SINGLE ION-CHANNEL CURRENT MEASUREMENTS FROM RAT BRAIN SYNAPTOSOMES IN PLANAR LIPID BILAYERS

MARK T. NELSON
Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

ROLAND REINHARDT
Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, Federal Republic of Germany

Because of their small size and inaccessibility, direct electrical measurements of ionic conductances from mammalian brain presynaptic nerve terminals have not been possible. Measurements using voltage-sensitive fluorescent dyes, however, indicate that the membrane potential of a preparation enriched in pinched-off and resealed presynaptic nerve terminals (synaptosomes) depends principally on K^+ conductances (1). To measure ionic conductances from the brain directly, synaptosomal ion channels were incorporated into lipid bilayers. We examined single K^+-channel currents and found four K^+-channels that can be distinguished by their single-channel conductances.

RESULTS AND DISCUSSION

After the addition of synaptosomes to the *cis* side of a lipid bilayer, the membrane conductance increased in discrete steps (Fig. 1). The single channel conductances in symmetric 100 mM KCl ranged from 2 to 14 pS. The lifetimes of these channels were not strongly voltage-dependent. In asymmetric KCl solutions (*cis*, 300 mM: *trans*, 100 mM), the current-voltage relationship of the macroscopic (channel-ensemble) currents exhibited rectification that could be described by the Goldman-Hodgkin-Katz equation (2, 6). Results from single-channel and macroscopic current measurements suggest that the nonlinearity of the *I-V* curve is conferred by the asymmetric K^+ concentrations across the bilayer and not by any intrinsic voltage-dependence of the conductance pathways.

Fig. 2 shows the voltage-dependence of the four K^+-dependent unit conductances that were usually observed. The four unit conductances were ~8 pS, 16 pS, 24 pS, and 32 pS (labeled 1, 2, 3, 4, respectively) and were independent of voltage (−100 to +100 mV). There was no measureable difference in the single channel conductances between 500 and 300 mM KCl. However, the single channel conductances were reduced by ~50% in 100 mM KCl (see Fig. 1), suggesting that the channels saturate with respect to K^+ between 100 and 300 mM. The differences in conductances between these channels were about equal (~8 pS) at every voltage tested. Furthermore, K^+ concentrations between 100–500 mM did not appear to affect this relationship between the unit conductances (i.e., the differences between single channel conductance levels were similar, regardless of the K^+ concentration). These observations suggest that these channels are related. However, there was no obvious simple relationship between the four unit conductances because one channel was able to open and close independent of the activity of other channels (e.g., Fig. 2, 3).

In asymmetric KCl solutions, the reversal potential for all four single channel currents follows the potassium equilibrium potential (E_K) (6), indicating that all the channels select for K^+ over Cl^-. In the experiment illustrated in Fig. 3, the selectivity of these channels for K^+ over Na^+ was tested. The *cis* side of the bilayer contained 500 mM KCl and the *trans* side contained 500 mM NaCl. If the channels select for K^+ over Na^+, then the reversal potential for the single channel currents would be negative to 0 mV; conversely, if the channels select for Na^+ over K^+, then the reversal potential would be positive to 0 mV. As shown in Fig. 3, at 0 mV, single channel current fluctua-

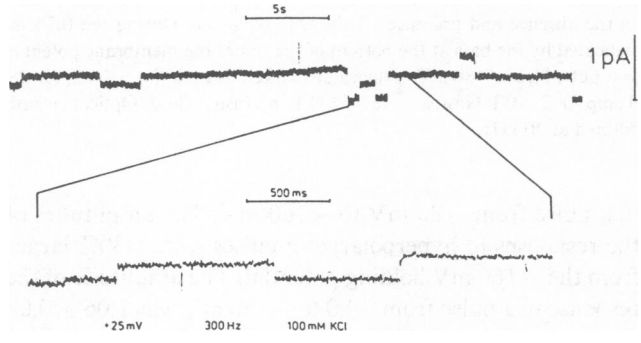

FIGURE 1 Ion channels from rat whole brain synaptosomes incorporated into a "folded" bilayer. Bilayer diameter, 40 μm. Bandwidth, 300 Hz. Symmetric 100 mM KCl, 0.1 mM $CaCl_2$, pH 7.0. Synaptosomes were prepared from whole rat brain and from rat median eminence homogenates by a differential and discontinuous sucrose density procedure (3, 6). The experimental procedure was to add synaptosomes (final concentration ~1 μg protein/ml solution) to one side (*cis*) that contained 100–500 mM KCl, 0.1 mM $CaCl_2$, pH 7.0 (cf. reference 5). The opposite side (*trans*) of the bilayer to the membrane vesicle addition was virtual ground. The bilayers ("painted" and "folded") were made from phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) or PE and phosphatidylserine (PS).

Dr. Reinhardt's present address is II. Physiologisches Institut, Universität des Saarlandes, 665 Homburg/Saar, Federal Republic of Germany.

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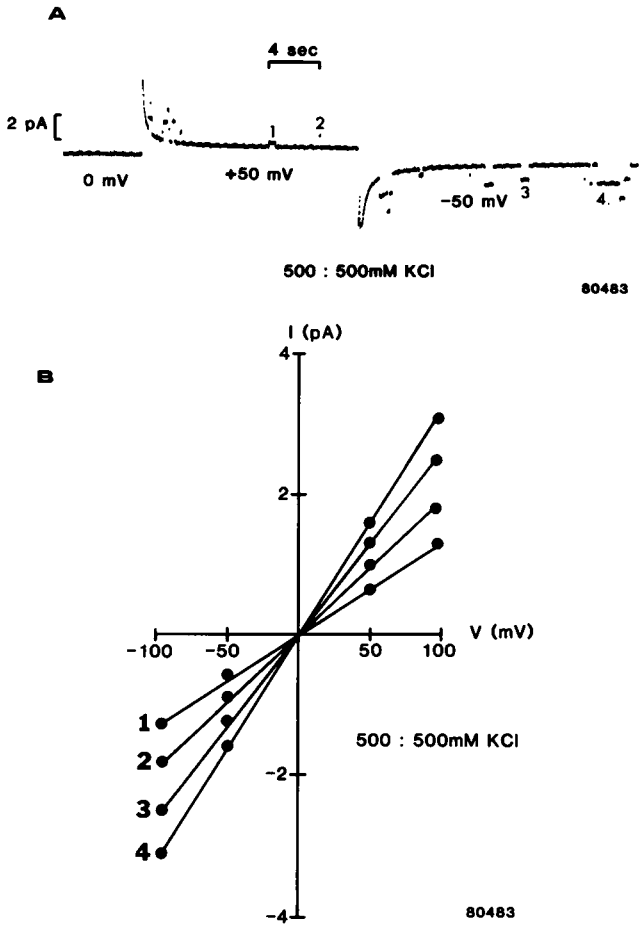

FIGURE 2 Identification and voltage-dependence of the single K^+-channel currents from rat median eminence synaptosomes. Bilayer ("painted") diameter, 250 μm. Playback, 60 Hz. Solutions: symmetric 500 mM KCl, 0.1 mM $CaCl_2$, pH 7.0. The four unit conductances are labeled 1, 2, 3, 4 (in ascending order of conductance size). *A*, original record of single-channel current fluctuations (0 mV, +50 mV, −50 mV). *B*, *I-V* relationships of the four unit conductances.

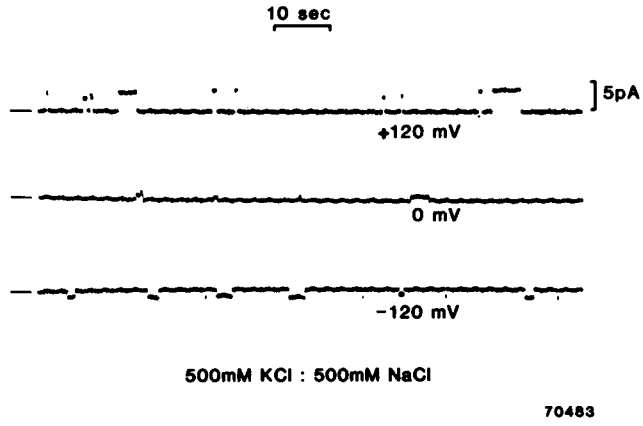

FIGURE 3 Ion selectivity of the single-channel currents from rat median eminence synaptosomes. Bilayer ("painted") diameter, 250 μm. Playback 60 Hz. Solutions: *cis*, 500 mM KCl; *trans*, 500 mM NaCl. The marks on the left-hand side of the record indicate the zero current level (i.e., all channels closed). The upward current deflections at 0 mV are in the direction of the K^+-gradient, indicating that K^+ ions are more permeable than Na^+ ions through these channels. Single-channel currents reversed between −30 and −40 mV. At −120 mV (+120 mV), Na^+ (K^+) should be carrying the current through these channels.

tions were in the direction of the K^+ gradient, indicating that K^+ passes through these channels more readily than Na^+ does. The reversal potential (−30 to −40 mV) for the single-channel currents suggests that the K^+/Na^+ permeability ratio is ~3:1–5:1. Furthermore, at very positive potentials the current through the single channels should be carried primarily by K^+. At very negative potentials, the single-channel currents should be carried by Na^+. A comparison of the single channel currents (or conductances) at +120 mV and −120 mV indicates that the single channels are about three times more conductive to K^+ than to Na^+.

These channels are not blocked by TEA (1 mM). In preliminary experiments, Barium (20 mM) from the *cis* side cause a voltage-dependent block of these channels.

Based on single-channel current measurements and macroscopic (multichannel) current measurements, we have observed, to date, the incorporation of two to several hundred channels. In addition to the channels described in this report we have been able to incorporate a number of other channels from brain into lipid bilayers, including the Ca^{2+}-activated K^+ channel that has been seen in other preparations (4, 7).

Because the channels described in this report select for K^+ over Na^+ and Cl^-, these channels may contribute the resting K^+ conductance of mammalian brain synaptosomes. Furthermore, these results suggest that this system can be readily extended to measurements of other ionic conductances in brain synaptosomes.

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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

PAMELA A. PAPPONE AND MICHAEL D. CAHALAN
Department of Physiology and Biophysics, University of California, Irvine, California 92717

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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