J. Physiol. (1985), **361**, *pp.* 441–457
With 11 *text-figures*
Printed in Great Britain

CALCIUM-ACTIVATED POTASSIUM CHANNELS IN ISOLATED PRESYNAPTIC NERVE TERMINALS FROM RAT BRAIN

BY D. K. BARTSCHAT AND M. P. BLAUSTEIN

From the Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201, U.S.A.

(*Received 18 June 1984*)

SUMMARY

1. ^{86}Rb efflux was examined in isolated presynaptic nerve terminals (synaptosomes) from rat brain in a study designed to assess K permeability (P_K) changes sensitive to alterations in internal Ca activity.

2. Rb efflux from ^{86}Rb-loaded synaptosomes into nominally Ca-free physiological saline (PSS) containing 5 mM-K was about 0·3–0·4 %/s. Raising extracellular K concentration ($[K]_o$), to depolarize the synaptosomes, stimulated the ^{86}Rb efflux. Addition of Ca to the 5 mM-K PSS had no effect, but Ca did further stimulate ^{86}Rb efflux into K-rich solutions. The effect of Ca was graded, with apparent half-maximal activation, $K_A \simeq 0{\cdot}5$ mM-Ca. These data fit the view that, during depolarization, Ca enters the terminals through voltage-regulated Ca channels, and that the rise in intracellular Ca concentration opens certain (Ca-activated) K channels.

3. The Ca-dependent stimulation of ^{86}Rb efflux was greatest during the initial seconds of incubation (component C_T), and then declined to a much lower rate (component C_S). Much of this change in rate could be attributed to inactivation of voltage-regulated Ca channels and reduced entry of Ca. The Ca-dependent increase in ^{86}Rb efflux was completely inhibited by 100 μM-La.

4. In the presence of Ca, but not in its absence, the Ca ionophore A23187 stimulated ^{86}Rb efflux both in 5 and 100 mM-K PSS. The effect in 100 mM-K was quantitatively greater, perhaps because of the increased outward driving force on Rb in depolarized synaptosomes.

5. When synaptosomes were suspended in media containing the voltage-sensitive fluorescent dye, DiS-C3-(5) (1,1′-dipentyl-2,2′-thiocarbocyanine), the addition of Ca + A23187 decreased the fluorescence intensity (= synaptosome hyperpolarization) when the media contained 5 mM-K but not 100 mM-K. This implies that in the presence of Ca + A23187, P_K was increased, and the membrane potential moved closer to the K equilibrium potential, E_K.

6. Quinine sulphate, a blocker of Ca-activated K channels, reduced the Ca-stimulated ^{86}Rb efflux with high affinity (apparent half-maximal inhibition, $K_I \simeq 1$ μM). Tetraethylammonium chloride, another agent known to block Ca-activated K channels, was also a relatively potent inhibitor of Ca-stimulated ^{86}Rb efflux ($K_I \simeq 0{\cdot}2$ mM).

7. The K-channel blocker, 4-aminopyridine, partially inhibited Ca-stimulated

^{86}Rb efflux at concentrations below 0·5 mM, but stimulated this efflux at concentrations $\geqslant$ 1 mM.

8. The results indicate that Ca-activated K channels are present in mammalian presynaptic nerve terminals, and that the physiological and pharmacological properties of these channels can be investigated in this preparation.

INTRODUCTION

Synaptic transmission plays a critical role in the integration and transfer of information in the central nervous system (C.N.S.). The importance of voltage-regulated Ca channels in this process is now widely accepted (Katz & Miledi, 1969; and see Llinas, Steinberg & Walton, 1980): a rise in the intraterminal free Ca concentration ($[Ca]_i$), as a result of Ca entry through these channels during depolarization, normally triggers neurotransmitter release. Immediately after neuronal activity, the Ca is rapidly buffered, sequestered and extruded (Blaustein, McGraw, Somlyo & Schweitzer, 1980; Blaustein & Rasgado-Flores, 1981; McGraw, Nachshen & Blaustein, 1982). 'Residual Ca' may remain in the cytoplasm following a period of activity, and may add to the Ca that enters during subsequent neuronal activity, to enhance transmitter release directly (facilitation and post-tetanic potentiation; see Katz & Miledi, 1968; Weinreich, 1971; Younkin, 1974; Kretz, Shapiro & Kandel, 1982).

A rise in $[Ca]_i$ in neurones may also influence a number of other physiological processes, including membrane excitability (Meech, 1978). Indeed (internal) Ca-activated K-selective channels have been observed in a wide variety of cell membranes (Meech, 1978; Schwartz & Passow, 1983; Petersen & Maruyama, 1984) and have been implicated as a modulator of membrane excitability in many excitable cell types (Meech, 1978) including mammalian central neurones (e.g. Krnjevic & Lisiewicz, 1972; Alger & Nicoll, 1980). However, until recently (Bartschat & Blaustein, 1983) evidence that these channels exist in the presynaptic nerve terminals from mammalian C.N.S. has been lacking, primarily because of the inaccessibility of these terminals to direct electrophysiological analysis.

Our data (Bartschat & Blaustein, 1983, 1985) suggest that at least some terminals have Ca-activated K channels and it is therefore conceivable that these channels play an important role in synaptic transmission. For example, it seems possible that the 'residual Ca' following nerve activity could, under some circumstances, contribute to *reduced* transmitter release by shortening the duration of depolarization. In fact, Kretz *et al.* (1982) have obtained evidence that the transient depression of transmitter release that follows the initial phase of post-tetanic potentiation may be due to delayed activation of Ca-dependent K conductance in *Aplysia* neurones.

The present report describes some of the physiological and pharmacological properties of Ca-activated ^{86}Rb efflux in isolated mammalian presynaptic nerve terminals (synaptosomes). The rationale for the use of ^{86}Rb as a tracer for K has been described (Bartschat & Blaustein, 1985). Our data suggest that ^{86}Rb efflux can be stimulated by Ca entry into the synaptosome, either through voltage-regulated Ca channels, or through the use of a Ca ionophore (A23187). This activation of ^{86}Rb efflux appears to be associated with an increase in K permeability, P_K, and with a hyperpolarization of the membrane potential when the terminals are incubated in

physiological saline containing 5 mM-K. Furthermore, the increase in ^{86}Rb efflux can be blocked by the Ca-activated K channel blockers quinine sulphate and tetraethylammonium (TEA). These properties are to be expected if Ca-stimulated ^{86}Rb efflux reflects Ca-activated K channel activity. A preliminary report of some of our findings has appeared in abstract form (Bartschat & Blaustein, 1983).

METHODS

Preparations of synaptosomes

Isolated presynaptic nerve terminals ('synaptosomes') were prepared and equilibrated with Ca-poor physiological saline (PSS) as described in the preceding article (Bartschat & Blaustein, 1985). Contaminating Ca in these solutions was estimated by Ca-selective electrodes to be 4–10 μM-free Ca (H. Rasgado-Flores & K. Gregerson, personal communications).

Measurement of ^{86}Rb efflux

^{86}Rb efflux from pre-loaded rat brain synaptosomes was measured with the rapid-quench technique described in the accompanying paper (Bartschat & Blaustein, 1985). Details of incubation solution composition are given in the Figure legends. In the present report, we define the Ca-dependent ^{86}Rb efflux as the increment in ^{86}Rb efflux observed when Ca was added to media of otherwise similar composition. Ca-dependent ^{86}Rb efflux can be separated into two components, C_T and C_S: component C_T is defined as the increase in the ordinate intercept of ^{86}Rb efflux when Ca is added to PSS containing elevated K concentrations; component C_S is defined as the increase in the rate of ^{86}Rb efflux (measured between 1 and 5 s) following the same manoeuvre. In those solutions containing no added Ca, Mg replaced Ca mole-for-mole (extracellular Ca concentration ($[Ca]_o$) + extracellular Mg concentration ($[Mg]_o$) = 2·0 mM).

Synaptosome membrane potentials

A voltage-sensitive fluorescent dye, DiS-C3-(5) (1,1′-dipentyl-2,2′-thiocarbocyanine) was used as a probe for changes of synaptosome membrane potentials as described by Blaustein & Goldring (1975), except that 0·25 μM-dye was used. The excitation wave-length was 622 nm, and emission was recorded at 670 nm. The DiS-C3-(5) was a generous gift of Dr Alan Waggoner.

Materials

Standard chemicals were all reagent grade. Sources of special drugs and reagents were: HEPES, TEA, TBA (tetrabutylammonium) and quinine sulphate, Sigma (St. Louis, MO); 4-aminopyridine, Aldrich (Milwaukee, WI); and A23187, Calbiochem (La Jolla, CA).

RESULTS

Effect of $[Ca]_o$ on ^{86}Rb efflux

The data presented in the preceding article (Bartschat & Blaustein, 1985; see Fig. 2) show that measurement of Rb efflux from ^{86}Rb-loaded terminals with a manual-quench technique may provide information about voltage-regulated K channels in synaptosomes. When the synaptosomes were incubated in PSS containing 5 mM-K (non-depolarizing solution) without Ca, there was only a slow efflux of ^{86}Rb (about 0·3–0·4 %/s); addition of 1 mM-Ca to this 5 mM-K solution did not alter the ^{86}Rb efflux. When the synaptosomes were exposed to a depolarizing solution of (nominally) Ca-free, K-rich media, ^{86}Rb efflux was stimulated, and at least two components could be identified that we have attributed to two (different) voltage-regulated K channels. When 1 mM-Ca was added to the K-rich solutions, there was a further increment in ^{86}Rb efflux (Fig. 2 in Bartschat & Blaustein, 1985). This Ca-dependent component of the ^{86}Rb efflux may represent efflux through Ca-activated K channels that are

present in the nerve terminal plasma membrane, and we have examined some of its properties.

The data in Fig. 1 illustrate the effect of varying the $[Ca]_o$ on the time course of ^{86}Rb efflux into PSS containing 100 mM-K. Two components of Ca-dependent ^{86}Rb efflux from the synaptosomes are observed. In the first place, there is an increase in

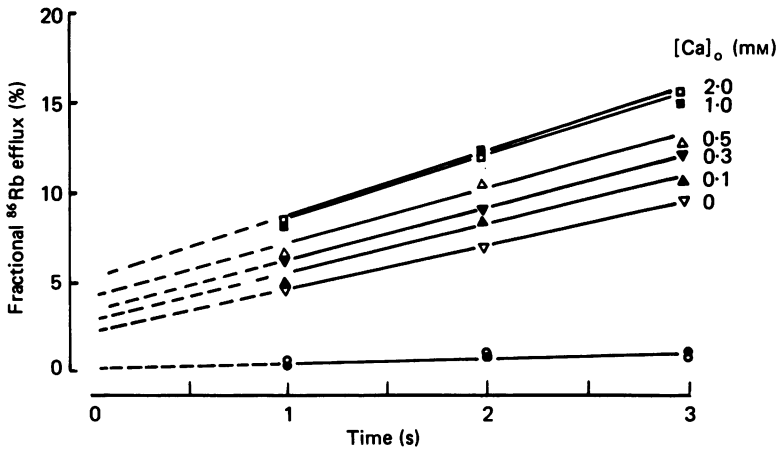

Fig. 1. Effect of Ca on K-stimulated ^{86}Rb efflux. The measurement of Rb efflux from ^{86}Rb-loaded synaptosomes placed on glass fibre filters was initiated by the addition of 5 mM-K PSS plus 2 mM-Mg (○) or 2 mM-Ca (●), or 100 mM-K with 2 mM-Mg, 0 Ca (▽) or progressively increasing concentrations of Ca (see Figure for concentrations). Where Ca was employed, Ca replaced Mg mole-for-mole. Data points indicate the means of four determinations. The lines were fitted by linear regression analysis. ^{86}Rb efflux component *B* (Bartschat & Blaustein, 1985) has been subtracted in this and all subsequent Figures.

the slope of the line (i.e. the rate of ^{86}Rb loss), between 1 and 3–5 s of incubation, which we define as the slow Ca-dependent component of ^{86}Rb efflux (C_S). Secondly, when these lines are extrapolated to zero time, the lines do not converge; i.e. there is another (transient) Ca-dependent ^{86}Rb efflux (C_T), that appears to inactivate within 1 s. As indicated in Fig. 1, component C_T is defined as the Ca-dependent increment in the ^{86}Rb efflux at the ordinate extrapolation.

The magnitudes of both Ca-dependent ^{86}Rb efflux components are graded with $[Ca]_o$ although, at saturating $[Ca]_o$, C_T is much larger than C_S (Fig. 2). The latter may have a somewhat greater sensitivity for Ca than C_T, but its small magnitude and its variation (in magnitude) from preparation to preparation has made this difficult to evaluate.

Synaptosomes have voltage-regulated Ca channels, and Ca enters the terminals rapidly when they are depolarized (Nachshen & Blaustein, 1980, 1982). This raises the possibility that the Ca-dependent ^{86}Rb efflux may be a manifestation of the activation of K-selective channels that are regulated by the rise in $[Ca]_i$. About 80% of the depolarization-activated Ca entry in the synaptosomes is inactivated within 1 s; thus, it is possible that the apparent inactivation of a large fraction of the Ca-dependent ^{86}Rb efflux may reflect the decline in Ca entry during a prolonged

incubation in K-rich solutions (cf. Nachshen & Blaustein, 1980; Nachshen, 1982). Because of this dilemma, as well as the fact that $[Ca]_i$ may change with time during prolonged depolarizations as a result of buffering and extrusion (McGraw *et al.* 1982), we are unable to resolve the kinetics of the Ca-dependent ^{86}Rb efflux with the present techniques. Nevertheless, the rapid-quench methods do enable us to study many other properties of this efflux.

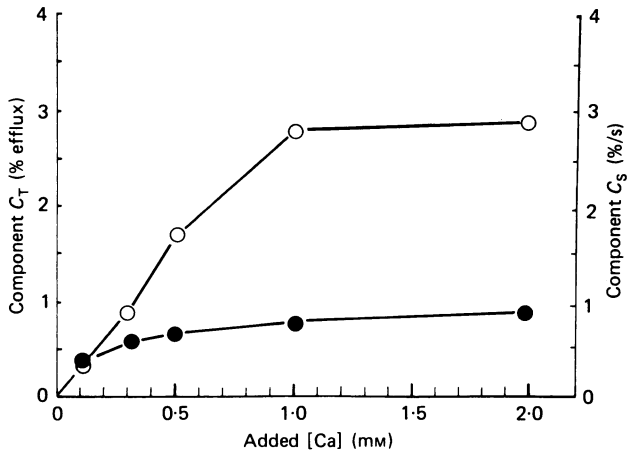

Fig. 2. Effect of $[Ca]_o$ on components C_T and C_S of the ^{86}Rb efflux. The initial phase (C_T) and the slow phase (C_S) of the Ca-dependent ^{86}Rb efflux data from the experiment of Fig. 1 are plotted as a function of $[Ca]_o$. The left-hand ordinate, corresponding to C_T (○), is expressed in the same units as the ordinate of Fig. 1; the right-hand ordinate (C_S, ●) is expressed as the percentage ^{86}Rb released/s. See Methods for a description of how C_T and C_S were quantitated.

Effect of external K and La on Ca-activated ^{86}Rb efflux

Synaptosomes appear to have membrane potentials that behave like K diffusion potentials (Blaustein & Goldring, 1975). The terminals should therefore be depolarized to various levels by altering the extracellular K concentration, $[K]_o$. In order to determine the role of the membrane potential (V_m) in the activation of Ca-dependent ^{86}Rb efflux, ^{86}Rb efflux was examined as a function of $[K]_o$ in the absence and presence of 1 mM-Ca. The 'zero-time' extrapolated values of the K-stimulated ^{86}Rb efflux, without and with Ca (i.e. component T (○), and $T+C_T$ (□), respectively) are plotted as a function of $[K]_o$ in Fig. 3*A*. Component T is observed at $[K]_o \geqslant 10$ mM (Fig. 3*A*), whilst C_T (Fig. 3*B*) first appears at $[K]_o \geqslant 20$ mM. The magnitudes of both components are graded with $[K]_o$, and do not appear to saturate at $[K]_o \leqslant 150$ mM. The relationship between C_T and $[K]_o$ (and, thus, V_m) is similar to that observed for the activation of Ca channels in synaptosomes by $[K]_o$ (Nachshen & Blaustein, 1980). These data are consistent with the idea that the Ca entry through voltage-regulated Ca channels is required to activate the Ca-dependent ^{86}Rb efflux. However, the Ca-activated Rb permeability, itself, may also display voltage sensitivity (cf. Barrett, Magleby & Pallotta, 1982).

The trivalent cation, La, is a potent inhibitor of Ca influx through voltage-regulated Ca-selective channels, with an apparent K_I of 0·2–0·3 μM (at $[Ca]_o = 20$ μM) for the inactivating Ca channels in synaptosomes (Nachshen & Blaustein, 1980). We therefore tested the effect of La on the K-stimulated ^{86}Rb efflux in the absence and presence of Ca. As illustrated in Fig. 3*A*, 100 μM-La reduced the K-stimulated ^{86}Rb

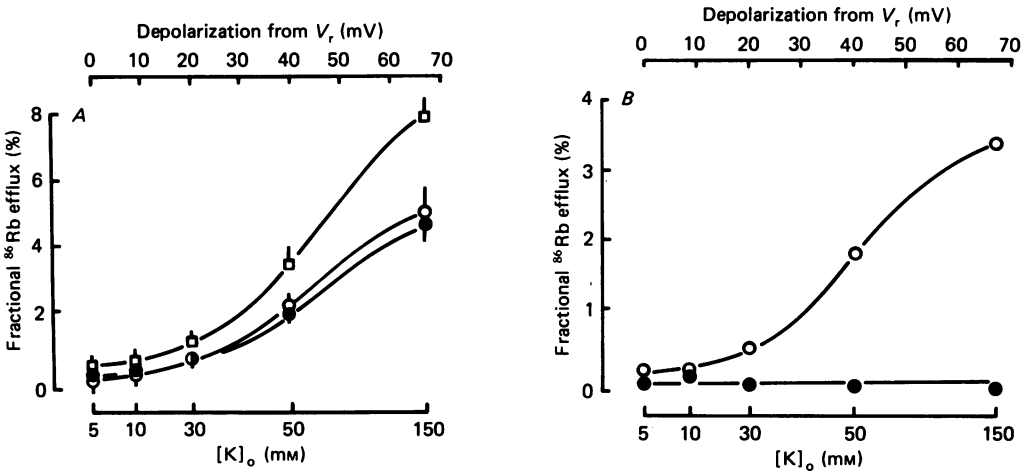

Fig. 3. Effect of La on ^{86}Rb efflux from synaptosomes. *A*, ^{86}Rb-loaded synaptosomes were trapped on glass fibre filters and were then exposed to PSS containing the K concentration indicated on the abscissa. In addition, the efflux media contained either no added Ca (○) (component *T*), 1 mM-Ca (□) (components $T + C_T$), or 1 mM-Ca and 0·1 mM-$LaCl_3$ (●). The upper abscissa scale refers to the calculated depolarization from the resting membrane potential (V_r) with increasing $[K]_o$ (see Blaustein & Goldring, 1975; Bartschat & Blaustein, 1985). *B*, data from *A* were used to calculate the increment in ^{86}Rb efflux above that seen in Ca-poor PSS (○ in *A*) in response to inclusion of 1 mM-Ca (○) (= component C_T) or 1 mM-Ca + 0·1 mM-$LaCl_3$ (●). The values are the means of four determinations ± S.E. of mean.

efflux in the presence of 1 mM-Ca to the level observed in the absence of Ca; 100 μM-La had little effect on the K-stimulated ^{86}Rb efflux in the absence of Ca (data not shown). This is additional evidence that Ca entry through voltage-regulated Ca channels is required to activate the Ca-dependent ^{86}Rb efflux that is observed in K-rich media.

Effect of Ca and A23187 on ^{86}Rb efflux

The Ca-selective ionophore, A23187, was used to determine whether Ca entry into synaptosomes in the absence of membrane depolarization could stimulate the release of ^{86}Rb. Fig. 4*A* shows the typical pattern of ^{86}Rb efflux into 5 and 75 mM-K media ± 1 mM-Ca in the absence of A23187; comparable fluxes, in the presence of 1 μM-A23187, are illustrated in Fig. 4*B*. Comparison of the data in Fig. 4*A* and *B* indicates that A23187 had no effect on the efflux of ^{86}Rb in either 5 mM-K or K-rich media in the absence of external Ca. However, with Ca present, the ionophore increased ^{86}Rb efflux into both 5 mM-K and 75 mM-K media. Thus, Ca entry into polarized or depolarized synaptosomes can activate the Ca-dependent ^{86}Rb efflux. Note that the ^{86}Rb efflux promoted by A23187 in the Ca-containing 75 mM-K solution

lasts for several seconds. This suggests that the early decline ('inactivation') in the Ca-dependent ^{86}Rb efflux, normally observed after 1 s (Figs. 1 and 4*A*; and see Fig. 2 in Bartschat & Blaustein, 1985), may be due largely to inactivation of voltage-regulated Ca channels (Nachshen & Blaustein, 1980) and reduction of Ca entry, and not to inactivation of the Ca-activated K channels.

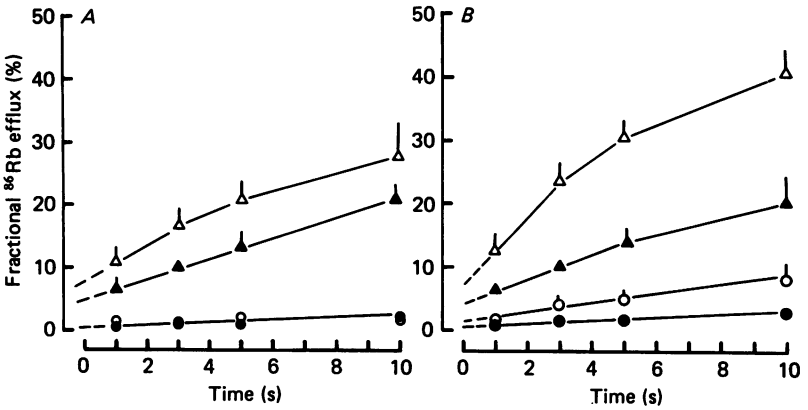

Fig. 4. Effect of Ca+A23187 on ^{86}Rb efflux. Rb efflux from ^{86}Rb-loaded synaptosomes was studied in 5 mM-K (circles) or 75 mM-K (triangles) in the presence (open symbols) or absence (filled symbols; 1 mM-Mg) of 1 mM-Ca. *A*, no A23187. *B*, in the presence of 1 μM-A23187. The wash solutions did not contain Ca, but the wash solution used for the synaptosomes in *B* did contain A23187. The values are the means of three determinations ± S.E. of mean.

Another observation that deserves comment is the quantitatively greater effect of the A23187 (+Ca) on the ^{86}Rb efflux in the K-rich, as compared to the 5 mM-K media. Possible explanations are: (i) membrane depolarization increases the outward driving force on Rb, so that a greater ^{86}Rb efflux should normally be observed in K-rich (*versus* 5 mM-K) media with no change in Rb permeability, and/or (ii) the Ca-activated channels may also be modulated by membrane potential (cf. Barrett *et al.* 1982). To distinguish between these possibilities, Rb permeability (P_{Rb}) was determined from the data of Fig. 4*B* by measuring ^{86}Rb efflux between 1 and 10 s in 5 mM-K PSS, or between 1 and 3 s in 100 mM-K PSS (see Fig. 2 in Bartschat & Blaustein, 1985, and related text). P_{Rb} was $1{\cdot}5 \times 10^{-7}$ cm/s in nominally Ca-free 5 mM-K media; inclusion of 1 mM-Ca increased P_{Rb} 1·9-fold to $2{\cdot}9 \times 10^{-7}$ cm/s. In 100 mM-K PSS, inclusion of 1 mM-Ca increased P_{Rb} from $2{\cdot}2 \times 10^{-7}$ to $6{\cdot}7 \times 10^{-7}$ cm/s, a 3·0-fold increase. These calculations suggest that both the changes in driving force and the intrinsic voltage dependence of the Ca-activated ^{86}Rb efflux pathway probably contribute to the quantitatively greater effect of Ca+A23187 in 100 mM-K as compared with 5 mM-K media.

Effect of Ca+A23187 on synaptosome membrane potentials

In order to determine whether or not the Ca+A23187-induced ^{86}Rb efflux reflects a selective increase in P_K, synaptosome membrane voltages were monitored with a

voltage-sensitive fluorescent dye, DiS-C3-(5) (Fig. 5). Fluorescence intensity of the dye plus synaptosomes increased with increasing K concentration, consistent with membrane depolarization (Blaustein & Goldring, 1975). The addition of A23187 to the synaptosome suspensions in nominally Ca-free media did not alter the fluorescence emission at any $[K]_o$. Subsequent addition of 1 mM-Ca decreased dye fluorescence in

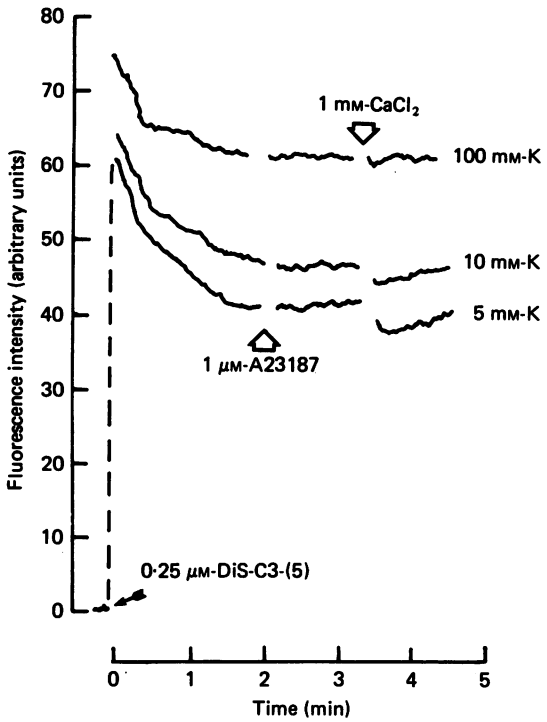

Fig. 5. Effect of Ca + A23187 on synaptosome membrane potentials. Membrane potentials were monitored with the voltage-sensitive fluorescent dye, DiS-C3-(5). Fluorescence intensity is expressed in arbitrary units where fluorescence intensity in the presence of synaptosomes but absence of dye (i.e. light scatter) is defined as '0' fluorescence intensity. 4 μl aliquots of A23187 (0·125 mM in ethanol) and 2 μl aliquots of 1 mM-$CaCl_2$ were added to the 2 ml synaptosome suspensions in the cuvette; the incubation solutions contained 50 μM-EGTA. Neither addition of Ca in the absence of A23187, nor ethanol alone, had a significant effect on dye fluorescence intensity (not shown). $[K]_o$ was varied by replacing K for Na in the PSS. All solutions contained 2 mM-Mg.

media containing 5 mM-K or 10 mM-K, but not 100 mM-K. This is to be expected if P_K is selectively increased because in 100 mM-K the membrane potential is probably at or very close to the K equilibrium potential, E_K, whereas in 5 or 10 mM-K the membrane potential is probably at some value more positive than E_K, due to the finite permeability to Na (cf. Blaustein & Goldring, 1975). Thus, a further increase in P_K should drive the membrane potential closer to E_K in 5 or 10 mM-K. The addition of Ca, alone (i.e. in the absence of A23187) had little effect on V_m, even at low $[K]_o$ (data not shown). However, valinomycin, which is a K-selective ionophore and should selectively increase P_K, reduced the fluorescence intensity (i.e. hyperpolarized the

synaptosomes) at low $[K]_o$, but had no effect at high $[K]_o$ (data not shown). In sum, these observations support the view that synaptosomes have Ca-activated K channels that can be opened when Ca enters the terminals either through depolarization-activated Ca channels, or through a carrier-mediated process when A23187 is added to the medium.

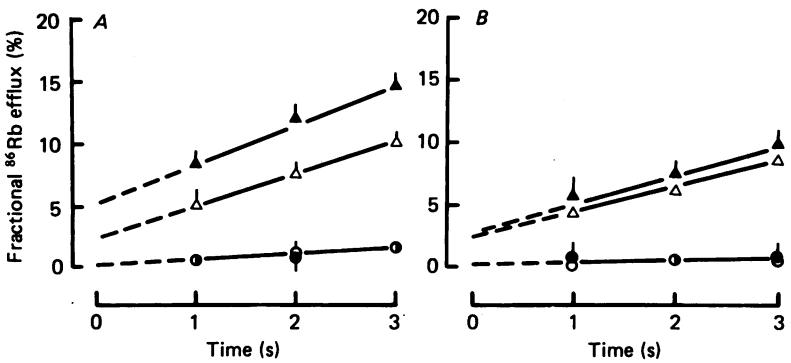

Fig. 6. Effect of quinine sulphate on the time course of ^{86}Rb efflux. Rb efflux from ^{86}Rb-loaded synaptosomes was measured as described in the legend to Fig. 1. *A*, data obtained in the absence of quinine sulphate. *B*, 10 μM-quinine sulphate was added to all wash and efflux solutions. These solutions were protected from light to prevent photodegradation. The lines were fitted by linear regression analysis. 5 mM-K, 0 Ca (○); 5 mM-K, 1 mM-Ca (●); 75 mM-K, 0 Ca (△); 75 mM-K, 1 mM-Ca (▲). Values are the means of six determinations ± S.E. of mean.

Pharmacology of Ca-dependent ^{86}Rb efflux

(*a*) *Quinine sulphate.* One way in which K channels in various cells have been characterized is by their sensitivity to pharmacological agents. The antimalarial, quinine, blocks Ca-activated K channels in a number of cells. For example, quinine sulphate blocks Ca-activated K channels in red blood cells (Armando-Hardy, Ellory, Ferreira, Fleminger & Lew, 1975; 0·1 mM blocks by 50%), in pancreatic β cells (Atwater, Dawson, Ribalet & Rojas, 1979), and in *Aplysia* neurones (Hermann & Gorman, 1981*c*). This drug is not specific, however: it blocks both Ca-activated and voltage-regulated K channels in molluscan neurones (Thompson, 1977; Hermann & Gorman, 1981*b*; Walden & Speckmann, 1981).

Fig. 6 shows data from an experiment in which we tested the effect of 10 μM-quinine sulphate on ^{86}Rb efflux into 5 and 75 mM-K PSS with and without 1 mM-Ca. The ^{86}Rb efflux into 5 mM-K PSS was not measurably affected by the drug, and efflux into 75 mM-K PSS in the absence of added Ca was only slightly reduced ($\sim$ 10%). However, this low concentration of quinine nearly abolished the increment in ^{86}Rb efflux induced by the addition of Ca to the K-rich solution.

The potency of quinine's blocking effect on the Ca-dependent ^{86}Rb efflux was determined by testing several concentrations of quinine on C_T. As shown in Fig. 7, quinine reduced this component of efflux (●) in a dose-dependent manner, with an apparent half-maximal inhibition, K_I of 1–2 μM. In control experiments, we found

that K-stimulated ^{45}Ca influx (Fig. 7 (○); cf. Nachshen & Blaustein, 1980) and ^{86}Rb efflux components T and S (data not shown; cf. Bartschat & Blausten, 1985) were blocked by quinine sulphate only at higher concentrations ($K_I > 50$ μM). Thus, the Ca-activated K channels in synaptosomes seem to be selectively blocked by low concentrations of quinine; these channels appear to be much more sensitive to quinine than are those in red blood cells ($K_I \simeq 100$ μM; Armando-Hardy *et al.* 1975).

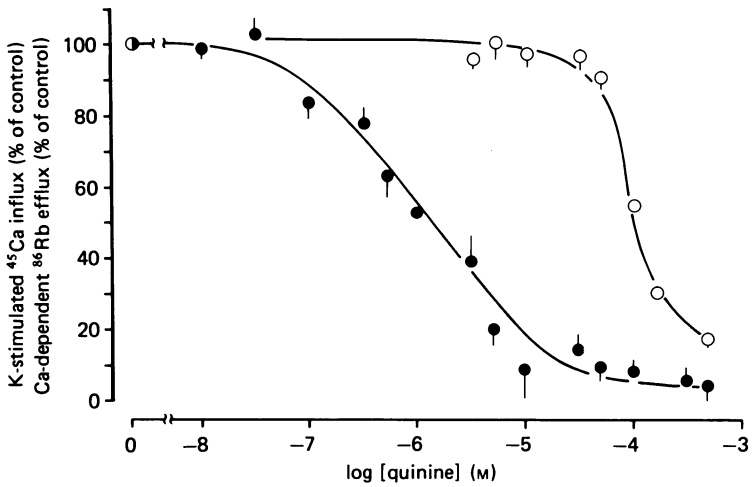

Fig. 7. Dose–response curves illustrating the effects of quinine sulphate on K-stimulated ^{45}Ca influx (○) and Ca-dependent ^{86}Rb efflux (●). The depolarization-activated ^{45}Ca influx (75 mM-K, 1 s incubation) was determined by the method of Nachshen & Blaustein (1980). The Ca-dependent ^{86}Rb efflux (100 mM-K, 1 s incubation) in this experiment consists primarily of component C_T, but is expected to be about 10% larger than C_T because of a small contribution from C_S (cf. Fig. 1). The symbols represent the means of five (●) or four (○) determinations ± S.E. of mean.

Quinine sulphate was also tested for its ability to inhibit the Ca + A23187-induced increase in ^{86}Rb efflux (Fig. 8). As in the experiment of Fig. 4, A23187 increased the efflux of ^{86}Rb into Ca-containing 5 mM-K and 100 mM-K PSS. About 90% of the Ca + A23187-stimulated efflux was blocked by 10 μM-quinine sulphate. This is further evidence that the Ca-dependent ^{86}Rb effluxes activated by K-rich media and by A23187 reflect the same conductance.

(*b*) *Tetraalkylammonium ions.* Several tetraalkylammonium ions, including TEA and TBA, block K channels in many excitable cells (Armstrong, 1975). As described in the preceding article (Bartschat & Blaustein, 1985), TEA and TBA block the K-stimulated (Ca-independent) ^{86}Rb efflux in synaptosomes. However, tetraalkylammonium ions have also been found to block some Ca-activated K channels. For example, TEA blocks the Ca-activated K conductance in molluscan neurones (Thompson, 1977; Hermann & Gorman, 1981*b*).

The dose–response curves in Fig. 9 show the effects of TEA and TBA on ^{86}Rb efflux component C_T. Note that both agents blocked this efflux, and that TEA was more potent, with an apparent K_I of 0·2 mM which is comparable to the value obtained by Hermann & Gorman (1981*b*) in *Aplysia* ($K_I = 0{\cdot}4$ mM). The relative potency,

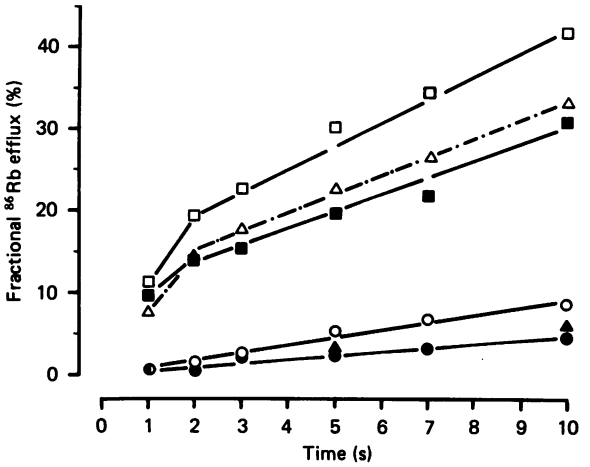

Fig. 8. Effect of quinine sulphate on Ca + A23187-stimulated ^{86}Rb efflux. ^{86}Rb efflux was measured in 5 mM-K (○, ●, ▲) or 100 mM-K (□, ■, △), in the absence (●, ■) or presence (○, △, ▲, □) of 1 μM-A23187. All efflux solutions contained 1 mM-Ca. In some experiments, 10 μM-quinine sulphate + 1 mM-Ca (triangles) was added to the efflux media, in order to assess the effect of this drug on Ca + A23187-stimulated ^{86}Rb efflux. The results are the means of four determinations.

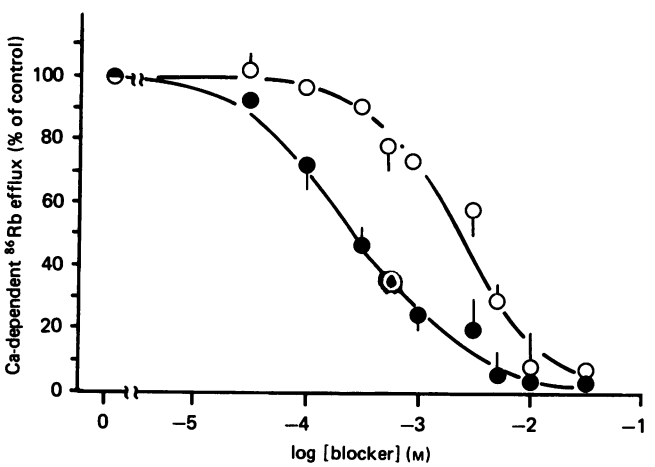

Fig. 9. Inhibition of Ca-dependent ^{86}Rb efflux by TEA and TBA. A protocol similar to that of the ^{86}Rb efflux experiment in Fig. 7 was used. The results are the means of four determinations ± S.E. of mean. TEA (●); TBA (○).

TEA > TBA, in synaptosomes is also consistent with the observations in *Aplysia* (Hermann & Gorman, 1981*b*).

(*c*) *4-aminopyridine (4-AP)*. Several aminopyridines, including 4-AP and 3,4-diaminopyridine, are potent blockers of some voltage-regulated K channels (Meves & Pichon, 1977; Thompson, 1977; Kirsch & Narahashi, 1978). However, 4-AP has been found to have either no effect (Hermann & Hartung, 1982) or, at high

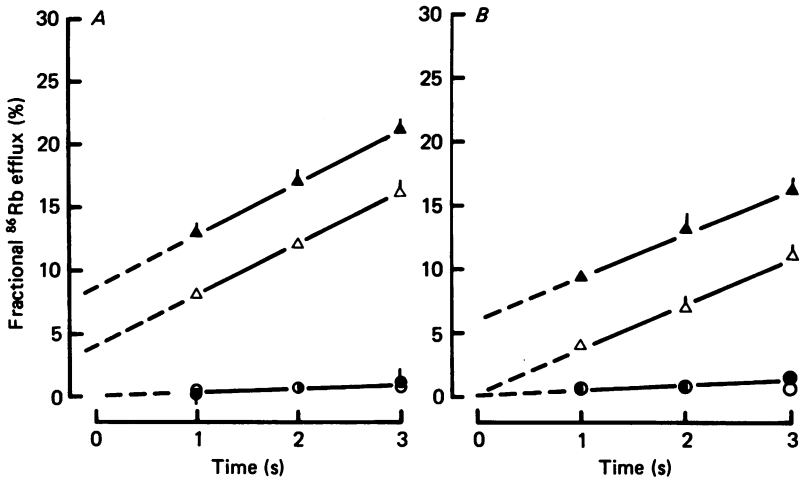

Fig. 10. Effect of 1 mM-4-AP on the time course of ^{86}Rb efflux. A protocol similar to that employed in the experiment of Fig. 6 was used. 5 mM-K, 0 Ca (○); 5 mM-K, 1 mM-Ca (●); 100 mM-K, 0 Ca (△); 100 mM-K, 1 mM-Ca (▲). The experimental values are the means of quadruplicate determinations in each of four separate experiments ±S.E. of mean. *A*, control; *B*, 1 mM-4-AP.

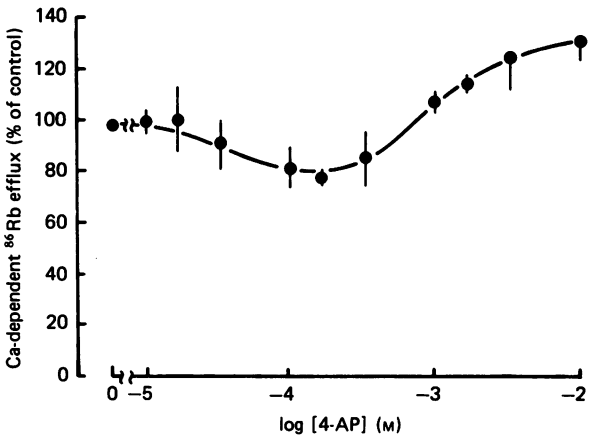

Fig. 11. Effect of various 4-AP concentrations on Ca-dependent ^{86}Rb efflux. A protocol similar to that of the ^{86}Rb efflux experiment in Fig. 7 was employed. The symbols represent the means±S.E. of triplicate determinations in each of three separate experiments.

concentrations, a stimulatory effect on Ca-activated K currents (e.g. Hermann & Gorman, 1981*a*). Fig. 10 illustrates the effects of 4-AP on ^{86}Rb efflux from synaptosomes in the absence and presence of 1 mM-Ca. Ca-insensitive component T was totally abolished by 1 mM-4-AP, but the Ca-dependent component was not inhibited.

A dose–response curve that illustrates the effect of 4-AP on ^{86}Rb efflux component C_T is presented in Fig. 11. Low concentrations (100–300 μM) of 4-AP inhibited the Ca-dependent ^{86}Rb efflux by approximately 20–30% but concentrations greater than

1–2 mM stimulated the efflux, in agreement with Hermann & Gorman's (1981*a*) observation in *Aplysia* (30 % stimulation of Ca-activated K currents by 5 mM-4-AP). These data raise the possibility that 4-AP may have two effects on Ca-activated K channels: at low concentrations it may block the channels, as it does voltage-regulated K channels, but at higher concentrations it may stimulate the Ca-activated K channels by an unknown mechanism. Thus the observed effects of 4-AP on Ca-activated K channels may depend on the concentrations of 4-AP tested.

DISCUSSION

Ca-activated K channels in synaptosomes. The small size of presynaptic nerve terminals from mammalian brain (usually < 1 μm diameter) precludes the use of standard electrophysiological techniques in these terminals. As described in this report and the preceding one (Bartschat & Blaustein, 1985), we have circumvented this obstacle by employing tracer flux methods to study some of the physiological and pharmacological properties of several K permeability mechanisms in isolated nerve terminals from rat brain. These flux methods have a unique advantage in that the unidirectional transport of specific ionic species are measured directly, rather than by the indirect methods required to separate the components of net current flow in electrophysiological experiments. A significant drawback to our tracer flux methods is the relatively slow time scale on which the experiments can be carried out; nevertheless, the introduction of manual rapid-quench techniques has enabled us to identify at least four different components of ^{86}Rb (and ^{42}K) efflux with a time resolution of 1 s.

The present report is focused on the Ca-dependent component(s) of the Rb efflux from ^{86}Rb-loaded synaptosomes. Our data lead us to conclude that this ^{86}Rb efflux is mediated by Ca-activated K channels on the basis of the following criteria:

(i) the Ca-dependent ^{86}Rb efflux (Fig. 1) parallels the Ca-dependent ^{42}K efflux, although the latter is about 20 % larger in double-label experiments (Bartschat & Blaustein, 1985). This is consistent with published reports that Rb passes through Ca-activated K channels in other cells nearly as well as K itself (e.g. Gorman, Woolum & Cornwall, 1982). Our data imply a Rb:K permeability ratio, $P_{Rb}:P_K$, of about 0·8, for the Ca-activated K channels (component C_T; Bartschat & Blaustein, 1985). ^{86}Rb has been used primarily because it has a much longer half-life than ^{42}K.

(ii) The Ca-dependent ^{86}Rb efflux is activated by conditions that promote Ca entry into the terminals: either addition of the Ca ionophore A23187 (Fig. 4), or depolarizing solutions (Fig. 3), which would open Ca-selective channels in the plasmalemma (Nachshen & Blaustein, 1980). Thus, the efflux appears to be mediated by a rise in $[Ca]_i$. High $[K]_o$ or the addition of A23187 in the absence of external Ca are ineffective, as is high $[K]_o$ in the presence of Ca and La (Fig. 3), a Ca channel blocker. This implies that the latter treatments do not release sufficient Ca from internal stores (cf. Blaustein *et al.* 1980), so that extracellular Ca is required to activate the K channels.

(iii) The activation of ^{86}Rb efflux by Ca + A23187 appears to be associated with a specific increase in P_K, as demonstrated by experiments in which the membrane potential was monitored with a voltage-sensitive fluorescent dye (Fig. 5). The hyperpolarization (manifested by reduced fluorescence) observed in the media with

5 mM-K implies that P_K was increased in the presence of Ca + A23187, and that V_m therefore approached E_K (which is probably more negative than V_m at low $[K]_o$ but equal to V_m at high $[K]_o$). In some excitable cells an increase in $[Ca]_i$ can activate a non-selective cation channel (e.g. Suarez-Kurtz, 1979; Colquhoun, Neher, Reuter & Stevens, 1981). This cannot be the case in the synaptosomes, however, because activation of a non-selective cation conductance in 5 mM-K PSS would be expected to produce a *depolarization* in association with an increase in Rb (or K) efflux.

(iv) The pharmacological properties of the Ca-activated ^{86}Rb efflux are also consistent with the view that this flux is mediated by Ca-activated K channels. This efflux is selectively blocked by low concentrations of quinine sulphate (Figs. 6–8), a known blocker of Ca-activated K channels (Atwater *et al.* 1979; Lew, Muallem & Seymour, 1982). The inhibition of the ^{86}Rb efflux by TEA and TBA (Fig. 9) and the stimulation by high concentrations of 4-AP (Fig. 11) are also consistent with the reported effects of these agents on Ca-activated K channels (Hermann & Gormann, 1981*a*, *b*).

The foregoing discussion has been concerned with the 'transient' component of the Ca-activated ^{86}Rb efflux, C_T (see Fig. 1 and related text). The slower Ca-dependent ^{86}Rb efflux observed after 1 s (i.e. component C_S) may be a consequence of the decline in Ca influx due to the inactivation of Ca channels (to about 20–25% of the influx observed in the initial 1 s; Nachshen & Blaustein, 1980). We have no information on whether or not some of the Ca-activated K channels inactivate within a few seconds; however, the fact that A23187 promotes a Ca-dependent ^{86}Rb efflux that does not appear to inactivate may indicate that the majority of Ca-activated K channels in synaptosomes do not inactivate within 3–5 s.

The pharmacology of Ca-activated K channels

An unexpected result of the present study is the observation that the Ca-activated K channels in rat brain nerve terminals are substantially more sensitive to quinine sulphate than are other Ca-activated K channels on which this agent has been tested (cf. Lew *et al.* 1982). This has obvious implications for the *in situ* investigation of K conductances with electrophysiological methods: by blocking certain K channels, quinine sulphate might enhance, or otherwise modify synaptic transmission in a specific manner (see below).

The fact that all Ca-activated K channels do not have identical pharmacological properties provides ample reason for carefully examining the pharmacological properties of the specific channels in the tissue of interest. An important problem that has not yet been studied concerns the localization of the various K channels: are they ubiquitously distributed? Or, do some terminals have only one or two types of K channels? A study of synaptosomes from selected brain regions may help to answer these critical questions.

Conclusions

As summarized in Table 1, our data from synaptosomes provide the first pharmacological profiles of several classes of K channels that are present in the rat brain presynaptic nerve terminals. This conclusion is based upon the evidence presented in this and the accompanying article (Bartschat & Blaustein, 1985) that the four ^{86}Rb

efflux components we have identified correspond to different classes of K permeability mechanisms: (i) the 'resting' K permeability is blocked only by high concentrations of TEA, TBA and 4-AP; (ii) the inactivating, voltage-regulated K channels are selectively blocked by low concentrations of 4-AP; (iii) the Ca-activated K channels are especially sensitive to quinine sulphate, and are stimulated by high concentrations of 4-AP; and (iv) the non-inactivating (or slowly inactivating), voltage-regulated K

TABLE 1. Pharmacological properties of synaptosome K channels

K permeability mechanisms	^{86}Rb efflux component	Sensitivity to K channel blockers*			
		QS	TEA	4-AP	PCP
1. Resting	R	0	+	+	0
2. Ca activated	C_T	+++	++	Stim.†	0
3. V_m regulated, inactivating	T	+	++	++	++
4. V_m regulated, non-inactivating	S	+	++	+	+++

* QS = quinine sulphate; PCP = phencyclidine (1-(1-phenylcyclohexyl)piperidine HCl); 0 = no effect; + = $K_I > 1000\ \mu M$; ++ = K_I 50–1000 μM; +++ = $K_I < 5\ \mu M$.

† Stim. = stimulation. (Data from this article, and from Bartschat & Blaustein, 1984, 1985.) As described in Bartschat & Blaustein (1984, 1985), the V_m-regulated, non-inactivating channels presumably correspond to the fraction of ^{86}Rb efflux component S that is not mediated by the 'resting' K permeability, and is sensitive to low concentrations of PCP.

channels are particularly sensitive to very low concentrations of phencyclidine (Bartschat & Blaustein, 1984), and moderately sensitive to 4-AP. The latter three classes of K channels are all blocked by significantly lower concentrations of TEA and TBA than is the resting K permeability. Appropriate application of these data to electrophysiological studies of neurotransmitter release (e.g. in rat brain slice preparations) may help to elucidate the influence of the various K channels on synaptic transmission. These pharmacological data may also be useful for biochemical identification and purification of the K channel proteins, and for the identification and pharmacological characterization of single K channels when plasma membrane fragments are incorporated into planar lipid bilayers (cf. Krueger, Worley & French, 1983; Nelson, Roudna & Bamberg, 1983).

We gratefully acknowledge Drs B. K. Krueger and B. E. Alger for helpful discussions and critical reading of this manuscript, and thank Ms R. K. Ickowicz for permission to include data from an unpublished experiment (Fig. 7 (○)). We also thank Ms A. Wilder for preparing the typescript. Supported by NIH grant NS-16106.

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