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CHEMICAL MODIFICATION OF POTASSIUM CHANNELS IN MYELINATED NERVE FIBERS

Treatment with TNBS or High pH Causes Resistance to Block by 4-Aminopyridine

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Excitable cells contain a wide variety of potassium channels that can be differentiated on the basis of their kinetics, conductance characteristics, block by ions, and sensitivity to pharmacological agents. At least three populations of K channels can be distinguished in frog myelinated nerve fibers by their distinct kinetic properties (Dubois, 1981). The fast phases of K-tail current are blocked completely by 1 mM 4-aminopyridine (4AP), while the slow component of the current is resistant to block by 4AP. In this paper we describe two procedures that alter the kinetics of K channels in myelinated nerve fibers: treatment of the membrane with the amino group reagent trinitrobenzene sulfonic acid (TNBS), and exposure of the nodal membrane to high pH solution. Both of these treatments slow the K-tail current by converting normally fast-closing channels into channels that close slowly after repolarization, with kinetics resembling those of the 4AP-resistant conductance of normal fibers. We have found, in addition, that these agents decrease the sensitivity of the K channels to block by 4AP.

Experiments were performed on myelinated nerve fibers from bullfrog (*Rana catesbiana*), voltage clamped using the vaseline gap method, and reacted with TNBS as described previously (Cahalan and Pappone, 1983). Fig. 1 *A* shows the time course of 4AP block of K current in a normal, untreated nerve fiber. When repolarized following an activating depolarization, the inward K-tail current showed both fast and slow components. Fitting the sum of two exponential functions to the tail currents in this fiber showed that 89% of the current decayed rapidly, with a time constant (τ) of 1.0 ms, and 11% decayed slowly with τ = 11.9 ms. At time zero, 1 mM 4AP was added to the external solution. The rate and extent of block of the K current were assessed with short pulses at one minute intervals to minimize the voltage-dependent unblock by 4AP, which occurs in nerve fibers with depolarization (Ulbricht and Wagner, 1976). 1 mM 4AP blocked 88% of the fast compnent of the K tail current within 270 s in this untreated fiber. Only 40% of the slow component of the tail current was blocked, consistent with Dubois' (1981) finding that the slow component is more resistant to block by 4AP.

Fig. 1 *B* shows the same experiment in a fiber that had been reacted with TNBS. TNBS is a membrane-impermeant, amino-group-specific reagent that reacts to convert normally titratable primary amino groups to neutral, trinitrophenylated derivatives. External TNBS treatment causes a dramatic slowing in the K-channel closing rates (Cahalan and Pappone, 1983). Before TNBS treatment 83% of the K-tail current decayed rapidly with a fast time constant, τ = 5.3 ms, and 17% of the current decayed with a slower time constant, τ = 31.4 ms. Following exposure to TNBS only 9% of the current decayed rapidly (τ = 10.9 ms) and the remainder of the current decayed very slowly (τ = 301 ms). The total current magnitude decreased 25% following the TNBS treatment, indicating that the channels that closed rapidly before modification had been converted into very slowly closing channels. In the experiments presented here, TNBS treatment resulted in a 6–13-fold decrease in the rate of K channel closing. Potassium channels also become resistant to block by 4AP following TNBS treatment. Fig. 1 *B* shows that even after 270 s of exposure to 1 mM 4AP, 78% of the slow tail current component in the TNBS-treated fiber remains. Clearly, the channels that were converted from rapidly closing to slowly closing by the TNBS treatment also became more resistant to the blocking effects of 4AP. The small, fast component of tail current that remained after modification was blocked completely within 90 s. In four untreated fibers 1 mM 4AP blocked all but 5% of the fast component of the tail current and 48% of the slow component in 270 s. Since two-thirds of the current decayed rapidly in these fibers, these results indicate that over 80% of the total current was blocked by 1 mM 4AP. In contrast, only 45% of the total current was blocked by 1 mM 4AP in four TNBS-treated fibers. TNBS-treated fibers were also resistant to higher concentrations of 4AP. A 150-s exposure to 10 mM 4AP resulted in a block of 0–80% of the

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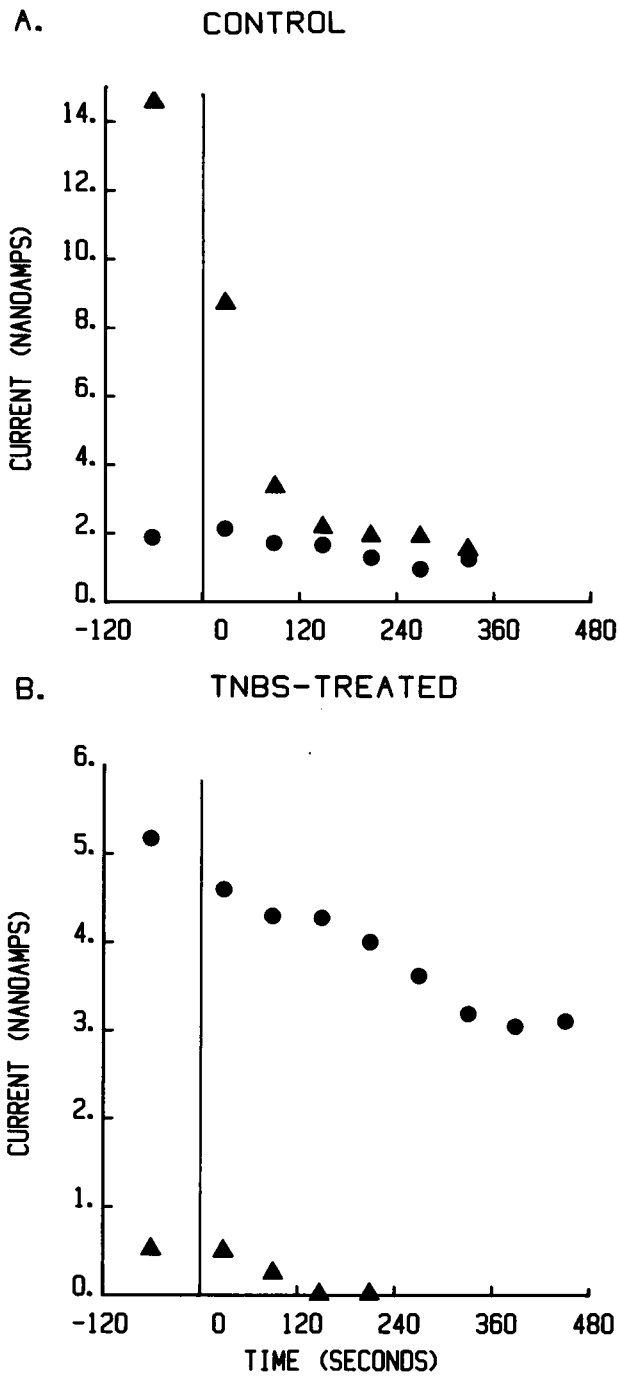

FIGURE 1 The time course of block of K-tail currents by 1 mM 4AP. K current was activated by a 40-ms depolarization to +10 mV from the holding potential of −110 mV. The time course of the decline of K-tail current upon repolarization to the holding potential was fitted by the sum of two exponential functions. ▲, amplitude of the rapidly decaying component. •, amplitude of the slowly decaying component. *A*, Normal fiber. *B*, Fiber treated with 20-mM TNBS for 2 min. The external solution was high-K Ringer's, consisting of 117.5 mM KCl, 1.8 mM $CaCl_2$, 5 mM TMA-MOPS, 200 nM tetrodotoxin (TTX), pH 7.4, with and without 1 mM 4AP. Fiber ends were cut in 120 mM KF, 2 mM TMA-HEPES, pH 7.4. 4AP solutions were made up daily from a 200-mM stock. TNBS and 4AP were purchased from the Sigma Chemical Co. (St. Louis, MO).

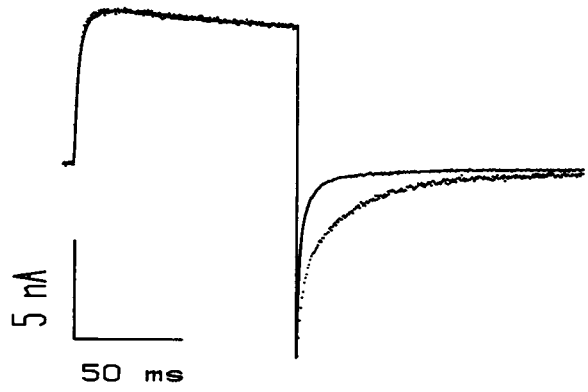

FIGURE 2 K current measured before (——) and 2 min after (···) a 7.5-min exposure to pH 11 solution. The potential was stepped from the holding potential of −110 to +30 mV. Both currents were recorded in pH 7.4, high-K Ringer's. The record after the high-pH treatment was scaled by a factor of 1.35 to match the peak amplitude in the control. The solution for the high pH treatment had the composition: 102.5 mM KCl, 1.8 mM $CaCl_2$, and 20 mM TMA-CAPS, pH 11.

slow component in modified fibers, while a 30-s exposure to 10-mM 4AP is sufficient to block all of the fast component of the K current in control fibers. Multiple reactive sites seem to be involved in the TNBS effect (Cahalan and Pappone, 1983), and the variability in the degree of block seen in modified fibers is probably due to increasing 4AP resistance with prolonged reaction with TNBS.

Treatment of nerve membranes with high-pH solutions has effects on K-channel kinetics similar to those of TNBS. The rate of K channel closing decreased within 15 s of exposure to a pH 11.5 solution. High pH treatment for 1.5–7.5 min slowed the closing rates of K channels up to

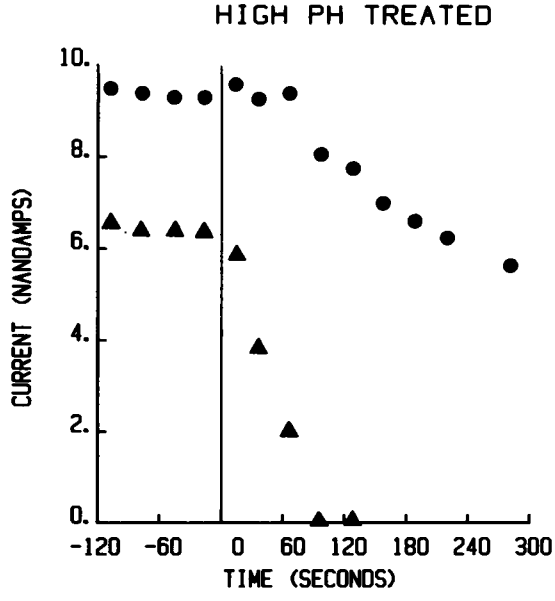

FIGURE 3 The time course of block of K-tail currents by 1 mM 4AP in a fiber pretreated with pH 11.5 solution for 2 min. Measurements were made in high-K Ringer's, pH 7.4. Procedures, solutions, symbols, and analysis as in Fig. 1.

35-fold when measured immediately following the return to normal pH solution. This effect is only partially reversible. Fig. 2 shows K currents recorded in pH 7.4 solution before and 2 min after a 7.5 min. exposure to pH 11. The closing of K channels remains slowed by the high-pH treatment. As with TNBS treatment, high pH seems to have its greatest effects on the closing rates of K channels. Activation rates are less affected by the procedure. The effects of high-pH treatment are larger and less reversible following long exposures. Following short exposure (<2 min), the K-channel closing kinetics return towards control on a time course of minutes. Like TNBS, high-pH treatment increases the resistance of K channels to block by 4AP. Fig. 3 shows the time course of block by 4AP in a fiber pretreated with pH-11.5 solution. Only 40% of the slow component of the tail current was blocked after 280 s. Since this fiber showed no slow component in the tail current before treatment with high pH, this result indicates that high-pH treatment induced 4AP resistance along with the kinetic changes.

These data suggest that 4AP normally blocks K channels by binding to a site accessible to modification by external TNBS and high pH. Since both of these agents have dramatic effects on the kinetics of K channel closing, and since the K channels that close slowly in untreated fibers are also resistant to 4AP, it seems possible that 4AP binds to a site involved in K-channel gating. Our results also raise the possibility that the various subpopulations of K channels in myelinated nerve fibers may be interconvertable. Fink and Wettwer (1978) have shown that exhaustion in skeletal muscle fibers changes the properties of the K conductance, converting the channels to a permanently open state that is more resistant to block by 4AP. The characteristics of K channels in myelinated nerve fibers may be regulated by similar mechanisms.

Received for publication 4 May 1983.

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CELL-TO-CELL CHANNELS WITH TWO INDEPENDENT GATES IN SERIES, REGULATED BY MEMBRANE POTENTIALS, BY pCa_i AND BY pH_i

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Junctional conductance (g_j) between cells in *Chironomus* salivary glands is modulated by membrane potentials, $[Ca^{2+}]_i$, and pH_i. In the studies we describe here, the conductance variation appears to arise from two gates in series in each cell-to-cell channel; we show that each such gate responds to all three modulators (1).

We studied g_j in cell pairs, using two independent voltage clamps to set the potentials of the cells and to measure g_j. We found (Fig. 1 *A Inset*) that when $E_1 = E_2 = E$, steady state g_j varies sigmoidally between an upper asymptote ($g_j$max) and zero.

When g_j is examined as a function of E_j ($E_j = E_1 - E_2$, in Fig. 2), it becomes evident that g_j varies widely at any given E_j, is not symmetric about $E_j = 0$ (this does not imply a rectifier), but depends on E_1 and E_2. Hence E_j plays little or no role in determining g_j. The curves suggest that the fixed potential imposes an upper limit on g_j however negative the other potential. This is expected if g_j is the product of an E_1-dominated function and an E_2-dominated function, both sigmoid.

This pattern of g_j dependence on membrane potentials suggests that each cell-to-cell channel has two voltage-sensitive gates in series, pertaining to the two cell membranes of the junction. We postulate that the steady state g_j is the resultant of two simultaneous but independent voltage sensitive open/closed equilibria, one within each population of gates (i.e., one on each side of the junction), with open gates occurring on each side with a probability $\{1 + \exp[A(E_k - E_0)]\}^{-1}$, where A and E_0 are constants and E_k is the potential of the respective side. This model fits well the data of experiments such as that of Fig. 1, where $E_1 = E_2$. Although the model takes no account of E_j, it fits the g_j data for $E = -E_2$ despite presence of substantial E_j values (Table I). This is expected if the potential determining the state of a given gate is the corresponding perichannel membrane potential (E_p)—the potential

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Volume 45 January 1984 64–66