SURVIVAL OF THE K^+ CHANNEL IN AXONS EXTERNALLY AND INTERNALLY PERFUSED WITH K^+-FREE MEDIA

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ABSTRACT In perfused squid giant axons, potassium channels irreversibly deteriorate when the internal K^+ is removed and replaced by impermeant ions. Under the same conditions in perfused *Myxicola* giant axons, the K^+ conductance is also irreversibly lost with a time constant of 10–15 min. In contrast, the K^+ conductance in *Myxicola* giant axons dialyzed with impermeant ions and bathed in monovalent cation free solutions does not deteriorate, even over 5–6 h. Thus we suggest that washout of some internal component may be an important additional factor in the deterioration of K^+ channels in perfused giant axons.

INTRODUCTION

In perfused squid giant axons potassium channels are rapidly and irreversibly lost when the internal K^+ is removed and replaced by impermeant cations such as Na^+ and tetramethylammonium (TMA^+) (Chandler and Meves, 1970; Almers and Armstrong, 1980). Such an irreversible deterioration was shown to be prevented either by high external concentrations of K^+, Cs^+, NH_4^+, and Rb^+ (Almers and Armstrong, 1980) or by internal Cs^+ (Chandler and Meves, 1970), and it has thus been suggested that the presence of a monovalent cation within the K^+ channel may be necessary to stabilize its structure (Almers and Armstrong, 1980). In the course of performing other experiments we discovered that the K^+ conductance in *Myxicola* giant axons dialyzed with Na^+ or TMA^+ and bathed in monovalent cation free solutions did not deteriorate, even over 5–6 h, whereas in axons perfused with the identical solutions the K^+ conductance was irreversibly lost with a time constant of 10–15 min. We suggest that removal of an internal cellular component with a molecular weight in excess of 5,000–6,000 may be an important additional factor in the disappearance of K^+ channels in perfused axons.

METHODS

Myxicola giant axons were dialyzed using methods previously described (Bullock and Schauf, 1978). Briefly, a length of cellulose acetate dialysis tubing (Bio Fiber 50, Bio-Rad Corporation, Richmond, CA) with an OD of 180 μm and a molecular mass cutoff of 6,000 daltons was inserted longitudinally into the axon. The internal electrodes were placed inside this tubing through which the internal perfusion solution flowed at rates of 50–100 μliter/min.

To perfuse the axon with minimal changes in technique, a modification of this procedure was developed in which not one, but two short segments of the cellulose acetate tubing were inserted into the axon. Initially these segments were left adjacent so that the majority of the internal perfusate flowed through the tubing, as in the conventional dialysis procedure. The internal electrodes were inserted as usual. After 15–20 min of dialysis with a K^+ glutamate solution, the two segments of the tubing were gradually withdrawn to opposite ends of the axon, resulting in a true perfusion of the 1.5 cm central region (the electrodes remain in place during this procedure). This took another 15–20 min, and the two segments of dialysis tubing served as inflow and outflow cannulae, respectively. To prevent leakage, a thread was tied around the tubing at each end. If this procedure was performed carefully no change in the maximum Na^+ and K^+ conductances ($\bar{g}_{Na}$ and $\bar{g}_K$) occurred, and there was no increase in leakage current over that measured initially before the tubing was separated. The flow of perfusion fluid remained stable with no tendency for the outflow tubing to clog with axoplasm.

Several external and internal solutions were used. The standard artificial sea water (ASW) contained 430 mM NaCl, 50 mM $MgCl_2$, 10 mM $CaCl_2$, and 20 mM Tris (hydroxymethyl) aminomethane. To record K^+ currents the external Na^+ was replaced by Tris (or in part by K^+, see below) with 10^{-6} M tetrodotoxin present in addition. The standard internal solution (K^+-SIS) contained 500 mM K^+ glutamate, 50 mM KF, and 5 mM HEPES. Other internal solutions (Cs^+-SIS, Na^+-SIS, TMA^+-SIS) were prepared by replacing all the K^+ with the indicated cation, the anion composition remaining constant. In a few cases the internal glutamate was replaced by fluoride. All solutions were buffered to pH 7.3 ± 0.05 and the axon was maintained at 5.0 ± 0.5°C.

The general procedure was as follows. Axons were initially perfused or dialyzed with K^+-SIS, and the K^+ were currents recorded. The internal solution was then changed to Cs^+-SIS, Na^+-SIS, or TMA^+-SIS for a variable period of time ranging from a few minutes to 5 h (various other experimental protocols, including, for example, gating current measurements, were carried out during this time). The internal solution was then changed back to K^+-SIS and the K^+ currents remeasured. Thus, except for a few instances in which the K^+ currents in K^+-SIS perfused or dialyzed axons were continuously monitored, a given axon yielded a single data point in Fig. 1. Data were included from all axons used in 1981 (~60) in which an appropriate protocol had been followed.

BIOPHYS. J. © Biophysical Society · 0006-3495/82/11/171/03 $1.00
Volume 40 November 1982 171–173

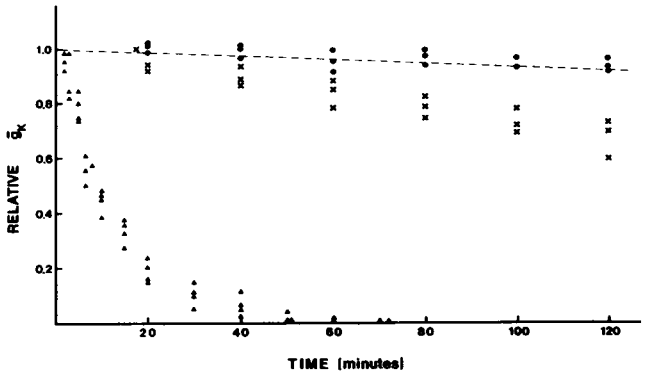

FIGURE 1 Effects of internal dialysis and perfusion with impermeant cations on the potassium conductance in *Myxicola* axons. Axons were either perfused or dialyzed with K^+-SIS, Na^+-SIS, or Cs^+-SIS. In the case of K^+-SIS perfusion and dialysis the K^+ currents were simply monitored as a function of time, converted to conductances, and the maximum conductance expressed as a fraction of the initial value. For the Na^+-SIS and Cs^+-SIS solutions the internal solution was returned to K^+-SIS after the exposure times indicated on the abscissa and the remaining I_K was measured after allowing 5 minutes for equilibration. Data for only the first two hours are shown, although exposure times as long as 5 h were used. The K^+ conductance in dialyzed axons slowly declines with time, but this rundown was the same for K^+, Cs^+, and Na^+ dialysis. The average rundown calculated from experiments on 15 dialyzed axons is illustrated by the dashed line. The data obtained from perfused axons are shown for K^+ (●), Cs^+ (×'s), and Na^+ (▲).

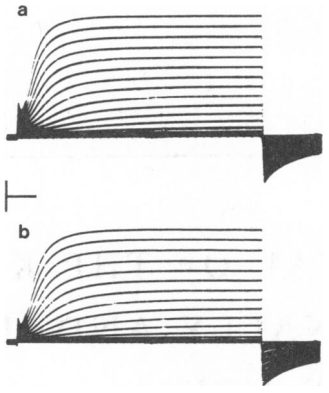

FIGURE 2 Potassium currents before (*A*) and following (*B*) 4 h of dialysis with 550 mM Na glutamate. Currents were recorded with K^+-SIS internally for depolarizations from −30 mv to +100 mv (10 mv increments). Calibration bars represent 0.75 ma/cm² and 2 ms, respectively.

RESULTS

The principal results of this study are illustrated in Fig. 1. Axons in Tris SW were initially perfused or dialyzed with K^+-SIS and the K^+ currents recorded and converted to conductances. Then, either g_K was simply monitored as a function of time, or the internal solution was changed to Na^+-SIS or Cs^+-SIS, and after exposure times ranging from a few minutes to 5 h K^+-SIS was readmitted and the remaining K^+ conductance determined. Axons dialyzed or perfused with K^+-SIS showed only a very slow rundown of g_K (4–6%/h over a period of 5 h). Internal dialysis with Cs^+-SIS or Na^+-SIS did not produce any additional deterioration, even with exposure times of 4–5 h. The average time course of the rundown in K^+-, Na^+-, or Cs^+-SIS dialyzed axons is illustrated by the dashed line in Fig. 1. As an example of the stability of dialyzed axons under all conditions, Fig. 2 shows the K^+ currents from a dialyzed axon recorded initially in K^+-SIS, and then following a 4 h exposure to Na^+-SIS. Recovery of the K^+ conductance is nearly complete and there is no change in its time course.

In contrast with dialyzed axons, axons perfused with Na^+-SIS irreversibly lost their K^+ currents with a time constant of 10–15 min (Fig., 1▲). For short exposure times, the voltage dependence and time course of the residual K^+ currents in Na^+-SIS perfused axons was the same as that seen initially with K^+-SIS, suggesting that K^+ channels simply become nonfunctional rather than modified. A very similar result was obtained with TMA^+-SIS. Axons perfused with TMA^+ irreversibly lose their K^+ currents with a time course not significantly different from that seen with Na^+-SIS perfusion, while the $\bar{g}_K$ in TMA^+-dialyzed axons was completely stable (not shown). Changes in internal anion composition had no effect on the overall results, except that rundown in K^+-SIS and Cs^+-SIS was more rapid in both perfused and dialyzed axons with high $[F^-]$.

This relatively rapid, irreversible loss of K^+ conductance did not occur in axons continuously perfused with K^+-SIS (Fig. 1, ●) or Cs^+-SIS (Fig. 1, ×), although in Cs^+-perfused axons the decline in $\bar{g}_K$ was somewhat greater than that seen either in Cs^+-dialyzed axons (---) or in axons perfused with K^+-SIS. Deterioration of the K^+ conductance could also be prevented by adding a small amount of K^+ to the Na^+-SIS in perfused axons. The concentration dependence was not measured in detail, but at least 20 mM internal K^+ was needed to provide any significant protection against irreversible inactivation during Na^+ perfusion, while axons perfused with a solution containing 100 mM K^+ were as stable as dialyzed axons. As previously noted in squid axons (Almers and Armstrong, 1980), $\bar{g}_K$ deterioration could also be prevented by a high external concentration of K^+ and Cs^+ (100–200 mM) in perfused *Myxicola* axons.

DISCUSSION

The behavior of perfused *Myxicola* axons is thus identical to that previously reported for squid axons (Almers and Armstrong, 1980). Exposure to K^+-free internal Na^+ or TMA^+ solutions, in the absence of external permeant cations, caused an irreversible loss of the K^+ conductance with a time constant of 10–15 min. This effect could be prevented if Cs^+ was the internal impermeant cation, if internal $[K^+]$ was >20 mM, or if external $[K^+]$ was 100 mM or more. When the loss of $\bar{g}_K$ was only partial, the remaining K^+ currents behaved normally, suggesting that K^+ channels are lost rather than modified.

In contrast to this behavior, the K^+ conductance in *Myxicola* axons dialyzed with Na^+ or TMA^+ is completely stable, even for a total exposure lasting many hours. This could be explained if the combined use of K^+ channel impermeant ions and internal perfusion allows the dissociation of a component whose presence is essential for the normal activation of the K^+ channel, and that cannot readily pass through a dialysis tubing. Because the dialysis tubing has a nominal cutoff at 5,000 daltons, this component may be relatively large. The fact that low concentrations of K^+ present internally can protect the K^+ channel from deterioration in Na^+- or TMA^+-perfused axons suggests that the presence of K^+ at some binding site is the other critical factor in maintaining K^+ channel stability.

To reach such a conclusion it is obviously necessary to rule out the possibility that dialysis does not adequately control internal ion composition so that, for example, significant residual levels of internal K^+ do not remain. The effectiveness of internal dialysis was tested by initially dialyzing an axon with 430 mM Na^+ glutamate and then after 30 min changing to a solution containing 10 mM Na^+ (Cs^+ was used as the other internal cation to control osmolarity). The reversal potential for the Na^+ channel was measured as a function of time following the onset of dialysis, and used to calculate internal [Na^+] after allowing for the finite permeability of Cs^+ ($P_{Cs}/P_{Na} = 0.037$, see Schauf and Bullock, 1980). Although the change in effective internal [Na^+] is not a single exponential, it is clear that the Na^+ concentration reaches 1–2 mM of that in the dialyzate within 20 min, and thus that the residual [K^+] in axons dialyzed with K^+-free solutions should be at least an order of magnitude smaller than those levels (20 mM) necessary to provide protection against irreversible K^+ channel deterioration.

This work was supported by USPHS Research Grant NS15741 and by the Morris Multiple Sclerosis Research Fund.

Received for publication 12 January 1981 and in revised form 10 May 1982.

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