductance is reduced, does not significantly differ from 0.5 for Li, Na, K, and Rb indicating that *N*-acetylation decreases channel permeability nonspecifically for all of these ions. For Cs,

Table 2. Comparison of single-channel conductances for *N*-acetyl-G and *N*-formyl-G. Conductance was measured in 1*M* chloride solutions, with 100 mV of applied potential.

Cation	Λ_N (pS)	Λ_G (pS)	Λ_N/Λ_G
Li	2.2	4.5	0.49
Na	12	22	0.55
K	27	45	0.60
Rb	39	79	0.49
Cs	28	82	0.34

in contrast, there is a significant specific reduction of the channel conductance: $\Lambda_N/\Lambda_G = 0.34$. This decrease in the conductance of *N*-acetyl-G channels relative to that of gramicidin A could be a result of the methyl groups increasing the relative stability of complex formation near the head-to-head junction for the larger Cs ion. In view of the complexities observed for gramicidin for the concentration dependence of Λ (*2*), however, such a suggestion can only be tentative. Further studies of the concentration dependence of Λ_N are necessary in order to establish the origin of the decreased channel conductance for Cs.

The most striking effect of the substitution of H by CH_3 at the amino terminal of gramicidin A is the reduction of the mean single-channel lifetime by a factor of 0.018 for all of the alkali metal cat-

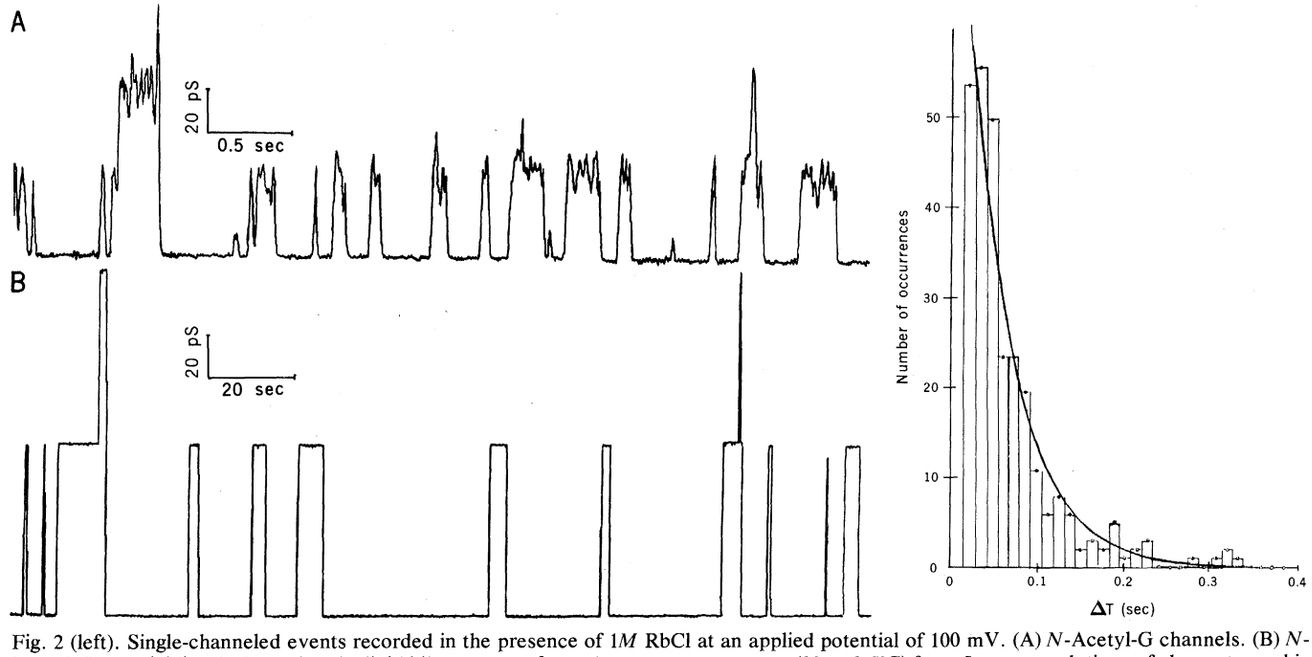

Fig. 2 (left). Single-channeled events recorded in the presence of 1*M* RbCl at an applied potential of 100 mV. (A) *N*-Acetyl-G channels. (B) *N*-formyl-G (gramicidin A) channels. The lipid bilayers were formed at room temperature (23 ± 0.5°C) from 5 percent solutions of chromatographically pure monoolein (Nu Check Prep) in hexadecane (Aldrich Gold Label, spectro grade) on a Teflon septum according to our usual techniques (*18*). Membranes of about 2.4×10^{-4} cm^2 area were voltage-clamped with silver chloride electrodes (asymmetry < 0.3 mV) and immersed in molar solutions (2 ml) of alkali metal chlorides. The electrical current through the membrane was monitored by a low-noise current amplifier and processed digitally by a PDP 11 computer. *N*-Acetyl gramicidin was added to the aqueous phases as a small portion (~ 1 μl) of a $10^{-8}M$ ethanol solution. The vertical calibration bars, which are the same for parts (A) and (B), correspond to a membrane current of 2×10^{-12} amperes as well as a conductance of 2×10^{-11} siemens. Fig. 3 (right). Distribution of channel lifetimes for 285 *N*-acetyl-G channels. 1*M* RbCl, 100 mV. The solid line was drawn for a nonlinear least-square fit of the data with a time constant of 52 msec.

ions, indicating that the rate of dissociation, $K_d = 1/\tau$ (*15*), of the conducting dimer is greatly increased by the presence of the CH_3 moiety. The magnitude of this reduction in channel stability, which corresponds to approximately 2.4 kcal/mole independently of the type of cation present in the channel, is consistent with the expected decrease of the energy of dimerization due to the presence of the bulky CH_3 moiety. When all six intermolecular hydrogen bonds are formed in the head-to-head dimerization of *N*-acetyl-G, there results a methyl-methyl steric crowding of several kilocalories per mole [see figure 14 in (*17*)]. This crowding could be relieved by shifting from six to four hydrogen bonds with water from the channel partially satisfying the two broken hydrogen bonds. The four hydrogen-bonded dimer, however, would not as adequately provide for lowering of the permeation barrier for an ion passing the junction between two molecules, and the conductance would decrease. Thus an oscillation between head-to-head dimeric structures of six and of four intermolecular hydrogen bonds would provide an explanation for the fluctuating conductance of a single *N*-acetyl-G channel. With respect to the double-stranded structures, it is particularly difficult to rationalize why they would be so stable with lifetimes measured in hours or days in polar organic solvents and yet have lifetimes of the order of tens of milliseconds within the less polar lipid membrane. However, the behavior of the *N*-acetyl-G derivative is predictable in terms of the conductive species being a head-to-head dimer.

The results obtained here for *N*-acetyl gramicidin, therefore, further support the head-to-head dimer structure for the ion-conducting channel. In general, any modification of the amino terminus—for example, *N*-pyromellitylization, *N*-succinylation, *N*-malonyl dimerization, or even this subtle substitution of methyl moiety for a hydrogen—causes dramatic and predictable changes in the channel properties, whereas similar derivatives made at the carboxy terminus causes relatively small but also predictable effects on the channel properties when the channel is considered in terms of the head-to-head dimer.

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Irradiance Modulation Used to Examine Sound-Radiating Cuticular Motion in Insects

Abstract. *A new, noncontact, optical technique for measurement of movements smaller than a few micrometers has been used to record cuticular motion of insects as they produce sound. The instrument described is highly sensitive to surface movement and offers good spatial resolution, broad dynamic range, tolerance of substantial background motion of the target surface, portability, and simplicity, and as such appears to hold promise for measuring other biologically interesting motions that have proved difficult to measure by other available techniques.*

In animals, production and reception of mechanical vibration constitutes a principal mode of communication and means of monitoring the environment. The development of hearing mechanisms throughout the animal kingdom attests to the importance of the airborne vibration we call sound. But production or reception of any type of vibration involves the motion, usually at minute amplitudes, of body surfaces. This surface motion has received more attention lately (*1*, *2*), but considerable difficulty is involved in measuring motion in many biological situations where one must avoid loading the structure being examined. Only recently have instruments capable of such measurement become available. The techniques used have included Mössbauer systems, laser interferometry, laser holography, capacitive probe, and modulation of reflected light (*3*).

As part of a study of insect sounds, I became interested in measuring the sound-radiating motion of the exoskeleton of intact and relatively unrestrained insects. Markl (*4*), through a consideration of the mechanics of the file and scraper system used by many stridulating insects, predicted the possible waveform of cuticular motion, but to my knowledge no one has observed this motion owing to inherent limitations of the available techniques. Described here is a simple method capable of making these measurements on hand-held insects. The technique involves sensing movement-induced light modulation over a small area of the insect's surface. Incorporated into the instrument is a continuous calibration of the output signal. The technique can be applied to many situations where vibration or position must be monitored, for instance in seismometry or barometry (*5*), but it is especially useful when noncontact measurement is desirable. Possible biological applications include measurement of substrate-borne sound, spider web vibration, water surface motion, and tympanic membrane motion (*2*).

Figure 1a shows a diagrammatic view of the motion-sensing elements of the measuring instrument. The target surface, normally the cuticle of an insect, is illuminated from the right of the figure by a high-intensity light beam. An opaque shield, made from a section of razor blade, partially occludes the light beam and casts a shadow on the target. When the target is properly positioned some of the light passing the shield strikes the target surface and is reflected through a narrow tube to the light detector, a miniature PIN (positive-intrinsic-negative) photodiode, whose output current is a linear function of irradiance (*6*). The narrow tube acts as a light guide serving to

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