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Chemically Induced K^+ Conduction Noise in Squid Axon

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Internal perfusion of tetraethylammonium ions (TEA) in squid axons produces a significant high frequency noise component. Although internal TEA suppresses the potassium conductance (G_K) noise at relatively low frequencies, it induces high frequency noise which exceeds the intensity of the normal potassium and sodium noise. In addition, the induced noise is dependent on the presence of internal potassium ions (K^+) suggesting that this source of noise arises from a modulation of the K^+ conductance due to the blocking and unblocking of the K^+ channel. The simplest model describing the TEA data is a two-step sequential pseudo-unimolecular reaction where TEA binds during an open conductance state. A unit channel conductance of 2 pS is estimated from the TEA data as well as noise induced by triethyldecylammonium (TEDA) ions. Thus, these data are consistent with the hypothesis that the channel is blocked whenever the quaternary ammonium ion binding site, located near or within the K^+ channel, is occupied.

Measurements of spontaneous ion conductance fluctuations in various cell membrane preparations have been interpreted as a manifestation of the steady-state statistical variation in the number of channels that conduct ions ("number fluctuations") at any given membrane potential. Furthermore, it is possible to distinguish between fluctuations of this type that are: (i) intrinsic to conducting membrane structures in the membrane in which the fluctuations occur and (ii) chemically induced by the interaction or modification of the inherent structures by molecular substances that originate from external sources. The first and most significant example of noise in the latter category is that produced by the transmitter substance acetylcholine (ACh) at the postsynaptic membrane of the neuromuscular junction (Katz & Miledi, 1972; Anderson & Stevens, 1973). More complicated phenomena involving the modulation by local anesthetics of the ACh-induced noise show complex spectra reflecting the binding of the anesthetic agent to the membrane site (Beam,

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1976; Adams, 1977; Ruff, 1977; Neher & Steinback, 1978). Induced noise has also been observed with amiloride in frog skin (Lindemann & Driessche, 1977; Hoshiko & Moore, 1978). Intrinsic noise was also observed in these preparations.

In axon membranes, intrinsic noises (Fishman, 1973; Siebenga, Meyer & Verveen, 1973; Conti, DeFelice & Wanke, 1975; Fishman, Moore & Poussart, 1975*a*; van den Berg, de Goede & Verveen, 1975; Fishman, Moore & Poussart, 1977*a*) as well as chemically-induced noises have been described (Fishman, *et al.*, 1975*a*; 1977*a*). Although chemically-induced noises are not a part of normal function in axonal membranes, they do, nevertheless, constitute new probes into the microscopic nature of the intrinsic conduction processes.

In this paper the fluctuations induced by quaternary ammonium (QA) ions added to the internal perfusate of squid axons are analyzed. The data suggest that QA ions bind to membrane sites to produce a reversible channel block and that fluctuations in K^+ conduction reflect the binding kinetics of this process. Estimates of channel conductance in the presence of QA ions were not significantly different from those obtained from intrinsic K channel conductance fluctuations. This result is consistent with the notion that during QA ion binding to a site located near or within a K^+ channel, current flow in the affected channel is blocked.

Materials and Methods

Preparation, Measurements, and Analysis

Single giant axons from squid (*Loligo pealei*), which were received live at the Marine Biological Laboratory, Woods Hole, Mass., were used in these experiments. The procedure for obtaining an experimental preparation from animals was the same as described previously (Fishman, Poussart & Moore, 1975*b*). The chamber, temperature control, monitoring, electrostatic shielding, and vibration isolation as well as the patch voltage clamp and associated instrumentation for noise analysis were the same as described in Fishman (1975) and Fishman *et al.* (1975*b*).

Data Analysis

For preliminary review the spectra presented in this paper were analyzed by spectrum analyzer (Honeywell, model 53A) during an experiment. Further detailed processing was done after the experiments from FM tape recordings of the original data and by a PDP 11/20 computer using a fast Fourier transform subroutine supplied by the DECUS program library of Digital Equipment Corporation, Maynard, Mass. (DECUS No. 11–16). The

power spectra that were calculated by computer gave good agreement with those determined with the spectrum analyzer. All model fitting was done with the power spectra generated by the computer.

The curve-fitting routines were based on a combination of the gradient search method of least squares and Marquardt algorithm as described by Bevington (1969). The model system generally used was either a single Lorentzian function $K_1[1+(f/f_a)^2]^{-1}$, or a single Lorentzian plus $K_2 f^{-1}$. The f^{-1} component was included in this analysis because it was generally observed in the normal controls. Other models, such as two Lorentzian functions with and without f^{-1}, did not give consistent or significantly improved fits of the data. If the f^{-1} component is ignored, some of the data can be fitted with two Lorentzian functions; however, this procedure was rejected since f^{-1} noise is observed in the absence of TEA.

Solutions

The standard external solution was filtered Woods Hole seawater (SW). The standard internal perfusate was 0.5 M KF buffered to pH 7.4 at 25 °C with 5 mM Tris-HCl. Tetraethylammonium (TEA) chloride and triethyldecylammonium (TEDA) bromide were the two QA compounds used in these experiments to induce noise. They were added to the standard perfusate in the concentrations specified in the text. TEA was synthesized from triethylamine and chloroethane; TEDA was synthesized from triethylamine and 1-bromodecane in order to avoid degradation which occurs with time. In some experiments pronase (0.1 mg/ml) was added to the perfusate to facilitate axoplasm clearance for 2–3 min before switching to standard perfusate without pronase. This treatment produced no measurable effects on membrane characteristics, and the results have been duplicated in axons in which initial pronase treatment was not required. The method of internal perfusion was described previously (Fishman, 1970). Isosmotic sucrose solution (0.8 M) was used for patch isolations (Fishman, 1975). The sucrose solution was passed through an ion-exchange column, and its conductivity measured to assure good patch isolations.

Results

TEA Induces High Frequency Noise

Current noise spectra obtained from steady-state fluctuations during voltage clamps of a patch of squid axon such as those shown in Fig. 1 have pronounced corners with little indication of an additional frequency component above 500 Hz. The peaking that is obvious at depolarizations of 20 and 40 mV from rest (spectra *a* and *b* in Fig. 1) is another extreme example of the sharp corners reported previously (Fishman, *et al.*, 1975*a*; 1977*a*) and clearly suggests a non-Lorentzian spectral component. These spectra have been related to the potassium conduction system (Fishman *et al.*, 1975*a*) by a variety of procedures including the use of tetraethylammonium (TEA) ion to block the potassium conductance (G_K). The effect of adding 50 mM TEA to the internal perfusate is shown in Fig. 2, where

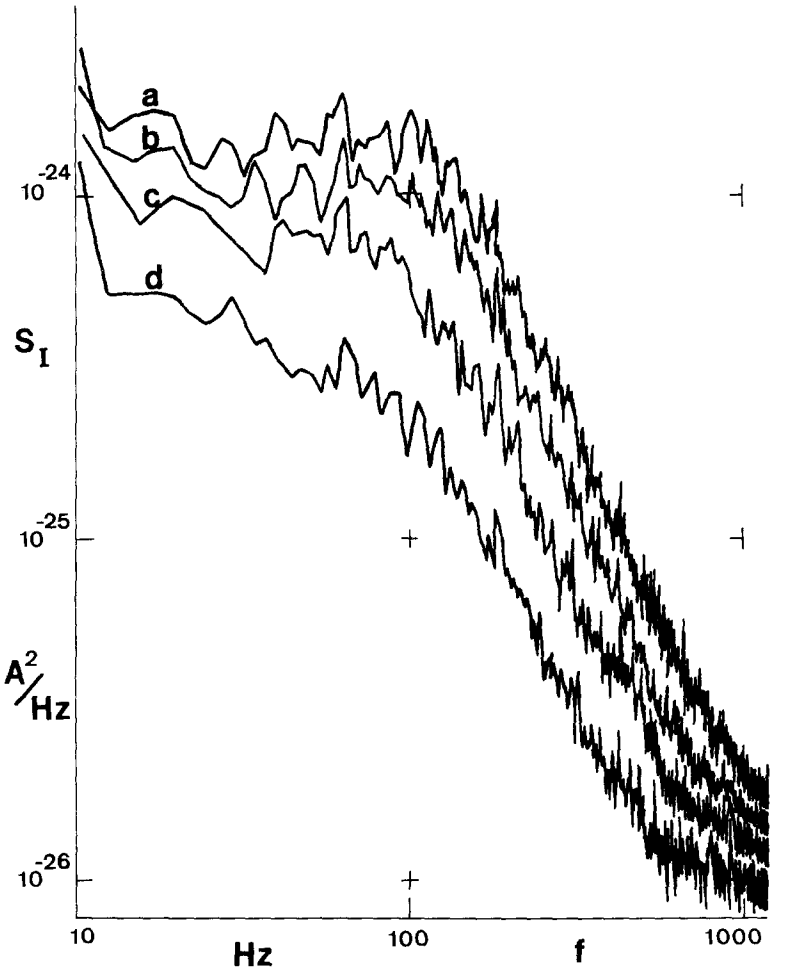

Fig. 1. Control spectra of current noise with 0.5 M $[KF]_i$. Displacements of the membrane potential from rest were as follows: $a = +40$ mV depolarization; $b = +20$ mV depolarization; c = resting fiber; $d = -20$ mV hyperpolarization

the corner region in the spectra of Fig. 1 at about 100 Hz is absent; however, a significant high frequency component becomes evident at increasing depolarizations. It has been shown previously (Fishman *et al.*, 1975*a*; 1977*a*) that, although internal TEA suppresses the G_K noise at relatively low frequencies, it induces a high frequency noise that has an intensity which is greater than the normal potassium and sodium noise. In addition, the induced high frequency noise specifically involves the interaction of TEA with conducting K channels since it does not occur when $[K^+]_i$ is reduced to 50 mM or less. The presence of a significant sodium noise in these experiments is ruled out by the spectra in Fig. 3

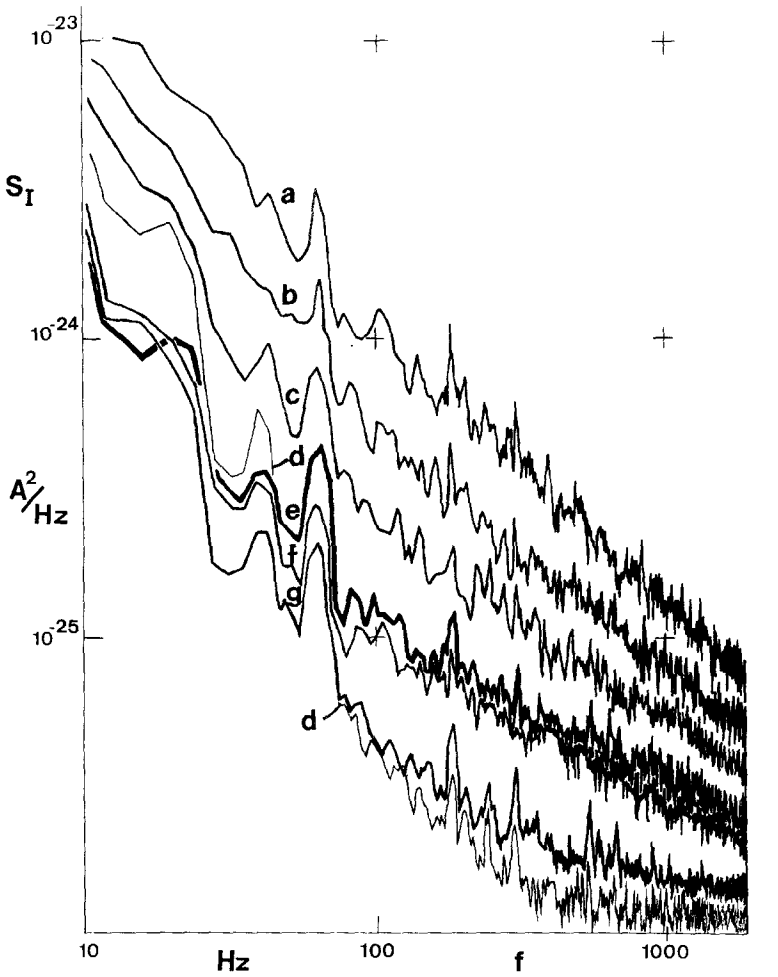

Fig. 2. Power density spectra of current noise with 50 mM $[TEA]_i$ and 0.5 M $[KF]_i$. Displacements of membrane potential from rest were as follows: $a = +100$ mV depolarization; $b = +80$ mV; $c = +50$ mV; $d = -51$ mV hyperpolarization; $e = +20$ mV depolarization; $f = 0$ mV; $g = -41$ mV hyperpolarization

which show potential insensitive spectra in an axon possessing an active sodium system but perfused with 50 mM $[K_i^+]$. Furthermore, other experiments have shown that the TEA-induced noise is unaffected by tetrodotoxin externally (Fishman *et al.*, 1977*a*).

Analysis of the TEA-Induced Noise

In general, the TEA experiments were analyzed by fitting f^{-1} and single Lorentzian functions to the difference spectra calculated by sub-

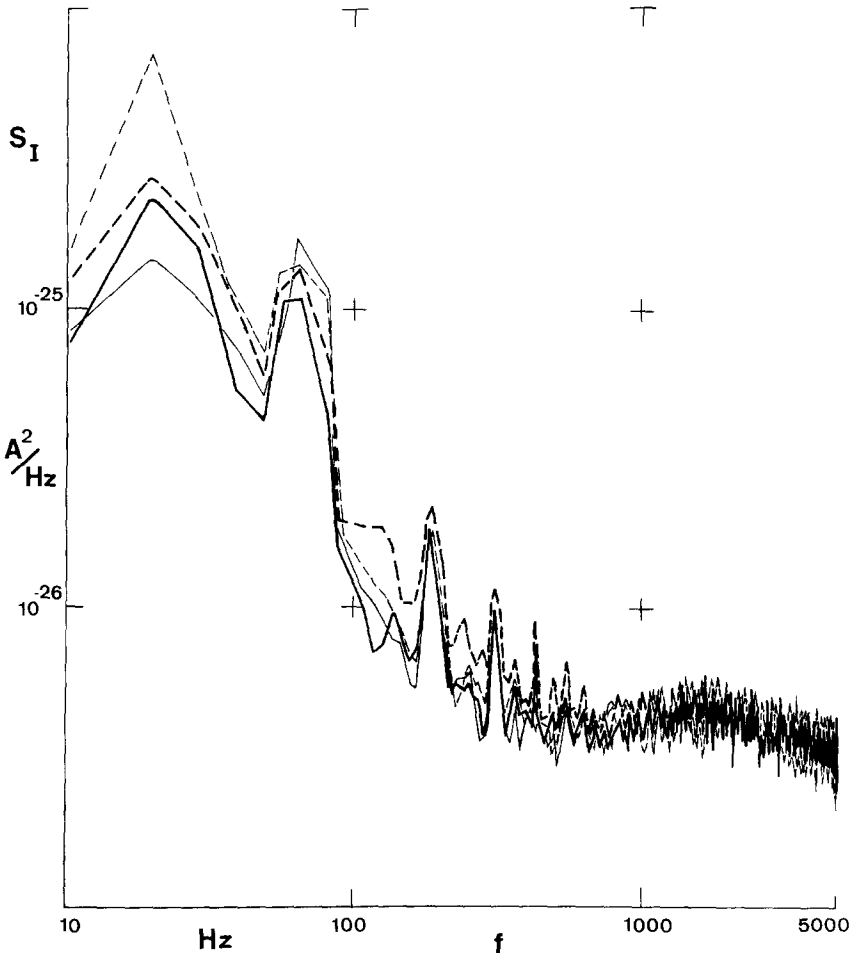

Fig. 3. Power density spectra of current noise with 50 mM $[KF]_i$. Displacements of membrane potential from rest from top to bottom were as follows: +34 mV, +20 mV depolarizations, resting membrane, and −23 mV hyperpolarization

tracting hyperpolarized from depolarized spectra. The difference spectra of Fig. 4 from an axon perfused with 10 mM TEA showed little f^{-1} behavior and were fitted with single Lorentzian curves.

Difference spectra of Fig. 5 measured with 5 mM TEA showed more f^{-1} noise and, consequently, were fitted with the function

$$A/f + B/[1 + (f/f_c)^2],$$

where A and B are constants, f_c is the corner frequency, and f is frequency. The single Lorentzian term also showed an increase in both B and f_c

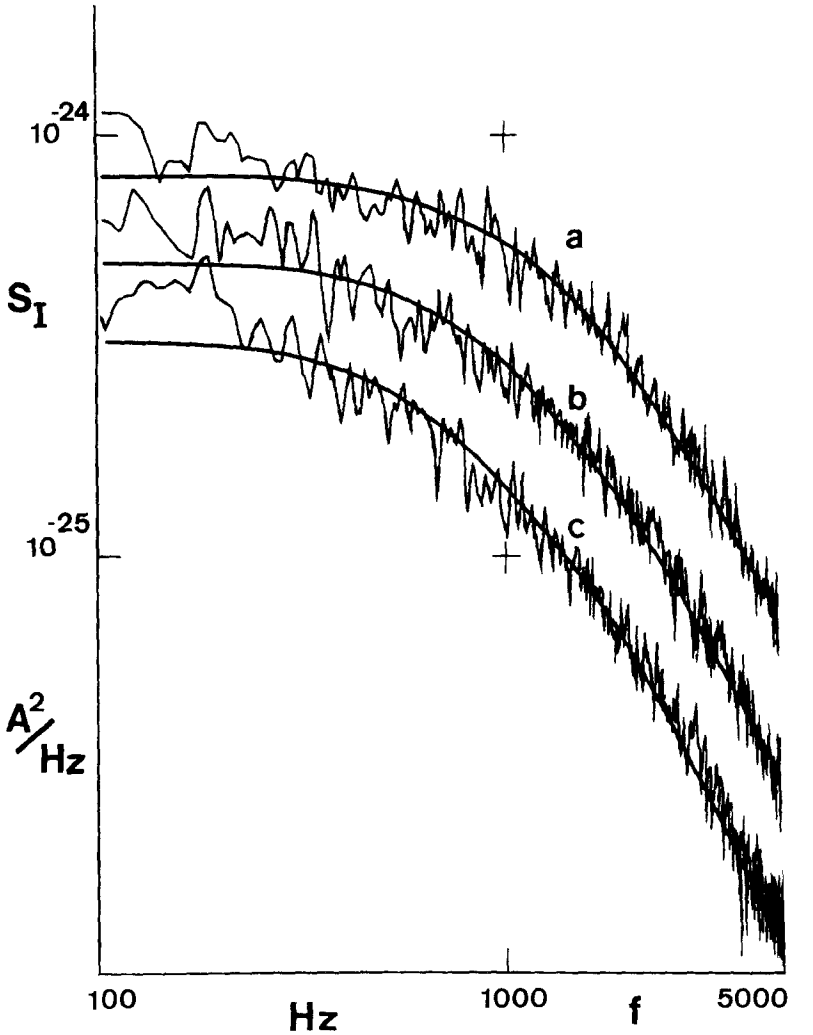

Fig. 4. Difference spectra of current noise with 10 mM $[TEA]_i$ and 0.5 M $[KF]_i$. Depolarizations of membrane potential from the holding level (−50 mV hyperpolarization) were as follows: $a=100$ mV; $b=80$ mV; and $c=60$ mV. The smooth curves are single Lorentzian functions and have corner frequencies of 1454, 1144, and 892 Hz for curves *a*, *b* and *c*, respectively

with potential. The f^{-1} portion of the difference spectra from axons perfused with less than 5 mM TEA may contain part of the normal K^+ conductance fluctuations mixed with a residual f^{-1} noise not removed when the hyperpolarized spectra were subtracted. In this respect, it is also apparent from Fig. 3 that the f^{-1} noise component evident in Figs. 1 and 2 is not present when $[K_i^+]$ is substantially reduced. Thus, a significant portion of the f^{-1} noise observed during normal ionic conditions

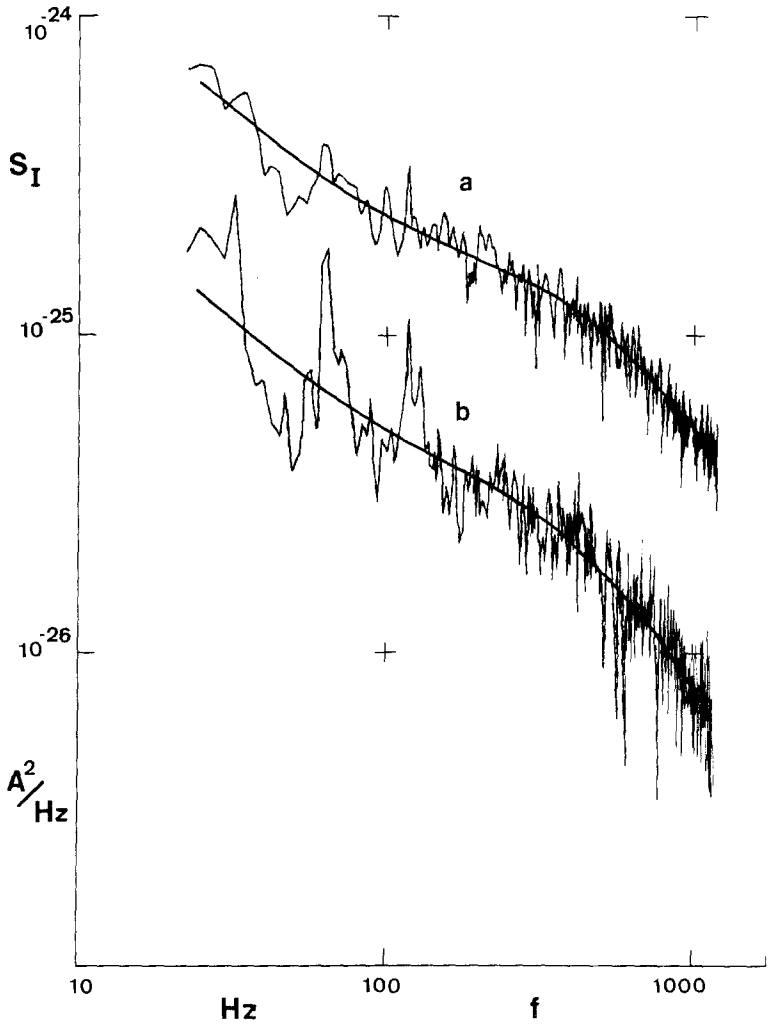

Fig. 5. Difference spectra of current noise with 5 mM $[TEA]_i$ and 0.5 M $[KF]_i$. The membrane potential depolarization was +24 mV for *a* and curve *b* was at rest. The smooth curves are combination f^{-1} and single Lorentzian functions which have corner frequencies of 732 and 511 Hz for curves *a* and *b*, respectively

is associated with K channels and cannot be attributed only to leakage current (Poussart, 1971).

The spectra observed in 0.5 mM TEA are more difficult to interpret because of a lesser effect on the normal K^+ conductance noise and the similarity of the TEA and K^+ channel relaxation times. The analysis of these data in Fig. 6 assumes A/f and a single Lorentzian function for the mixture of the unmodified K gating and the TEA induced noise. In addition, these spectra were fitted to a damped second order model

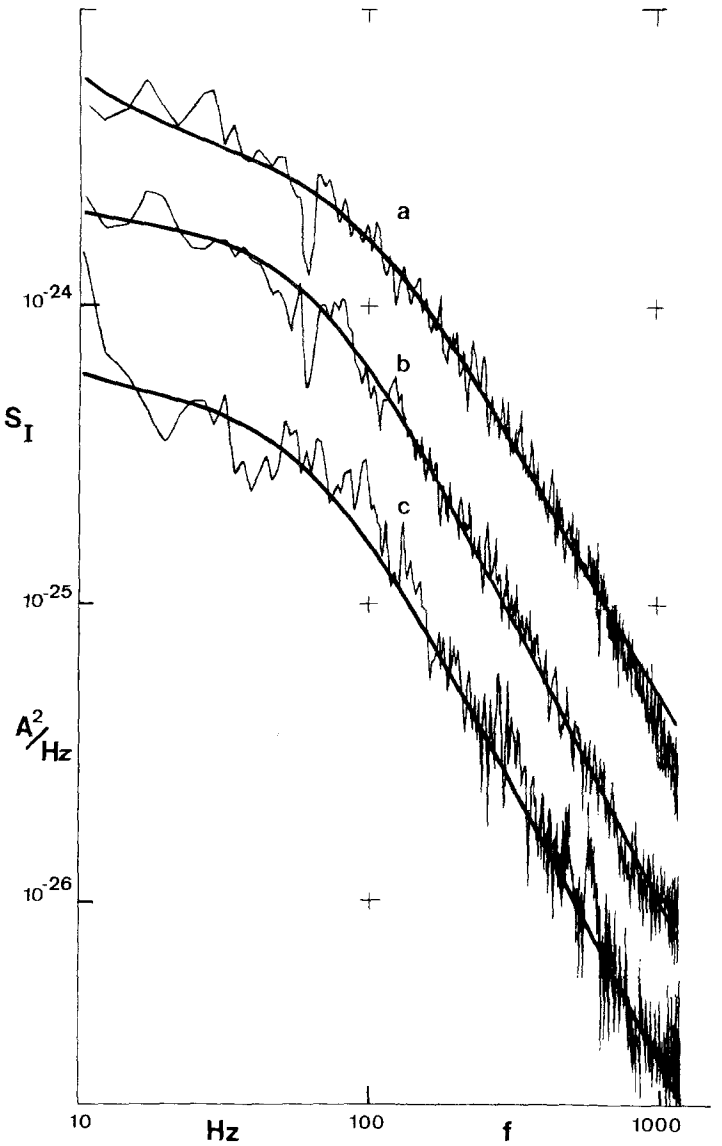

Fig. 6. Difference spectra of current noise with 0.5 mM $[TEA]_i$ and 0.5 M $[KF]_i$. Membrane potential displacements from rest were +18, −2, and −22 mV for *a*, *b*, and *c*, respectively. The smooth curves are combination f^{-1} and Lorentzian curves which have corner frequencies of 101, 68, and 65 Hz for *a*, *b*, and *c*, respectively

giving a Lorentzian component with similar corner frequencies. Either analysis leads to low corner frequencies for TEA concentrations below 1 mM. Thus for low concentrations of TEA, it does not appear possible to distinguish a unique component related to TEA-induced noise since the magnitude of the normal K conductance noise dominates the

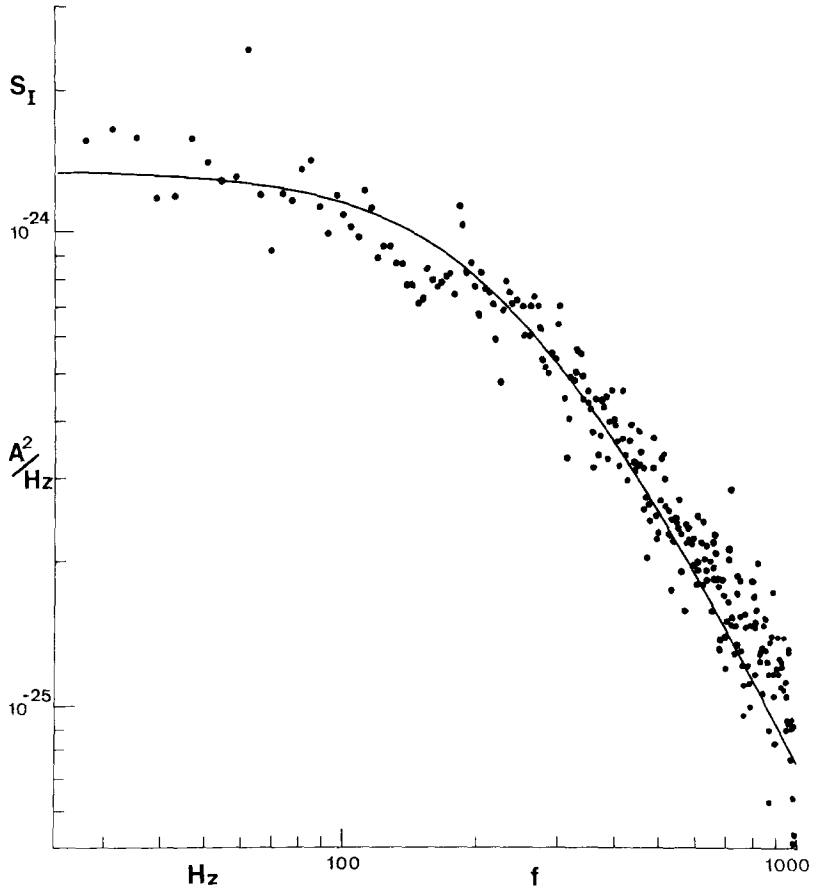

Fig. 7. Difference spectrum of current noise with 0.1 mM triethyldecylammonium $[TEDA]_i$ and 0.5 M $[KF]_i$. The membrane potential displacement from rest was 40 mV depolarized. The smooth curve is a single Lorentzian function with a corner frequency of 242 Hz. The original data are shown in Fig. 12 of Fishman *et al.*, 1975*a*

power spectra. In general, the TEA-induced fluctuations are observed to dominate the spectra at increasingly higher frequencies as the TEA concentration is raised.

The TEA-induced noise is a property of QA compounds in general as can be demonstrated by use of other TEA analogs such as that shown in Fig. 7 for 0.1 mM triethyldecylammonium (TEDA) ion in the perfusate. The observed noise is unlikely to be the normal K noise since the potassium currents are significantly reduced at this concentration of TEDA (Armstrong, 1971; *see* Fig. 12, Fishman *et al.*, 1975*a*). These data were fitted with a single Lorentzian function having a corner frequency of 242 Hz which is, as expected from voltage-clamp time constants (Arm-

strong, 1971), considerably below the [TEA] value for a comparable conductance blocking dose (Armstrong, 1966).

Discussion

These experiments show that QA ions, which suppress outward K^+ current flow in squid axons, do not abolish K^+ conductance fluctuations. Instead, the power spectra indicate that, in the presence of internal QA ions, substantial K^+ conductance noise persists, but its spectral character is different from that observed under normal ionic conditions, reflecting the interaction of QA ions with membrane sites that control K^+ outward flow.

It is interesting to note that the peaking power spectra observed at depolarized potentials in Fig. 1 and reported previously (Fishman *et al.*, 1975*a*; 1977*a*) are not observed with QA ions internally. Thus it appears that suppression of outward K^+ current flow eliminates this spectral feature as well as the usual low-frequency spectral component associated with K^+ conductance fluctuations. In this respect the observation of peaking in noise spectra is consistent with low-frequency impedance measurements (Fishman *et al.*, 1977*b*) which show a feature that is not contained in the linearized Hodgkin-Huxley equations and which may be a consequence of K^+ accumulation in the Schwann cell space during outward K^+ current flow. A recent analysis of K^+ accumulation (Grisell & Fishman, 1979) suggests that this mechanism could play a role in producing the low-frequency impedance behavior. Since K accumulation would have the effect of modifying the apparent K^+ conduction kinetics in squid axons to produce a nonequilibrium kinetic scheme, it is a plausible alternative explanation to that proposed by Chen (1975; 1977) for peaking spectra.

The simplest kinetic scheme consistent with the TEA noise data is a two-step model in which TEA binds to an open conductance state (S_2) as follows (Armstrong, 1966):

$$S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S_2 \underset{k_{-2}}{\overset{k_2[\mathrm{TEA}]_i}{\rightleftharpoons}} S_3.$$

Thus, S_1 is a closed resting conductance state, S_2 is the conducting state, S_3 is the TEA bound, nonconducting state and the k's are forward and reverse rate constants for the two steps.

This model ignores the more complex potassium kinetics usually described by a power (n^4) relationship, but it is consistent with recent small step clamp relaxation data on the K system (Moore, Poussart, Fishman, 1978) which indicate that at the amplitude level of spontaneous noise the observed kinetics are described by the linearized Hodgkin-Huxley equations (n^1) for the potassium conductance (Fishman, 1973; Fishman *et al.*, 1975a).

At internal concentrations of TEA above 5 mM, the model is simplified by the finding that the second step is much faster than the first. That is, the corner frequencies of the TEA-induced noise are much higher than the normal K kinetics. At 5 mM $[TEA]_i$ the spectra shown in Fig. 5 appear to contain a low-frequency component possibly associated with the normal K gating kinetics. However, this interpretation is not easily reconciled with the fact that the steady-state potassium current is reduced by more than 90% at comparable TEA concentrations (Armstrong, 1966). Such data would suggest that most channels are blocked at 5 mM TEA, and, consequently, the amplitude of the normal gating fluctuations should be insignificant. The most likely explanation of the low-frequency component is that it is a $1/f$ noise associated with the potassium channels which becomes less noticeable when the $[TEA]_i$ is increased to 10 mM, as illustrated in Fig. 4. Therefore, the data of Figs. 4 and 5 are consistent with the predictions of the model, namely, that the predominant Lorentzian component has a corner frequency which is proportional to the internal TEA concentration.

Estimates of unit channel conductances, γ, can be made from the induced noise. If the binding of the QA^+ produces a closed state, then the unit conductances should be unaffected by the QA species. However, if the QA-site complex partially occludes the channel such that ion transport is still possible, but restricted, then either the two-state model is not applicable or the estimated unit conductance value would be altered. Estimates of the control unit potassium conductance values from our data (Fishman *et al.*, 1975*a*) and others (Neher & Stevens, 1977) suggest a γ of 1–10 pS. This estimate assumes a two-state conductance model valid for high TEA concentrations and was calculated from the equation

$$\gamma = \frac{\sigma_I^2}{\bar{I}(\Delta V)} = \frac{S(0)\,\pi f_c}{2\bar{I}(\Delta V)}$$

where σ_I^2 is the variance of the current, I is the mean current, ΔV is the potential driving force, $S(0)$ is the low-frequency asymptote of a Lorentzian fit of the noise data and f_c is the corner frequency.

The experiment illustrated in Fig. 4 showed essentially constant γ values for five depolarizations of 20, 40, 60, 80, and 100 mV from the holding level. The mean currents ($\bar{I}$) used were assumed to be 0.1 of the measured total steady-state current based on a measured patch resistance of 1.1 MΩ (Fishman, 1975). The estimates of γ, which ranged from 1.2 to 2.1 pS, showed no trend associated with the potential step. The mean value of γ for the above five depolarizations was 1.6 pS which is within the range of γ's expected from the normal K conductance, but on the low side.

The estimate of γ using triethyldecylammonium ions rather than TEA was 1.8 pS. This was calculated using the fit of the difference spectrum of Fig. 7, where the leakage corrected mean current was 7 nA for a 40 mV depolarization.

In conclusion, these data show that the estimate of γ is the same based on either fluctuations arising from the normal open $\rightleftharpoons$ closed (rest) kinetics or those due to open $\rightleftharpoons$ blocked kinetics. Furthermore, the γ estimate appears independent of which quaternary ammonium blocking ion is used. Thus, these data are consistent with a single open $\rightleftharpoons$ blocked kinetic scheme which suggests that the blocking site is located within or near the channel.

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