TABLE I
CHARACTERISTICS OF VDAC AFTER MODIFICATION UNDER VARIOUS CONDITIONS

Electric field	Low	High positive		High negative	
	cis or *trans*	*cis*	*trans*	*cis*	*trans*
Succinic anhydride addition					
G^*_{max}	0.6	0.6	1.0	0.9	0.3
Opening during S.A. addition	—	NO	YES	NO	YES?
Conductance increase when field was dropped	NO	YES	NO	YES	NO
Voltage-dependent closure after S.A.	NONE	SOME SLOW‡	NONE	SOME	SOME
Symmetry§	YES	YES	YES	YES	YES

*Maximum conductance seen as a fraction of the conductance before modification.
‡The closure rate was > 10X slower than that of unmodified VDAC.
§Refers to behaviour at positive and negative fields.

of these experiments, the conductance of the open channel was virtually the same as that of the unmodified channel. In the other two treatments (*cis* addition, positive field; *trans* addition, negative field) the maximum conductance observed after modification was quite a bit lower (Table I) and some voltage-dependent closure was observed. In these two treatments, the anhydride may have modified VDAC conductance and kinetics without altering the gating mechanism.

This work was supported by grant GM28450 from the National Institutes of Health.

Received for publication 29 April 1983.

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NONLINEAR SINGLE-CHANNEL SODIUM-CONDUCTANCE IN SQUID AXON

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Spectral analyses of Na current fluctuations in squid axon (1) and frog node of Ranvier (2, 3), have required the removal of background noise before curves could be fitted. In both studies, the spectral data in tetrodotoxin (TTX) were subtracted from the data before TTX was applied (TTX difference spectrum) at the corresponding membrane potential to obtain a presumedly background-corrected spectrum. We show here that this procedure can produce significant distortion in the corrected spectrum and consequently in model fits and noise-variance estimates with which single channel conductance is calculated. The proper background current-noise spectrum, S_{IB}, can be obtained from the relation

$$S_{IB} = S_V |Y|^2, \quad (1)$$

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Volume 45 January 1984 46–49

where S_V is the power spectrum of voltage noise from that part of the voltage-clamp system that measures membrane potential (electrodes and subsequent amplifiers), and $|Y|^2$ is the squared magnitude of the membrane admittance measured at the membrane potential for which a current noise spectrum is obtained. With this method for determination of background noise, sodium current noise spectra obtained from squid axons (voltage clamped over the range -55 to -5 mV) were corrected and then curve fitted with a double Lorentzian function. The variance of the noise calculated from the spectral fits, together with measurements of mean Na conductance and reversal potential, yield a single-channel sodium conductance that is voltage-dependent and hence nonlinear.

METHODS

Measurements were made on the central 4-mm portion of a 3-cm length of squid (*Loligo pealei*) axon using an internal axial-electrode voltage clamp system. The partitioned central region of axon was isolated from the ends by 6-mm lengths of axon in air gaps on both sides of the central region. Admittance and noise measurements were made in the time interval 0.3–12 s after step voltage-clamp changes in membrane potential. Details are described in (4), where it is also shown that steady-state conditions prevail in this interval. The external solution was an artificial sea water (ASW) composed of 430 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, and 5 mM Tris·Cl. The internal perfusate consisted of 275 mM Cs glutamate, 20 mM CsF, 330 mM sucrose, and 5 mM Tris·Cl. Both solutions were adjusted to pH 7.4 at 20°C.

RESULTS AND DISCUSSION

Because background noise becomes significant with respect to ion-conductance noise at frequencies above several hundred hertz, correction for background is crucial in spectral analysis of ion (e.g., Na) conductance fluctuations producing significant energy at these frequencies. The background noise arises from the conversion of extraneous voltage noise into current noise by membrane admittance (5). Most membrane noise studies neglect the effects on membrane admittance and background noise due to changes of membrane voltage or suppression of ion conductances. However, the voltage- and time-dependent membrane ion conductances dominate membrane admittance in the 1–5000-Hz frequency range (6, 7). In addition, the Na conduction system produces both a steady-state negative conductance and a substantial frequency dependence of the membrane admittance (8). Consequently, whenever membrane ion conductances are altered or suppressed, both the membrane admittance and the background noise will change (4).

Spectral correction for background noise therefore must take into account the membrane admittance that exists under the ion conductance conditions prevalent during a noise measurement. This point is demonstrated in Fig. 1. The spectral determinations were made during a voltage clamp to -40 mV from a holding potential of -60 mV and under internal Cs perfusion to suppress K-channel conduction. A "raw" current noise spectrum was corrected for

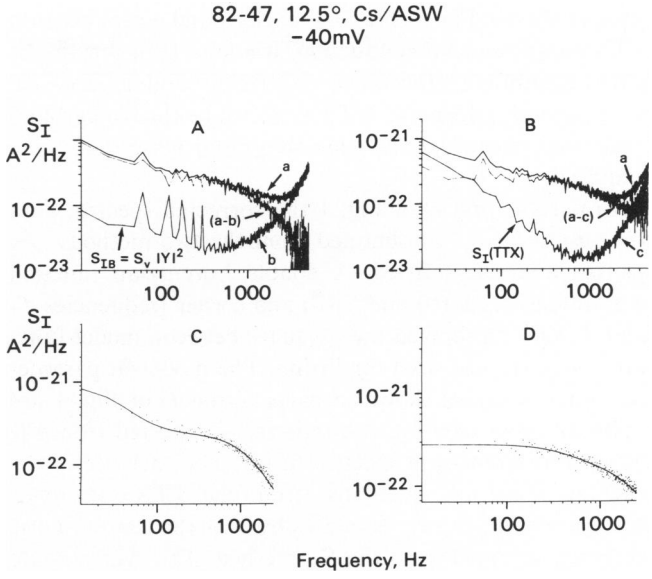

FIGURE 1 Comparison of two methods of removing background noise from a sodium current noise spectrum in squid axon. In *A*, curve *a* is a "raw" spectrum obtained by averaging 128 power spectra of current fluctuations analyzed continuously in the time interval 0.3–12 s after a step clamp to -40 mV from a holding potential of -60 mV. Curve *b* is the background current noise, S_{IB}, at -40 mV. S_{IB} is the product of electrode-plus-primary (voltage-clamp) stage voltage noise, S_V, and the magnitude square of admittance, $|Y|^2$, measured at -40 mV (Eq. 1). The "spikes" in S_{IB} are line-related artifacts which occurred in S_V. Curve $(a-b)$ is the corrected spectrum. In *B*, curve *a* is the same "raw" spectrum as in *A*. Curve *c* is the spectrum at -40 mV after addition of 1 μM TTX to the ASW, clearly showing that curve *a* reflects sodium current noise. Curve $(a-c)$ is the TTX difference spectrum. *C* is a replot of background-corrected spectrum $(a-b)$ data points in *A* with a double Lorentzian curve fit: $S_1(0) = 6.85 \times 10^{-22}\ A^2/Hz$, $f_1 = 31.9$ Hz, $S_2(0) = 2.34 \times 10^{-22}\ A^2/Hz$, $f_2 = 1294$ Hz, $\sigma_1^2 = 5.1 \times 10^{-19}\ A^2$. *D* is a replot of TTX difference spectrum $(a-c)$ data points in *B* with a double Lorentzian curve fit: $S_1(0) = 1.21 \times 10^{-22}\ A^2/Hz$, $f_1 = 449$ Hz, $S_2(0) = 1.59 \times 10^{-22}\ A^2/Hz$, $f_2 = 2422$ Hz, $\sigma_1^2 = 6.9 \times 10^{-19}\ A^2$. The TTX difference spectrum is not properly corrected for background noise. It yields different spectral parameters and overestimates $\sigma_{I_{Na}}^2$ by 35% due to change in $|Y|$ after TTX (4, 8), which makes background noise different from the background prevailing during measurement of curve *a*.

background in the two ways shown in Fig. 1 *A* and *B*. The intensity of the noise at low frequencies in TTX appeared to be dependent on the "freshness" of the axon. In the particular case of Fig. 1 *B*, the TTX spectrum (curve *c*) was obtained ~30–40 min after the data in curve *a*. This elapsed period was typical of the overall time necessary to acquire sets of control noise and admittance data at five membrane potentials, apply TTX, have the TTX take effect, and acquire data in TTX. Fresh axons in TTX showed less low-frequency noise, but the intensity steadily increased with time. Thus, even if the TTX is added immediately after the first noise measurement, it is not at all clear that the resulting low-frequency noise corresponds to the background present during the time the initial noise measurement was made. Furthermore, apart from effects due to instability of the preparation, curve *c* will differ

from curve *b* in Fig. 1 *A* (actual background noise) because TTX suppresses Na conduction, which in turn drastically alters membrane admittance and background noise (4, 8). Consequently, the noise in TTX cannot be the background noise that existed at the time the initial noise data were acquired.

In parts *C* and *D* of Fig. 1 the corrected spectral data $(a - b)$ and $(a - c)$ obtained from the two methods, are plotted and curve fitted. A double Lorentzian function with intensities $S_1(0)$ and $S_2(0)$ and corner frequencies f_1 and f_2 that minimized the χ-square between model function and data was used for fitting. The model-fit parameters for the curves shown in parts *C* and *D* of Fig. 1 are quite different (see Fig. 1 caption), as expected from the obvious differences in spectral forms. The variance of the current noise, σ_I^2, calculated from the TTX difference spectrum method, overestimates by 35% the current-noise variance obtained from the S_{IB} method. The overestimate of the variance at -40 mV was greater than at any other potential over a 50-mV range from holding; this was typical for the group of axons studied. Clearly, a correction for background noise must take into account the membrane admittance that exists during the measurement of ion-conductance noise in order to prevent distortion of the corrected spectrum.

Sodium current-noise spectral data were corrected for background noise by Eq. 1, as in Fig. 1 *A*, curve fitted as in Fig. 1 *C* and the variances computed and scaled to an area of 0.058 cm^2 for three axons in the voltage range from -55 to -5 mV (Fig. 2). The mean sodium conductance, $\langle g_{Na} \rangle$, was obtained from model fits of the complex admittance data at each corresponding voltage before and after TTX (4), and is also shown scaled to a membrane area of 0.058 cm^2. The curves of $\langle g_{Na} \rangle$ and $\sigma_{I_{Na}}{}^2$ vs. voltage are drawn through the average value of the three sets of data at the same potentials. A single-channel sodium conductance was then calculated from the relation

$$\gamma_{Na} = \sigma_{I_{Na}}{}^2 [\langle g_{Na} \rangle (V - V_r)^2]^{-1}, \quad (2)$$

where V_r, the reversal potential, was determined from step clamp data to occur in the membrane potential range between 85 and 95 mV. The plot of γ_{Na} vs. V_m data in Fig. 2 shows a strong voltage dependence, which may be expressed by the equation

$$\gamma_{Na} = 0.09 + 5.77 \times 10^{-5} (V_m + 60)^{2.5} \quad (3)$$

for the curve, where γ_{Na} is in pS and V_m in mV.

This nonlinear relation for γ_{Na} confirms our previous results obtained in squid axon (4, 9). Furthermore, this finding is consistent with the report of a calcium-dependent nonlinear single-channel sodium current-voltage, I_{Na}–V, relation in neuroblastoma cells (10) and a calcium-dependent nonlinear "instantaneous" I_{Na}–V relation in squid axon (11). Alternatively, it could be due to multiple conductance states arising from the gating process or to heterogeneity in the population of sodium channels.

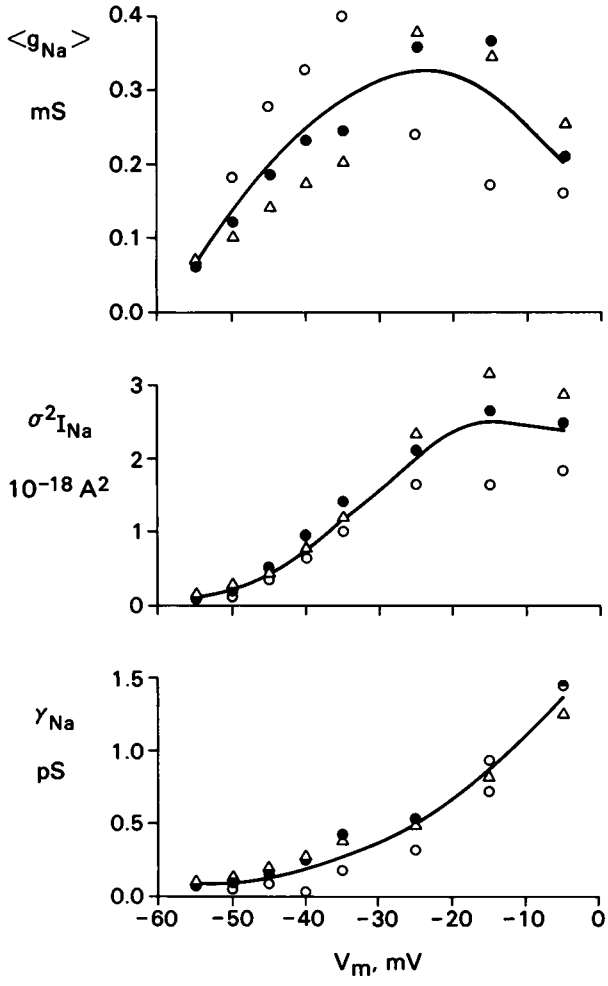

FIGURE 2 The mean sodium conductance, $\langle g_{Na} \rangle$, the variance of sodium current fluctuations, $\sigma_{I_{Na}}{}^2$, and the single-channel sodium conductance, γ_{Na}, vs. membrane potential, V_m. Data from three squid axons are indicated by different symbols. Data sets for $\langle g_{Na} \rangle$ and $\sigma_{I_{Na}}{}^2$ (82–48, •; 82–47, o) have been scaled to the area (0.058 cm^2) of axon 82–51 (Δ). The $\langle g_{Na} \rangle$ and σ_I^2 curves are drawn through average values of data points at each potential. The γ_{Na} curve is the function given by Eq. 3.

We thank the Marine Biological Laboratory, Woods Hole, Massachusetts for the use of facilities.

This work was supported by grant NS11764 from the National Institutes of Health to Harvey M. Fishman and by grant A-5274 from the Canadian Research Council to Denis Poussart.

Received for publication 4 April 1983.

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CHANNEL-MEDIATED Tl^+ FLUXES IN SARCOPLASMIC RETICULUM VESICLES

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The sarcoplasmic reticulum K^+ channel is unusual in that its properties are better known in the reconstituted than in the native membrane. The channel was identified by fusing sarcoplasmic reticulum (SR) vesicles into planar phospholipid bilayers (4). It has been thoroughly studied in this system, and today its cation selectivity, voltage-dependent gating, single-channel conductance, asymmetric orientation in the membrane, and other characteristics are well documented (1–4). The presence of the channel in SR vesicles has been suggested by radioisotope fluxes of monovalent cations (5), but so far, its characterization in the native state has not been possible. Given the high channel conductance in planar bilayers (1), the half time for K^+ flux in SR vesicles is expected to be ~1 ms, much faster than the time resolution attainable by conventional flux techniques. Moreover, the SR membrane in situ is not accessible to direct electrophysiological study. Hence, it still remains to be established whether the channel has been modified by the reconstitution process or how its behavior correlates to the known functions of the SR membrane.

In this report, we apply to SR vesicles a method introduced by Moore and Raftery (6) for monitoring monovalent cation fluxes on the millisecond time scale. A hydrophilic fluorescent probe, pyrenetetrasulfonate (PTS), is trapped inside of SR vesicles. These are then mixed with a solution containing Tl^+, a K^+ analogue that quenches the PTS fluorescence as it enters the vesicles. Given that Tl^+ permeates the SR channel as well as K^+,[1] its rate of entry will be limited by the permeability of other ions in the system, ions that must move to maintain electroneutrality. In the experiments described here, care has been taken to exclude rapidly permeant anions like Cl^-, so that the influx of Tl^+ is determined by the efflux of the cation with which the SR vesicles have been loaded.

METHODS

Isolated SR vesicles (20 mg prot/ml) from rabbit skeletal muscle were equilibrated overnight in a solution containing 100 mM glucose, 100 mM Li^+ (or other cation) glutamate, 10 mM PTS (tetracholine salt), 10 mM MOPS, pH 7.0. Prior to the experiment, the external PTS was removed by passing the SR suspension through a Dowex anion exchange column (Dow Corning Corp, Midland, MI). The vesicles were then diluted to a protein concentration of 0.1–0.2 mg/ml with the same medium, except that PTS was omitted and glucose was added to maintain osmolarity. Immediately after dilution, the vesicles were mixed in a stopped-flow apparatus with a solution of the same composition as the dilution medium, with 50 mM Li^+ replaced by Tl^+.

RESULTS AND DISCUSSION

A typical time course of fluorescence decrease is shown in Fig. 1. Clearly, when the SR vesicles have been loaded with Li^+, most of the Tl^+/Li^+ exchange is completed in the first 100 ms, while if choline is used as the compensating cation, the rate of fluorescence quenching is far slower. It is also observed that in the presence of a blocker of the K^+ channel in planar bilayers, 1,10-bis-guanidino-*n*-decane (bisG10), the initial fluorescence of Li^+ loaded vesicles is larger and decays more slowly.

Fig. 2 shows Tl^+ influx into SR vesicles loaded with different monovalent cations. It is observed that when K^+ is present inside the vesicles, at least 50% of the influx is completed within the mixing time of the stopped-flow apparatus (3 ms). With Li^+-loaded vesicles however, fast and slow relaxations are clearly observed. When the vesicles are made nonselectively permeable to cations by the ionophore gramicidin A, Tl^+ equilibrates with all the

[1]Coronado, R., and C. Miller. Unpublished results.