BRIEF COMMUNICATION

INHIBITION OF POTASSIUM CONDUCTANCE WITH EXTERNAL TETRAETHYLAMMONIUM ION IN *MYXICOLA* GIANT AXONS

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ABSTRACT In voltage clamp experiments, externally applied tetraethylammonium ion (TEA) was found to have minimal effects on transient sodium currents and to suppress steady-state potassium currents in *Myxicola* giant axons by causing a specific decrease in the maximum potassium conductance $\bar{g}_K$. The dose-response curve suggests a one-to-one stoichiometry for TEA- receptor binding with an apparent dissociation constant of 24 mM. The suppression of I_K is essentially reversible. Experiments performed on high external potassium ion concentrations indicate that both outward and inward I_K were blocked by external TEA. The results thus suggest the presence of TEA receptors on the outer surface of *Myxicola* axonal membrane similar to those reported in the frog node.

Tetraethylammonium ion (TEA) is known to cause a fairly specific decrease in the maximum potassium conductance in many biological preparations. In myelinated frog nerve fibers (Hille, 1967), crab nerve fibers (Burke et al., 1953), molluscan neurons of *Onchidium verraculatum* (Hagiwara and Saito, 1959) and *Tritonia diomedia* (Thompson, 1977), and frog skeletal muscle fibers (Hagiwara and Watanabe, 1955), TEA is effective on external application. In the frog nodes of Ranvier, internal TEA also blocks potassium channels (Koppenhöfer and Vogel, 1969; Armstrong and Hille, 1972), but appears to act by a qualitatively different mechanism from external TEA. However, in the squid giant axon, TEA is effective only when introduced inside (Tasaki and Hagiwara, 1957) and exhibits "anomalous rectification"; it completely blocks outward potassium current (I_K) while leaving inward I_K virtually unchanged (Armstrong and Binstock, 1965). In *Myxicola*, Schauf et al. (1976) found that injected TEA blocks outward I_K in a manner similar to that observed in squid. We report here that externally applied TEA also suppresses maximum potassium conductance ($\bar{g}_K$) in *Myxicola* axons. The effects of external TEA on membrane conductances are different from TEA injected inside the axon. We postulate the presence of TEA receptors on the outer surface of the *Myxicola* giant axon, similar to that proposed for the frog node (Hille, 1967). A preliminary report of some of this work has been given (Wong and Binstock, 1980).

Myxicola infundibulum were obtained from Maritime Research Associates, Deer Island,

New Brunswick. Experiments were performed on intact axons by conventional voltage clamp techniques at 8°C. Methods for preparing and voltage clamping the axon were as described by Binstock and Goldman (1967, 1969). The composition of the artificial sea water (ASW) was: 340 mM NaCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, 105 mM tris (hydroxymethyl) aminomethane, pH 7.4. Tetraethylammonium chloride (Eastman Kodak Co., Rochester, N.Y.) was substituted for tris, leaving the total concentration of sodium ions constant. Compensated feedback was used to reduce the effects of series resistance. A 100-mV hyperpolarizing prepulse from the holding potential lasting 20 ms was used to remove resting sodium inactivation and to assure uniform initial conditions.

The resting membrane potential was depolarized 24.8 ± 3.5 mV (n = 9) in the presence of 100 mM external TEA. The depolarization is not due to an inward current of TEA, because no inward current was observed in isotonic TEA at potentials depolarized from rest. Instead, the depolarization is attributed to the blocking of resting I_K and the corresponding shifting of the resting potential to the apparent reversal potential for leakage current which is depolarized with respect to rest in ASW (Wong et al., manuscript in preparation). The smaller depolarization observed in squid with internal TEA is probably due to a different mechanism of action as well as species difference. Under current clamp, the descending phase of the action potential was only slightly prolonged, usually two to three times the normal duration, and the amplitude slightly decreased (~10%). The rising phase of the action potential was not

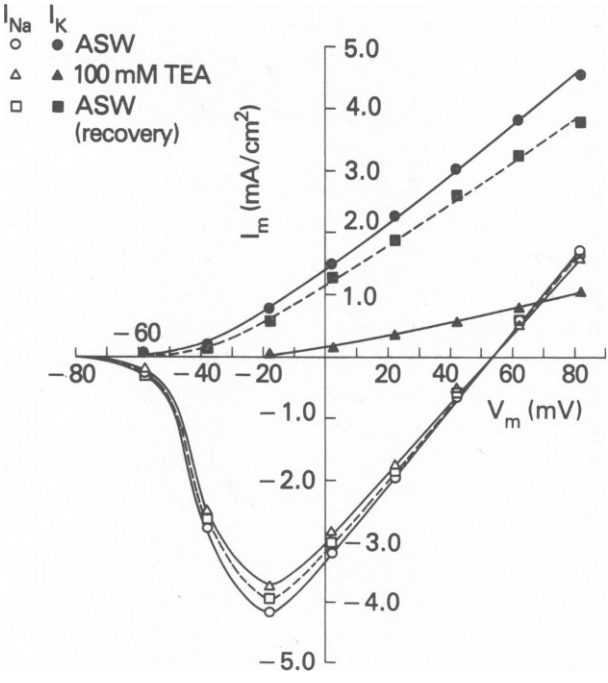

FIGURE 1 Current-voltage curves for an intact *Myxicola* axon in ASW (circles), 100 mM TEA-ASW (triangles), and recovery in ASW (squares). The holding potential was −76 mV between step depolarizations which were applied at 15-s intervals. I_{Na} and I_K represent transient sodium and steady-state potassium currents, respectively. Temperature 8°C.

affected. Under voltage clamp, 100 mM TEA was found to have a minimal effect on I_{Na}, reducing I_{Na} by ~10%. The corresponding decrease in I_K was ~80%. A typical current-voltage curve is shown in Fig. 1. Linear leakage conductance approximated by hyperpolarizing pulses has been subtracted from the total current. The leakage conductance ranged between 0.7–1.2 mmho/cm^2 and was 0.9 mmho/cm^2 for the axon shown in Fig. 1. However, rectification is known to occur in the leakage current-voltage curve in *Myxicola* (Goldman and Binstock, 1969; Wong et al., manuscript in preparation). Thus, the correction for leakage by this method, though convenient, may produce an underestimate of I_K inhibition, especially for large depolarization potentials. Although I_{Na} was decreased slightly, the kinetics for sodium activation were unaffected when the time-to-peak I_{Na} in ASW and 100 mM TEA-ASW as a function of membrane potential was compared. The effect of 100 mM TEA on steady-state sodium inactivation (h_∞) was also examined using the two pulse method. A 3–4 mV shift in the hyperpolarizing direction was observed.

To study the effect of external TEA on the rate of potassium activation, experiments were performed in the presence of 10^{-6} M tetrodotoxin (TTX) to selectively block sodium currents. The time required for I_K to reach 50% of its steady-state value was measured in ASW and 100 mM TEA-ASW and over the potential range of −35 to +85 mV. Values found under the two

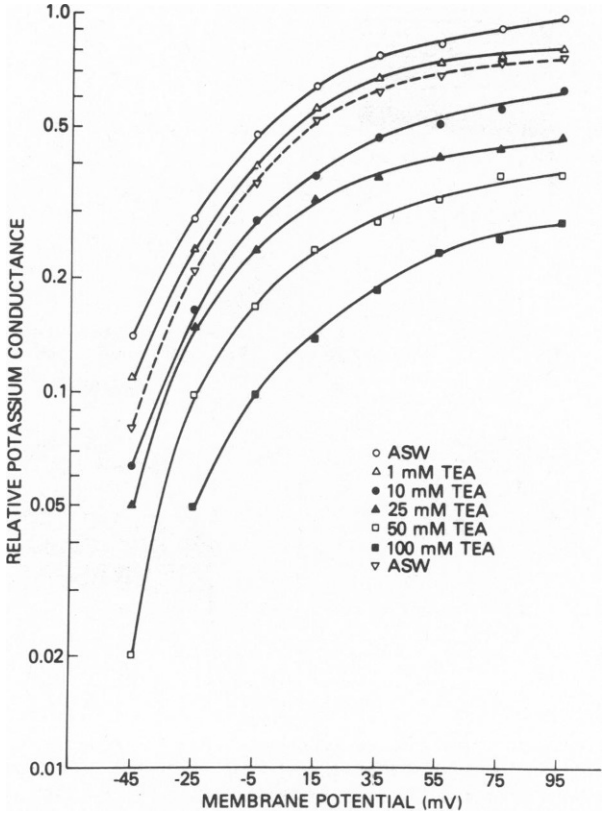

FIGURE 2 Relative potassium conductance with respect to ASW as a function of membrane potential in the presence of various amounts of external TEA. Temperature 8°C.

conditions agreed to within 6%, suggesting that the kinetics for potassium activation are not appreciably affected in the presence of TEA, and that the decrease in I_K is due to a depression of $\bar{g}_K$.

The relative potassium conductance as a function of membrane potential in the presence of various amounts of external TEA is shown in Fig. 2. Potassium conductance was determined by $g_K = I_K/(V_m - V_K)$, where V_m is the membrane potential and V_K is the equilibrium potential for I_K. V_K was assumed to be -80 mV. The decrease in I_K is due to a specific decrease in the maximum potassium conductance $\bar{g}_K$ as demonstrated by an approximately vertical shift of g_K plotted as a function of membrane potential (Fig. 2). For 100 mM TEA, a 8–10 mV shift of g_K in the depolarized direction was also observed. The suppression of I_K is essentially reversible, as demonstrated by a consistently observed 80% recovery in I_K after the external TEA has been removed by washing with normal ASW (Fig. 1). The recovery, in general, reached equilibrium within 10 min. In a few experiments, I_{Na} was observed to be slightly decreased upon washing with normal ASW. This, however, can be explained by the labile nature of the sodium channels in *Myxicola* (Goldman and Schauf, 1972). Experiments performed on an external solution with high potassium ion concentrations (340 mM K^+)

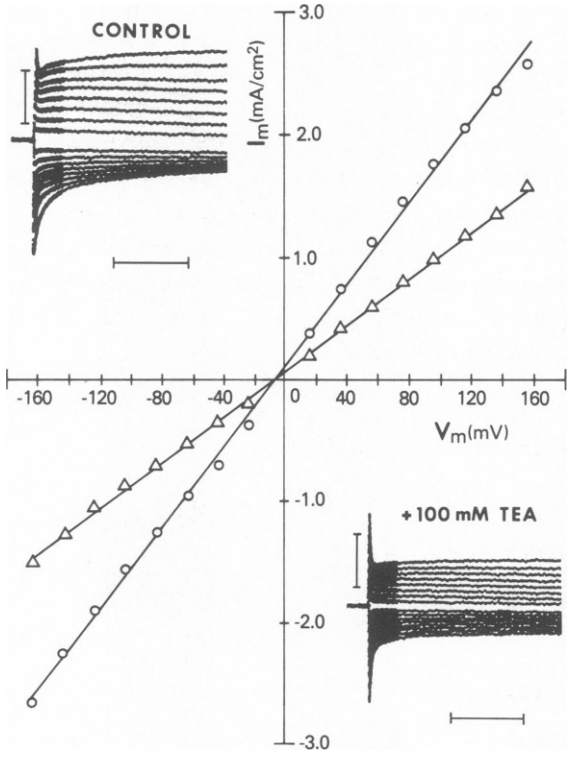

FIGURE 3 Quasi-instantaneous potassium currents measured at 0.3 ms from the beginning of each pulse of an intact *Myxicola* axon bathed in high potassium (340 mM) sea water (circles) and in 100 mM TEA high potassium sea water (triangles). Insets are membrane currents in control (340 mM K^+-ASW) and in control + 100 mM TEA in response to eight step depolarizations and eight step hyperpolarizations in increments of 20 mV from a holding potential of -5 mV. Leakage currents are included in the current traces. The vertical and horizontal bars in both insets represent 2 mA/cm² and 5 ms, respectively.

indicate that both outward and inward I_K were suppressed (Fig. 3), similar to the effect observed by Koppenhöfer (1967) on *Xenopus laevis* nodes. The potassium currents plotted in Fig. 3 are quasi-instantaneous values measured 0.3 ms from the beginning of each pulse. The amount of block in response to 100 mM TEA in 340 mM K^+-ASW is ~45%, which is considerably less than the 80% observed in normal ASW. This may be due to some interaction between potassium ions and TEA, such as extracellular potassium's ability to displace TEA from the receptor site, as well as differences in the number of open potassium channels due to the different holding potentials used.

The dose-response for suppression of I_K by external TEA in ASW at a constant membrane potential of +50 mV is shown in Fig. 4. The data are from six different axons. The apparent dissociation constant for the TEA-receptor complex was estimated to be 24 mM from the double reciprocal plot. The corresponding theoretical Langmuir adsorption isotherm dose-response curve was drawn in Fig. 4. The Hill's plot analysis, log $V/(1 - V)$ vs. log C, where V represents the percentage suppression of maximum $\bar{g}_K$ and C the TEA concentration, yielded a slope of 0.93, indicating a one-to-one stoichiometry for TEA-receptor binding.

The ease of reversibility would suggest that the receptor is on the outer surface of the axonal membrane. It is also probably distinct from the inner TEA receptor because external TEA suppressed both inward and outward I_K more or less equally. Internally, TEA has been shown, both in squid (Armstrong and Binstock, 1965) and in frog nodes (Armstrong and Hille, 1972), to suppress outward I_K only. Also, at least with squid, internal TEA is not totally specific for potassium channels. Armstrong and Binstock (1965) reported that the amplitude of I_{Na} was reduced ~50% under injected TEA, but the remaining I_{NA} had apparently normal sodium channel kinetics. The smaller effect on I_{Na} in *Myxicola* with external TEA suggests that TEA may have a higher specificity when applied externally.

In summary, as in most other preparations except squid, external TEA is effective in reducing I_K in *Myxicola* giant axons. Its effects can be well described by a specific decrease in the maximum potassium conductance $\bar{g}_K$, together with a translational shift of the conductance curve in the depolarized direction.

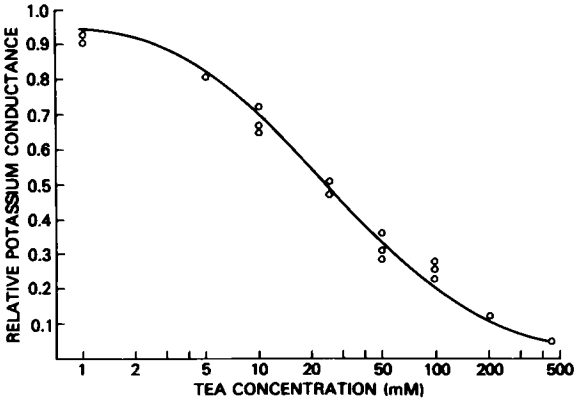

FIGURE 4 Dose response to external TEA. The relative potassium conductance with respect to ASW as a function of TEA concentration at a constant membrane potential of 50 mV. Results from six different axons were pooled. The solid line is a plot of the Langmuir adsorption isotherm with a one-to-one stoichiometry for TEA-receptor binding and an apparent dissociation constant of 24 mM.

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