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POTASSIUM CHANNELS IN ISOLATED PRESYNAPTIC NERVE TERMINALS FROM RAT BRAIN

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SUMMARY

1. ^{86}Rb efflux from pinched-off rat brain presynaptic nerve terminals (synaptosomes) was used to measure the K permeability of the terminals. Synaptosomes were pre-loaded with ^{86}Rb and the suspensions were then filtered on glass fibre filters. The terminals trapped on the filters were superfused with 'efflux solutions', and the effluent and filters were then counted.

2. ^{86}Rb efflux into physiological saline (PSS) containing 5 mM-K and 145 mM-Na was about 0·4 % of the ^{86}Rb load per second (component '*R*'). Increasing extracellular K concentration ($[K]_o$), or adding veratridine *and* sea anemone toxin, stimulated efflux; presumably by depolarizing the nerve terminals. The K-stimulated ^{86}Rb efflux was a graded function of $[K]_o$.

3. High $[K]_o$ evoked at least three components of efflux: a 'fast phase' (*T*) that apparently inactivated in less than 1 s, a 'slower phase' (*S*) that was linear for 3–5 s, and a Ca-dependent phase (*C*). Some, but not all, of the slow phase ^{86}Rb efflux (component *S*) may be attributable to increased efflux mediated by the 'resting' K permeability mechanism when the driving force is increased by depolarization.

4. K efflux was also studied and was found to be qualitatively similar to ^{86}Rb efflux. ^{86}Rb:^{42}K permeability ratios were 0·6–0·8 for most components of the efflux.

5. Raising the Mg concentration in the efflux solution shifted the ^{86}Rb efflux *versus* $[K]_o$ curve in the direction of increased $[K]_o$. This shift may be the result of screening of surface charges by Mg.

6. Several agents that block various K channels in other preparations inhibited K-stimulated ^{86}Rb efflux in synaptosomes: tetraethylammonium (TEA), tetrabutylammonium (TBA), and 4-aminopyridine (4-AP).

7. The fast component (*T*) of high $[K]_o$-stimulated ^{86}Rb efflux was selectively blocked by low concentrations of 4-AP (apparent half-maximal inhibition, K_I = 0·1–0·2 mM); it was also blocked by TEA (K_I = 0·6 mM) and TBA (K_I = 0·8–1·0 mM). Dose–response curves for inhibition of component *T* by all three agents were monophasic.

8. The slow component (*S*) of the K-stimulated ^{86}Rb efflux was much less sensitive to all three agents, than was component *T*; the broad dose–response curves were consistent with the view that two (or more) different K conductances may contribute to component *S*.

9. The data suggest that rat brain synaptosomes contain at least four classes of

K channels or permeability mechanisms: a 'resting' K permeability and a non-inactivating (or slowly inactivating) voltage-regulated K channel, both of which are included in ^{86}Rb efflux component S; an inactivating, voltage-regulated K channel (component T); and a Ca-activated K channel (component C).

INTRODUCTION

Hodgkin and Huxley, in their classical study of excitability in squid axons (cf. Hodgkin, 1967), showed that the rising phase of the nerve action potential was due to a selective, transient increase in plasma membrane Na conductance. The spike was terminated by a delayed, selective increase in K conductance (cf. Hodgkin, 1967). Both conductance changes could be explicitly described by sets of voltage- and time-dependent parameters; in this respect, the conductance changes appeared to be relatively stereotyped. Subsequent work on a large variety of excitable cells has revealed that a number of K conductances do not conform to the simple voltage- and time-dependent mechanism that occurs in squid axons. For example, in many types of cells, some K conductances are activated by a rise in the free (ionized) Ca concentration in the cytoplasm (cf. Meech, 1978). Also, in some cells, K conductances may be modulated by cyclic nucleotides, for example, as a consequence of neurotransmitter action (Castellucci, Kandel, Schwartz, Wilson, Nairn & Greengard, 1980; De Peyer, Cachelin, Levitan & Reuter, 1982). Klein & Kandel (1980) have demonstrated that modulation of K conductances, due to transmitter action on presynaptic neurones in *Aplysia*, may have profound behavioural effects. In fact, the bizarre schizophrenia-like behavioural effects of intoxication with phencyclidine in man and animals have been attributed to blockade of certain presynaptic K channels in the central nervous system (C.N.S.) (Albuquerque, Aguayo, Warnick, Weinstein, Glick, Maayani, Ickowicz & Blaustein, 1981; Blaustein & Ickowicz, 1983; Bartschat & Blaustein, 1984). Presumably, by altering the duration of the presynaptic action potential, Ca entry and, therefore, neurotransmitter release, are altered.

Much information has been obtained on K channel function in the neurones of lower animals. Less is known about K channels in the mammalian C.N.S., and no detailed studies of nerve terminal K channels have been published. The small size of the terminals (< 1 μm diameter), renders them unsuitable for micro-electrode penetration. Therefore, we developed methods to assess K channel activity based on the use of radioactive tracer fluxes in an isolated presynaptic nerve terminal (synaptosome) preparation from rat brain. Such an approach has previously been applied to the study of presynaptic Na channels (Krueger & Blaustein, 1980) and Ca channels (Nachshen & Blaustein, 1980, 1982).

The present report describes experiments in which tracer techniques were used to examine some properties of the K fluxes activated by conditions designed to depolarize the synaptosomes. In most experiments ^{86}Rb was employed as a tracer for K because it has a much longer half-life than ^{42}K. The use of ^{86}Rb is justified because: (i) Rb is actively accumulated by cells via the ouabain-sensitive Na–K pump (e.g. Baker, Blaustein, Keynes, Manil, Shaw & Steinhardt, 1969; and see Results), and (ii) Rb passes through various types of neuronal K channels almost as well as K itself (cf. Hille, 1975; Reuter & Stevens, 1980; Gorman, Woolum & Cornwall, 1982).

Our data show that depolarizing conditions stimulate ^{86}Rb efflux from tracer-loaded synaptosomes. This stimulated efflux is blocked by a number of 'typical' K channel blockers including tetraalkylammonium ions and aminopyridines. The differential effects of various pharmacologic agents indicates that there may be *at least* four classes of pharmacologically distinct K effluxes, each possibly representing distinct K channels or permeability mechanisms, in our heterogeneous synaptosome population. Some of the K channels appear to be activated by Ca. Preliminary reports of some of our findings have been published (Blaustein & Ickowicz, 1982; Bartschat & Blaustein, 1983).

METHODS

Preparation of synaptosomes

Synaptosomes were prepared from the forebrains of 125 g female albino rats by a one-step sucrose gradient method (Krueger, Ratzlaff, Strichartz & Blaustein, 1979). The 0·8 M-sucrose fraction containing the synaptosomes was equilibrated slowly with three volumes of ice-cold solution (145 mM-Na + 5 mM-K) which consisted of (mM): NaCl, 145; KCl, 5; $MgCl_2$, 2·0; $CaCl_2$, 0·02; glucose, 10; HEPES buffer, 10, titrated to pH 7·4 with NaOH.

Loading of synaptosomes with ^{86}Rb

The equilibrated synaptosomes were centrifuged at 20000 *g* for 6 min at 5 °C. The supernatant solution was discarded and the pelleted synaptosomes were resuspended in 145 mM-Na + 5 mM-K containing tracer ^{86}Rb (~ 20 μCi/ml) and 0·1 mM-unlabelled RbCl; the protein concentration (Lowry, Rosebrough, Farr & Randall, 1951) in these suspensions was about 45 mg/ml. The suspensions were incubated for 30 min at 30 °C to load the synaptosomes with ^{86}Rb.

Measurement of ^{86}Rb efflux

In order to measure ^{86}Rb efflux at short incubation times, a manual quench method was developed, similar to the one employed by Drapeau & Blaustein (1983) in their study of dopamine efflux. Aliquots (50 μl) of ^{86}Rb-loaded synaptosomes were pipetted into the wells of a filtration apparatus (Amicon) containing 2 ml of 'wash solution' at 30 °C, and suction was then applied, trapping the synaptosomes on a glass fibre filter (Schleicher & Schuell, 25 mm diameter, no. 25 or no. 30). This procedure was repeated four more times with the same solution (for a total of five wash–suction cycles) to remove residual extracellular ^{86}Rb. The wash solution (unless otherwise specified) contained (mM): NaCl, 145; KCl, 5; $MgCl_2$, 2; RbCl, 0·1; glucose, 10; and HEPES buffer, 10, at pH 7·4. The entire wash procedure took about 20 s. In drug experiments the appropriate drug concentration was added to the wash solution to facilitate equilibration of the drug with the synaptosomes.

The measurement of ^{86}Rb efflux was begun 15–20 s after the final wash cycle by the addition of 1·5–2·0 ml of reaction (efflux) medium (30 °C) to the filter well, followed by the rapid addition of an equal volume of 'stopping solution' (also 30 °C) 1–10 s later; the incubations were timed with an electronic metronome. Suction was then applied, and the entire filtrate was collected into a plastic vial; liquid scintillation cocktail was added, and the amount of ^{86}Rb released was then determined. The radioactivity remaining on the filter was also evaluated by scintillation spectroscopy. ^{86}Rb efflux was expressed as the percentage of the total ^{86}Rb appearing in the filtrate at each time point; thus,

$$\%\ ^{86}\text{Rb efflux} = \frac{^{86}\text{Rb in filtrate}}{^{86}\text{Rb in filtrate} + ^{86}\text{Rb on filter}} \times 100.$$

Since the filter wells contained no bulk wash solution after the final wash cycle, exposure of the synaptosomes to the reaction medium should have been virtually instantaneous. Also, to facilitate mixing of the 'stopping solution' with the reaction medium in the wells, the 'stopping solution' was introduced as a stream, under pressure, directly on to the trapped synaptosomes. While the exposure of the synaptosomes to the 'stopping solution' was not 'instantaneous', mixing of the solutions should have occurred in substantially less than 1 s. Indeed, in control experiments (data

not shown) ^{86}Rb efflux was measured for several seconds before, and again after the introduction of 'stopping solution' (i.e. suction was applied at various times after this solution was added). The data indicate that high K-stimulated ^{86}Rb efflux was reduced to negligible rates within 0·2–0·3 s after the addition of 'stopping solution'. Thus, while the duration of the initial 'one second' may be slightly underestimated, the subsequent intervals are likely to be quite accurately timed.

The reaction media (Physiological saline; PSS) contained (mM): NaCl, 0–145; KCl, 5–150 (NaCl + KCl = 150); $MgCl_2$, 2; RbCl, 0·1; glucose, 10; and HEPES buffer, 10, pH 7·4. In some experiments, $CaCl_2$ was substituted mole-for-mole for Mg. Additional details on the composition of the reaction media are given in the Results section.

The 'stopping solution' contained (mM): tetraethylammonium chloride (TEA), 145; RbCl, 5; tetrabutylammonium chloride (TBA), 1; $MgCl_2$, 5; $NiCl_2$, 10; and HEPES buffer, 20, pH 7·4.

Drugs and other reagents

Standard reagents were all reagent grade. Sources of special drugs, reagents and tracer were: HEPES, Tris base, TEA, TBA and quinine sulphate, Sigma (St. Louis, MO); 4-aminopyridine (4-AP), 3,4-diaminopyridine (3,4-DAP) and veratridine, Aldrich (Milwaukee, WI); tetrodotoxin (TTX), Calbiochem (San Diego, CA); ^{86}Rb, New England Nuclear (Boston, MA). We thank Dr B. K. Krueger for kindly providing purified sea anemone (*Anthopleura xanthogrammica*) toxin (= AxTX).

RESULTS

^{86}Rb accumulation by synaptosomes

Data from several laboratories (e.g. Escueta & Appel, 1969; Blaustein & Goldring, 1975) indicate that synaptosomes are able to accumulate K and extrude Na by an ouabain-sensitive, metabolically dependent route (presumably the 'Na pump'). Like most animal cells, they are able to maintain large K and Na concentration gradients (intracellular K concentration ($[K]_i$) $\gg$ extracellular K concentration and intracellular Na concentration ($[Na]_i$) $\ll$ extracellular Na concentration ($[Na]_o$)). Experiments with voltage-sensitive fluorescent dyes (Blaustein & Goldring, 1975) and lipid-soluble tracer cations (e.g. Ramos, Grollman, Lazo, Dyer, Habig, Hardegree, Kaback & Kohn, 1979) indicate that synaptosomes have membrane potentials that behave like K diffusion potentials. We reasoned that it should be possible to evaluate the properties of voltage-sensitive K channels in presynaptic terminals by comparing K (or Rb) efflux from controls and from synaptosomes exposed to depolarizing conditions (e.g. elevated $[K]_o$). As noted in the Introduction, we used ^{86}Rb as a tracer for K in most experiments. Arner & Stallcup (1981) have employed comparable methods in their studies of K channels in cultured neuroblastoma cells.

In an earlier study (Blaustein & Goldring, 1975), about two-thirds of the ^{42}K accumulated by synaptosomes was observed to be ouabain sensitive. As illustrated in Fig. 1, similar results are obtained with ^{86}Rb. In this experiment, about three-fourths of the ^{86}Rb accumulated during a 30 min incubation was ouabain sensitive; the quantitative difference between this and our earlier results probably reflects recent improvement in our experimental methods. If K and Rb accumulation by synaptosomes are identical (Blaustein & Goldring, 1975; and M. P. Blaustein, unpublished data), the data in Fig. 1 can be used to calculate the ouabain-sensitive K concentration in the synaptosomes. The value obtained is 380 nmol K/mg synaptosome protein. If the intraterminal volume is 3·5 μl/mg protein (Blaustein & Goldring, 1975) and K (or Rb) is distributed uniformly, this ouabain-sensitive ^{86}Rb accumulation corresponds to a $[K]_i$ of about 110 mM. We used the ouabain-sensitive uptake of ^{86}Rb

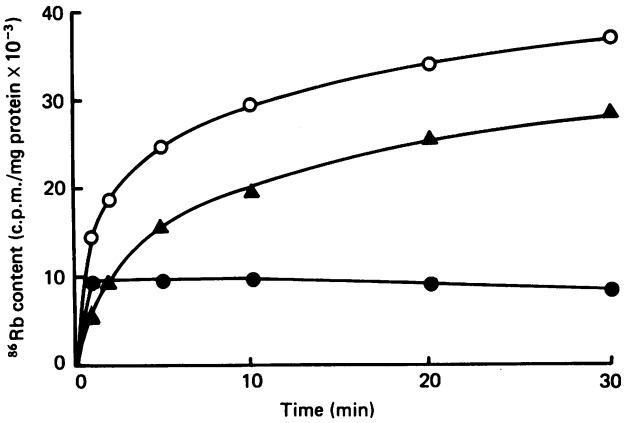

Fig. 1. Time course of ^{86}Rb uptake by synaptosomes in the absence and presence of ouabain. Aliquots (10 μl) of equilibrated synaptosome suspensions were added to 10 μl 145 mM-Na + 5 mM-K solutions containing tracer ^{86}Rb; some solutions (●) also contained ouabain (concentration after dilution = 1 mM). The suspension was incubated at 30 °C. At the indicated times ^{86}Rb uptake was terminated by dilution with 4 ml ice-cold 5 mM-K + 120 mM-Na + 25 mM-TEA (tetraethylammonium chloride). The samples were filtered and washed as described in Methods. Total ^{86}Rb uptake (○); ouabain-sensitive ^{86}Rb uptake (▲).

as an estimate of $[K]_i$ because this parameter should reflect the K content of metabolically competent nerve terminals only.

The time course of ^{86}Rb efflux

In order to measure the time course of Rb efflux from ^{86}Rb-loaded nerve terminals, a manual rapid-quench method was developed. An aliquot of ^{86}Rb-loaded synaptosomes was placed on a glass fibre filter, was washed free of extracellular ^{86}Rb, and was then 'pulsed' with an efflux solution of desired composition. Efflux was rapidly terminated by the addition of quench solution containing high concentrations of the K channel blockers TEA and TBA, followed immediately by application of suction to the filters. By collecting the filtrate, and subjecting both filter and filtrate to liquid scintillation counting, it was possible to determine the fraction (or percentage) of previously accumulated ^{86}Rb that was released. This method can resolve ^{86}Rb effluxes as small as 0·2–0·3 %/s of the total tracer accumulated by the synaptosome with an error of ± 3 % or less.

When experiments of this type were carried out, the pattern of release illustrated in Fig. 2 was seen. Efflux into PSS containing 5 mM-K (i.e. the slope of the least-squares regression line drawn through the circles) was about 0·4 %/s. This component of efflux ('*R*', Fig. 2) was unaffected by the presence or absence of Ca in the 5 mM-K medium. Component *R* probably represents ^{86}Rb efflux mediated by the system(s) responsible for the 'resting' K permeability of the nerve terminals (Blaustein & Goldring, 1975).

Extrapolation of component *R* to the ordinate ('zero time') revealed the presence of another component of efflux (component '*B*', Fig. 2). However, most of component *B* probably also represents ^{86}Rb efflux through the resting K permeability mechanism(s) because the synaptosomes were exposed to residual 5 mM-K wash solution

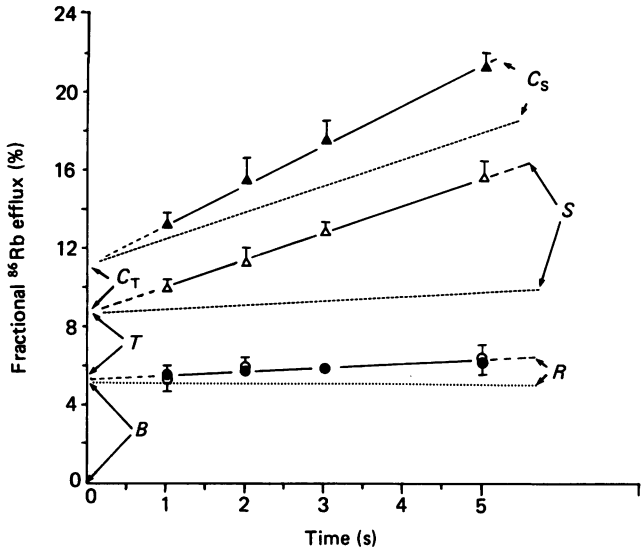

Fig. 2. Time course of ^{86}Rb efflux from ^{86}Rb-loaded synaptosomes showing the effects of Ca and elevated $[K]_o$ (rapid-quench method). 50 μl of an ^{86}Rb-loaded synaptosome suspension was trapped on a 25 mm glass fibre filter, and was washed five times with PSS; the measurement of ^{86}Rb efflux was initiated by addition of PSS with (in mM): 5 K, 0 Ca (○); 5 K, 1 Ca (●); 100 K, 0 Ca (△); or 100 K, 1 Ca (▲). Efflux was terminated by the addition of 2 ml quench solution (see Methods) followed by collection of the filtrate. The ordinate indicates the percentage of accumulated ^{86}Rb released at each time point. The lower and upper dashed lines represent the slopes of ^{86}Rb efflux in nominally Ca-free, 5 mM-K and 100 mM-K solutions, respectively. Each symbol indicates the mean ± S.E. of four determinations. The various components of the ^{86}Rb efflux, *B*, *R*, *T*, *S*, C_T and C_S, are described in the text.

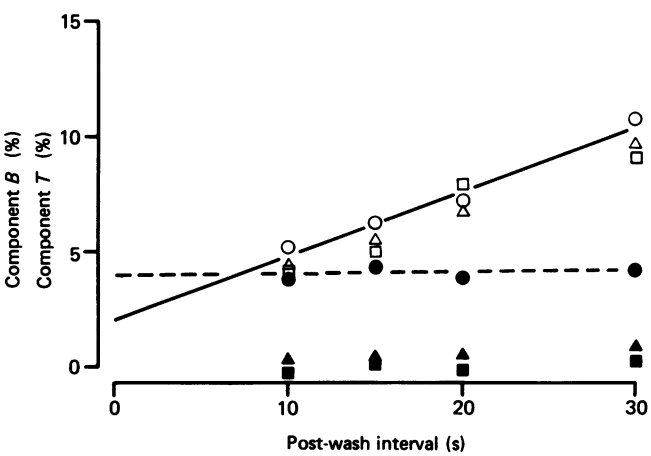

Fig. 3. The effects of the post-wash delay on components *B* and *T*. The interval between the final wash–suction cycle and the introduction of the reaction medium (absissa) is plotted *versus* the magnitude of component *B* (open symbols) or component *T* (filled symbols). The magnitudes of *B* and *T* were determined as in Fig. 2. The circles indicate data obtained in the absence of drugs. In some instances, 1 mM-4-AP (triangles) or 10 mM-TEA (squares) was included in the wash and reaction solutions. The values are the means of six determinations. The slope of the continuous line (component *B*, control was 0·3 %/s, similar to the rate of efflux through component *R*, determined in other experiments (see text).

during the 15–20 s interval (the time was constant within each experiment) between the end of the final wash cycle and the introduction of the reaction (efflux) solution. Evidence supporting this conclusion is presented in Fig. 3 (below).

The mean rate constant for ^{86}Rb efflux in 5 mM-K (i.e. 'resting' efflux or component R) was $0{\cdot}004 \pm 0{\cdot}0003$/sec ($n = 12$). This value can be used to calculate the K (Rb) efflux and resting K (Rb) permeability, P_K (Carmeliet, Horres, Lieberman & Vereeke, 1976), with the following assumptions: (a) intracellular K = 110 mM (see above); (b) the synaptosomal surface area-to-volume ratio is 8×10^4/cm (Blaustein, 1975); and (c) ^{86}Rb traverses the plasmalemma through the resting K permeability mechanism(s) as well as K itself (see below). With these limitations, the resting Rb and K permeabilities were calculated to be $1{\cdot}9 \times 10^{-7}$ and $2{\cdot}4 \times 10^{-7}$ cm/s, respectively, in good agreement with other excitable tissues (Carmeliet *et al.* 1976). The resting Rb and K effluxes were 5·5 and 7·2 pmol/cm^2 . s, respectively.

Exposure of the synaptosomes to nominally Ca-free PSS containing 100 mM-K markedly stimulated ^{86}Rb efflux (△, Fig. 2); the release occurred in two phases. Between 1 and 5 s, 100 mM-K-evoked ^{86}Rb release appeared to be linear, and was about 6–8 times more rapid than release into 5 mM-K solutions. This linear component of efflux did not extrapolate to the 'ordinate' value for efflux seen under 'resting' (5 mM-K) conditions; the extrapolation exposes an additional component of efflux (component 'T', Fig. 2). Thus, exposure of the synaptosomes to Ca-free K-rich media may activate two separate K effluxes: a fast, transient K efflux, T, (which terminates within 1 s), and a slower, steady-state K efflux which is maintained over 1–5 s (component 'S').

Addition of 1 mM-Ca to PSS containing 5 mM-K had no effect. However, when Ca was added to K-rich media, a greater ^{86}Rb efflux was seen than with elevated K and no added Ca (Fig. 2, ▲). This Ca-dependent increment in ^{86}Rb efflux in K-rich media, could also be divided into two components: a small, somewhat variable slow component, corresponding to the increment in ^{86}Rb efflux between 1 and 5 s ('C_S' in Fig. 2), and a fast transient component, corresponding to the extra Ca-dependent ^{86}Rb efflux when the efflux is extrapolated back to 'zero time' ('C_T' in Fig. 2). Since Ca is ineffective in 5 mM-K, but requires depolarizing conditions to act, Ca probably exerts its action by entering the synaptosomes through voltage-regulated Ca channels. This should lead to an increase in the internal free Ca concentration, thereby activating ^{86}Rb efflux. Thus, our observations are consistent with the presence of Ca-activated K channels in the synaptosomes; this possibility is addressed in greater detail in the accompanying article (Bartschat & Blaustein, 1985).

In control experiments, ouabain-treated or glucose-depleted synaptosomes were allowed to accumulate ^{86}Rb, and were then exposed to efflux media as described above. Under these conditions, high $[K]_o$-evoked ^{86}Rb efflux was not seen (data not shown). These results indicate that only metabolically active synaptosomes contributed significantly to the pattern of ^{86}Rb efflux shown in Fig. 2.

Fig. 3 shows data from experiments designed to test whether the magnitudes of components B and T were a function of the time interval between the final synaptosome wash cycle and the beginning of the efflux measurement period. These data indicate that the magnitude of B (○) increased linearly as the interval between the final wash and the introduction of the efflux solution was lengthened, whereas component T (●) remained virtually unchanged. The continuous line in Fig. 3 has

a slope of 0·3 %/s, which is comparable to the magnitude of component R ($\sim$ 0·4 %/s, see above). The implication is that most of component B can be attributed to ^{86}Rb efflux through the resting K permeability mechanism(s); this is not surprising, since the terminals were exposed to residual 5 mM-K wash solution during the post-wash interval. Extrapolation of B to the ordinate suggests that a small fraction of this component may be independent of the wash cycle–efflux measurement interval; this may be a result of incomplete removal of extracellular ^{86}Rb during the wash, or it may represent lysis of a small fraction of the trapped synaptosomes.

The K channel blockers 4-AP (1 mM) and TEA (10 mM) were also tested for possible effects on components T and B. These agents had very little effect on B, but abolished component T (Fig. 3). These results provide additional evidence that components B and T involve separate K efflux mechanism(s).

Effects of veratridine plus sea anemone toxin on ^{86}Rb efflux

Many excitable cells have voltage-regulated K channels that open when the cells are depolarized, and that are permeable to Rb as well as K (e.g. Hille, 1975; Gorman *et al.* 1982). Therefore, the increased ^{86}Rb efflux that occurs when synaptosomes are presumably depolarized by raising $[K]_o$ (cf. Blaustein & Goldring, 1975) could be attributed to ^{86}Rb exit through voltage-regulated K channels. The fact that the K-stimulated ^{86}Rb efflux can be blocked by a variety of selective K channel blockers such as TEA, aminopyridines and quinine sulphate (Fig. 3; also see below and the accompanying paper), supports the view that the effect of external K is due to its depolarizing action.

More direct evidence that depolarization of the terminals, *per se*, triggers the ^{86}Rb efflux comes from experiments with veratridine plus sea anemone (*Anthopleura*) toxin, AxTX. These two agents act synergistically to open TTX-sensitive Na channels in synaptosomes (Kreuger & Blaustein, 1980). By opening Na channels, they should make the resting membrane potential approach the Na equilibrium potential, E_{Na}. When the medium contains 100 or 145 mM-Na, and $[Na]_o \gg [Na]_i$ the synaptosomes should depolarize in the presence of veratridine and AxTX; but when the external medium contains only 3 mM-Na, and $[Na]_o < [Na]_i$, there should be little depolarization (see Blaustein & Goldring, 1975; Krueger & Blaustein, 1980).

Fig. 4*A* and *B* show data from an experiment in which we tested the effects of veratridine *plus* AxTX on ^{86}Rb efflux from ^{86}Rb-loaded synaptosomes. ^{86}Rb efflux was quite low when the synaptosomes were incubated in media containing 3 mM-Na without the toxins, or in media with 145 mM-Na *plus* veratridine, AxTX *and* TTX. In the latter case the Na channels should have been blocked. When the Na channels are opened in the presence of 3 mM-Na (with veratridine and AxTX but *not* TTX), so that little or no depolarization would be expected (B. K. Krueger, personal communication), there is, nevertheless, a small but significant stimulation of ^{86}Rb efflux. The fact that this efflux is reduced in the presence of TTX, suggests that it involves Rb movement through TTX-sensitive Na channels. Indeed, other investigators have shown that Na channels opened by veratridine are significantly more permeable to Rb and K than are normal Na channels (Lazdunski, Balerna, Barhanin, Chicheportiche, Fosset, Frelin, Jacques, Lombet, Pouyssegur, Renaud, Romey, Schweitz & Vincent, 1980).

When the ^{86}Rb-loaded synaptosomes are treated with veratridine *plus* AxTX, and $[Na]_o$ is increased from 3 to 145 mM so that the terminals should be depolarized, there is a further increment in ^{86}Rb efflux comparable to that seen with K depolarization. This extra increment was partially blocked by 2 mM-4-AP or 10 mM-TEA (Fig. 4*B*). These data support the view that ^{86}Rb efflux is increased by depolarization, and that this ^{86}Rb efflux is mediated by K channels.

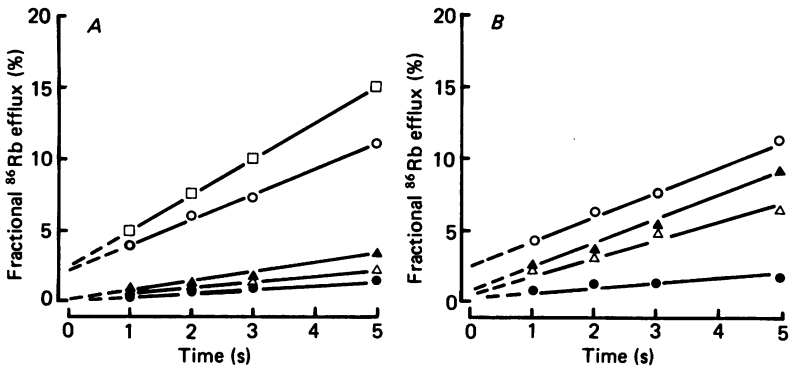

Fig. 4. Effects of veratridine and sea anemone toxin (AxTX) on ^{86}Rb efflux from synaptosomes. *A*, ^{86}Rb-loaded synaptosomes were trapped on glass fibre filters and were washed with Ca-free PSS containing (mM): KCl, 5; NaCl, 3; and choline chloride, 142. Some of the washed synaptosomes were then exposed for varying lengths of time to an identical solution containing: no additions (●), 50 μM-veratridine and 1 μM-AxTX (▲), or 50 μM-veratridine, 1 μM-AxTX, and 10 μM-tetrodotoxin (TTX; △). Other washed synaptosomes were exposed to 5 mM-K, 145 mM-Na PSS containing veratridine and AxTX (○), or were exposed to 100 mM-KCl and 50 mM-NaCl (□). *B*, synaptosomes trapped on glass fibre filters were washed with PSS as in *A*, and were then exposed to PSS containing 5 mM-KCl, 145 mM-NaCl, 50 μM-veratridine, 1 μM-AxTX and no additions (○), 2 mM-4-AP (▲), 10 mM-TEA (△), or 10 μM-TTX (●). When the efflux media contained pharmacological agents, the same agents were also added to the wash solution. The values are the means ± S.E. of mean of four determinations. For convenience, efflux component *B* (see Fig. 2) has been subtracted and is not shown.

Comparison of ^{86}Rb and ^{42}K efflux

The assumption that ^{86}Rb efflux can be used to study the K conductances of the nerve terminals is justified by electrophysiological observations that Rb is 0·70–0·95 times as permeant through various types of K channels as K itself (Hille, 1975; Reuter & Stevens, 1980; Gorman *et al.* 1982). The experiment of Fig. 5 is a direct test of this assumption. Using dual-labelling techniques, ^{86}Rb and ^{42}K effluxes were studied in synaptosomes pre-loaded with both isotopes under 'resting' (i.e. 5 mM-K) and depolarizing conditions, both in the presence and absence of 1 mM-Ca. There was good qualitative agreement between these effluxes. Table 1 shows the magnitudes of the various components of the ^{86}Rb efflux and comparable ^{42}K efflux (cf. Fig. 2 and related text), as well as the ^{86}Rb:^{42}K efflux (permeability) ratio for each of the components. In general, Rb was 0·6–0·8 times as permeant as K. The exeption was component C_S: in this case Rb was about 1·3 times as permeant as K; however, due to the small size of component C_S, it is uncertain whether this represents a real difference in the

properties of the two Ca-dependent components of efflux, or is simply due to experimental error (see Bartschat & Blaustein, 1985). Taken together, the results suggest that the ^{86}Rb and ^{42}K effluxes are mediated by similar, if not identical pathways.

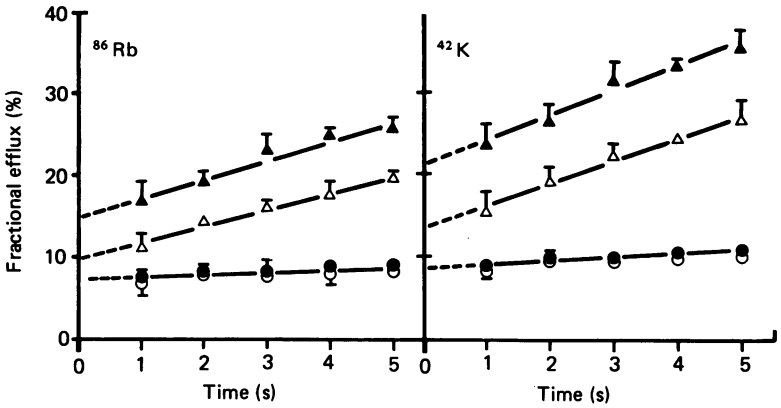

Fig. 5. Time course of ^{86}Rb and ^{42}K efflux in the same synaptosome preparation. Synaptosomes, previously loaded with both ^{86}Rb and ^{42}K, were treated as in the experiment of Fig. 2. The radioactivity in the filters and filtrates ($^{86}Rb + ^{42}K$) was determined by liquid scintillation techniques immediately after the experiment. The ^{42}K was allowed to decay (> 15 half-lives) and the samples were then recounted for ^{86}Rb. The differences in radioactivity between the two determinations was attributed to ^{42}K. The values represent the means of four determinations, ±S.E. of mean: (○) 5 mM-K, 0 Ca; (●) 5 mM-K, 1 mM-Ca; (△) 100 mM-K, 0 Ca; and (▲) 100 mM-K, 1 mM-Ca.

TABLE 1. Comparison of ^{86}Rb and ^{42}K effluxes

Efflux component*	Efflux (as % or %/s)† ^{86}Rb	Efflux (as % or %/s)† ^{42}K	Efflux ratio $^{86}Rb:^{42}K$
R	0·35 %/s	0·45 %/s	0·78
T	2·71 %	4·70 %	0·58
S	2·01 %/s	2·80 %/s	0·72
C_T	4·37 %	7·65 %	0·57
C_S	0·32 %/s	0·24 %/s	1·33

* See Fig. 2 and related text for nomenclature.

† Fluxes for components T and C_T were extrapolated to 'zero time' and are expressed as percentages (see text).

The effects of the membrane potential on ^{86}Rb efflux

The relationship between $[K]_0$ and ^{86}Rb efflux. As indicated above, it is not K, *per se*, but rather depolarization of the terminals, that stimulates the ^{86}Rb efflux. Therefore, in view of the well-established relationship between $[K]_0$ and membrane potential in many cells, including synaptosomes (Blaustein & Goldring, 1975; Ramos *et al.* 1979), we examined the dependence of ^{86}Rb efflux in Ca-free media on $[K]_0$ (Fig. 6, *A–C*). ^{86}Rb efflux was increased when $[K]_0$ was elevated above 5 mM, and was graded with increasing K concentrations (Fig. 6*A*). Note also that both the fast (T) and slow (S) components of the K-stimulated efflux were increased by this manoeuvre.

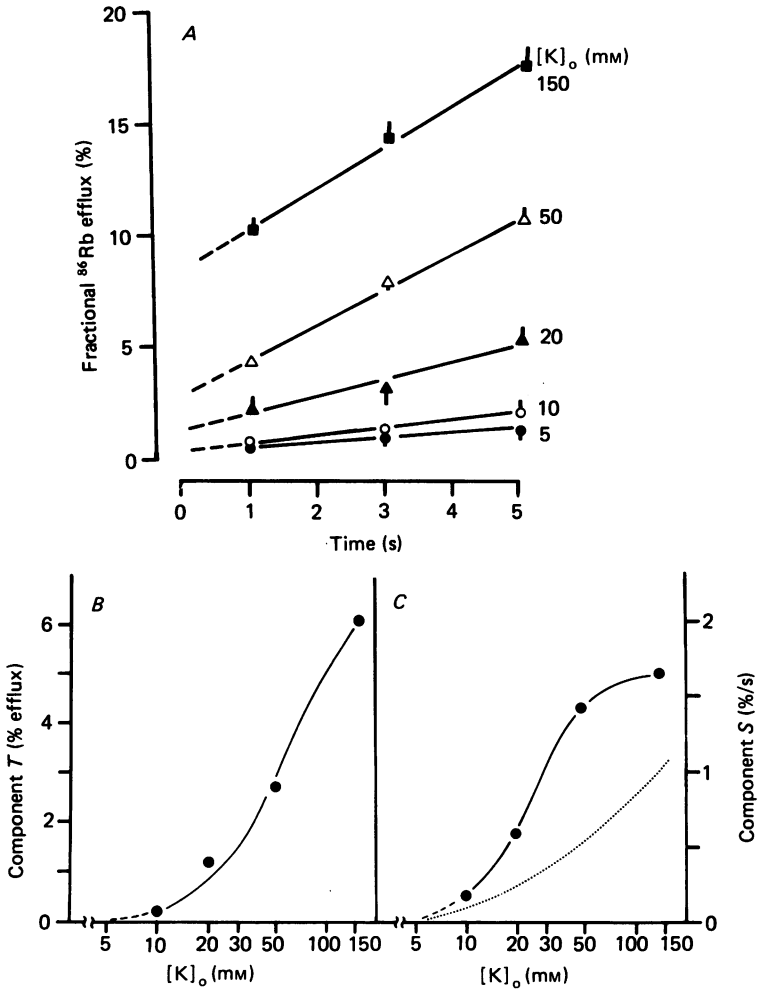

Fig. 6. Effect of $[K]_o$ on ^{86}Rb efflux from synaptosomes. *A*, ^{86}Rb-loaded synaptosomes trapped on glass fibre filters were exposed to the $[K]_o$ indicated beside each line for 1–5 s. The values are the means of four determinations ± S.E. of mean. The lines were fitted by least-squares analysis. *B*, the data from *A* were used to plot the magnitude of the extrapolated 'zero-time' intercept (component *T*) on the ordinate as a function of $[K]_o$. The line was drawn by eye. *C*, the magnitude of component *S*, determined from the slopes of the least-squares fit lines from *A*, minus the slope of ^{86}Rb efflux in 5 mM-K (component *R*), is plotted as a function of $[K]_o$. The dotted line represents the increase in efflux through component *S* predicted by electrodiffusion effects, assuming no voltage-dependent activation of component *R* (see text for details). The values are the means of four determinations. In this Figure, and Figs. 7–10, component *B* (see Fig. 2) has been subtracted and is not shown.

The relationship between component *T* (cf. Fig. 2) and $[K]_o$ is illustrated in Fig. 6*B*, whilst that between component *S* (cf. Fig. 2) and $[K]_o$ is illustrated in Fig. 6*C*. Inspection of these curves shows that the relationships between $[K]_o$ and the magnitudes of these two components of ^{86}Rb efflux are different. This may reflect a real difference in the voltage dependence of these components. However, this

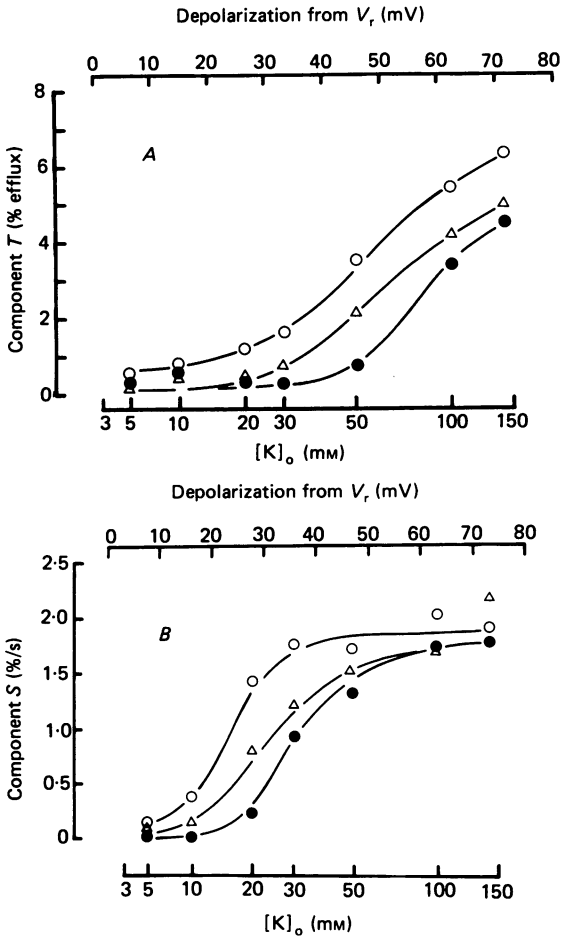

Fig. 7. Effect of Mg on ^{86}Rb efflux synaptosomes. A protocol similiar to that in Fig. 6 was employed, using $[K]_o$ of 3–150 mM and $[Mg]_o$ of 2 mM (○), 5 mM (△), or 15 mM (●). When less than 15 mM-Mg was used, appropriate amounts of sucrose were added to maintain osmolarity. *A*, the magnitude of component *T*, determined from the extrapolated 'zero-time' values is plotted as a function of $[K]_o$ for each $[Mg]_o$. *B*, the magnitude of component *S*, determined from the slope of ^{86}Rb efflux between 1 and 5 s is plotted as a function of $[K]_o$ for each $[Mg]_o$. The upper abscissa scales indicate the expected depolarizations from the 'resting' membrane potential, V_r, in the K-rich solutions (cf. Blaustein & Goldring, 1975). The curves were drawn by eye. The values are the means of six determinations.

interpretation is complicated by the contribution of the 'resting' K permeability to component *S* (see below) and by the difference in the way *S* and *T* are measured (slope *versus* difference in intercepts, respectively; cf. Fig. 2).

The stimulation of ^{86}Rb efflux by K-rich media is a result of the increased $[K]_o$ (and probably a consequence of depolarization), and is not due to the reduction of $[Na]_o$: partial or complete replacement of Na by choline did not stimulate ^{86}Rb efflux (data not shown, but see Fig. 4). Therefore, in most experiments when $[K]_o$ was increased, K replaced Na, mole-for-mole, with Na + K = 150 mM.

A noteworthy point is that depolarization of the nerve terminals *per se* (e.g. by increasing $[K]_o$) would be expected to increase the ^{86}Rb efflux even without increasing P_K, if Rb fluxes obey the independence principle, because depolarization increases the outward driving force on Rb (e.g. Carmeliet *et al.* 1976). Accordingly, we calculated the 'resting' permeability to Rb in 5 mM-K solution (see above), and used this value to estimate the expected ^{86}Rb efflux at various membrane potentials (or $[K]_o$ values), assuming that P_K (or P_{Rb}; Rb permeability) did not change, and that $[K]_i$ was 110 mM (see above) and P_K/P_{Na} (Na permeability) was 20 (Blaustein & Goldring, 1975); this expected efflux is indicated by the dotted line in Fig. 6*C*. The changes in driving force can account for a large fraction (but not all) of the increased ^{86}Rb efflux observed between 1 and 5 s in K-rich media. Thus, whilst component *T* of ^{86}Rb efflux is most likely a true voltage-regulated K conductance, the slower component, *S*, is complex, and appears to include both a non-inactivating (or slowly inactivating) voltage-regulated K conductance and an electrodiffusion contribution from the 'resting' K permeability. The pharmacological data described below appear to support this view.

The influence of Mg on K-stimulated ^{86}Rb efflux. A number of K conductances are sensitive to neutralization of surface charges by various cations (e.g. Frankenhaeuser & Hodgkin, 1957). For example, Mg, by screening membrane surface charges in the vicinity of voltage-regulated K channels, can shift the activation curve for the voltage-gated systems to more positive voltages along the voltage ($[K]_o$) axis. A larger depolarization (or higher $[K]_o$) is therefore needed to activate the K conductance mechanism in high extracellular Mg concentration ($[Mg]_o$). A clear Mg-induced voltage shift for the activation of ^{86}Rb efflux would be consistent with a voltage-gated conductance. Fig. 7 shows data from an experiment designed to test whether altering $[Mg]_o$ influences high K-evoked ^{86}Rb efflux. Both the transient (Fig. 7*A*) and the slow components of efflux (Fig. 7*B*) were affected, so that a higher $[K]_o$ was required to promote the same level of efflux when the $[Mg]_o$ was raised from 2 to 5 or 15 mM. This was not an osmotic effect because the solutions were maintained isosmotic by the addition of appropriate amounts of sucrose to the solutions that contained only 2 or 5 mM-Mg. Increasing $[Mg]_o$ shifted the $[K]_o$ *versus* ^{86}Rb efflux curves to the right for both the slow and fast components; but, whilst the peak flux was not altered in the case of the slow component, the maximal conductance through the fast component ('*T*') decreased as $[Mg]_o$ was increased. This may be due to a direct depression of this K conductance by Mg or, alternatively, it may be a consequence of alterations in voltage-dependent activation and/or inactivation rates. The present methods do not allow us to test this latter possibility. However, it does seem that efflux through the fast pathway, and at least some through the slow pathway, is regulated by voltage-gated conductance mechanisms.

The effects of various K channel blockers on K-stimulated ^{86}Rb efflux

Tetraalkylammonium ions. In the preceding sections we provided evidence that synaptosomes contain at least two classes of Ca-independent, voltage-regulated K channels. It is relevant to determine how potent various inhibitors are in blocking these K channels. TEA is known to be a selective blocker of K channels in many excitable cells (Stanfield, 1983). In squid axons TEA acts at the inner surface of the

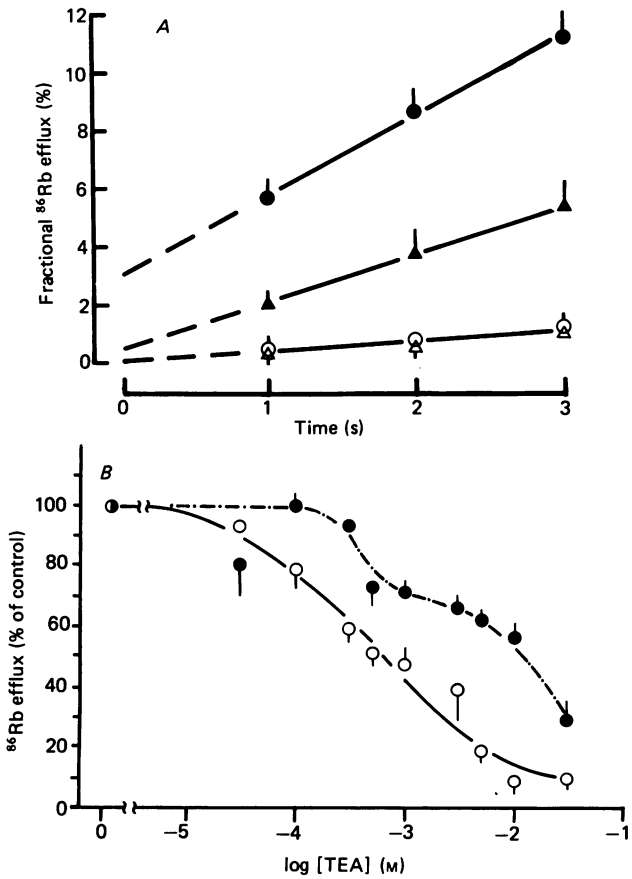

Fig. 8. Effect of TEA on ^{86}Rb efflux. *A*, a protocol similar to the experiment of Fig. 3 was employed, except that these solutions contained no added Ca. TEA (10 mM) was added to the appropriate wash solution to faclitate equilibration with synaptosomes. 5 mM-K, 0 TEA (○), 100 mM-K, 0 TEA (●), 5 mM-K + 10 mM-TEA (△), 100 mM-K + 10 mM-TEA (▲). Note that component *T* is inhibited to a greater degree than component *S*. *B*, dose dependence of the inhibition of component *T* (○) and component *S* (●) by TEA. Symbols indicate the means ± S.E. of mean of six determinations.

plasma membrane, and not at the outer surface (cf. Armstrong, 1977). However, in other cells, such as frog myelinated axons and molluscan neurones, TEA is effective whether applied internally or externally (e.g. Koppenhoffer & Vogel, 1969; Hermann & Gorman, 1981*b*), although the actions at the two surfaces are quantitively different (Armstrong & Hille, 1972). Fig. 8*A* shows that external application of TEA inhibits K-stimulated ^{86}Rb efflux effectively in the synaptosome preparation; although not illustrated here, nearly complete inhibition occurs with 50–60 mM-TEA. The implication is that TEA, at sufficiently high concentration, can block all of the components of K-promoted ^{86}Rb (and K) efflux as well as the 'resting' K permeability in synaptosomes.

Fig. 8*A* suggests that, at 10 mM-TEA, the fast component of efflux is totally inhibited, whilst the slow component is inhibited only by about 50%. In order to

examine this differential sensitivity further, we tested the effects of various concentrations of TEA on both the slow (*S*) and fast (*T*) components of ^{86}Rb efflux (Fig. 8*B*). Component *T* was very sensitive to external TEA: it was approximately 50% inhibited at 0·6 mM. Furthermore, the dose–response curve for this component appears to be monophasic. In contrast, the dose–response curve for component *S* is not a simple monophasic relationship. The latter observations fit the idea that (at least) two K conductances, with different sensitivities to TEA, contribute to component *S*.

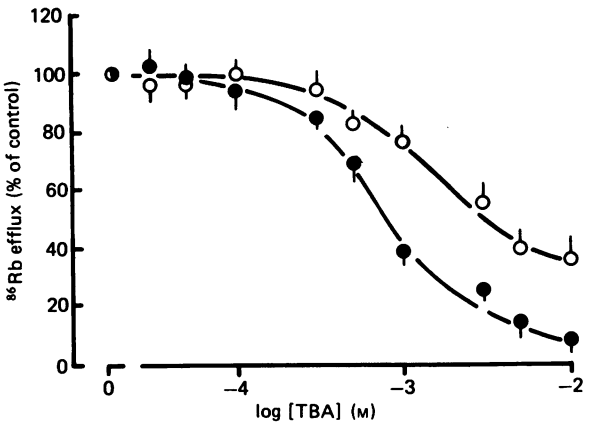

Fig. 9. Effect of TBA on ^{86}Rb efflux. See Fig. 8 legend for details of the experimental protocol. Symbols indicate the means±S.E. of mean of six determinations. Component *T* (●); component *S* (○).

A number of other tetraalkylammonium ions also block K channels (e.g. Armstrong, 1977; French & Shoukimas, 1981). We tested the effect of TBA ions on ^{86}Rb efflux from synaptosomes. The dose–response curves in Fig. 9 indicate that this agent, too, can block virtually all of the K-stimulated ^{86}Rb efflux. Again, the fast component was more sensitive (50% inhibition at 0·8–1·0 mM) than the slow component, and appeared to be inhibited monophasically. As with TEA, the inhibition of component *S* by TBA is probably more complex because the broad dose–response curve does not correspond to a simple monophasic relationship.

Aminopyridines. Several aminopyridines, including 4-AP and 3,4-DAP, block voltage-regulated K channels in a variety of neuronal preparations (e.g. Llinas, Walton & Bohr, 1976; Thompson, 1977; Kirsch & Narahasi, 1978; and see Hermann & Gorman, 1981*a*). Fig. 10*A* illustrates the effect of 1 mM-4-AP on the time course of ^{86}Rb efflux: note that its primary effect is the virtually complete block of component *T*. Fig. 10*B* shows a dose–response curve for this agent on the fast and slow components of ^{86}Rb efflux. The fast component is very sensitive to this drug (50% inhibition at 0·1–0·2 mM), and is monophasically blocked. As is the case with TEA, the slow component is much less sensitive to 4-AP; the broad dose–response curve suggests that two or more permeability mechanisms, with different sensitivities to block by 4-AP, may contribute to component *S*. The latter observation is consistent with the aforementioned evidence that both a 'resting' K permeability and a voltage-regulated K efflux mechanism may contribute to component *S*.

In important control experiments (data not shown), we tested the effects of 4-AP

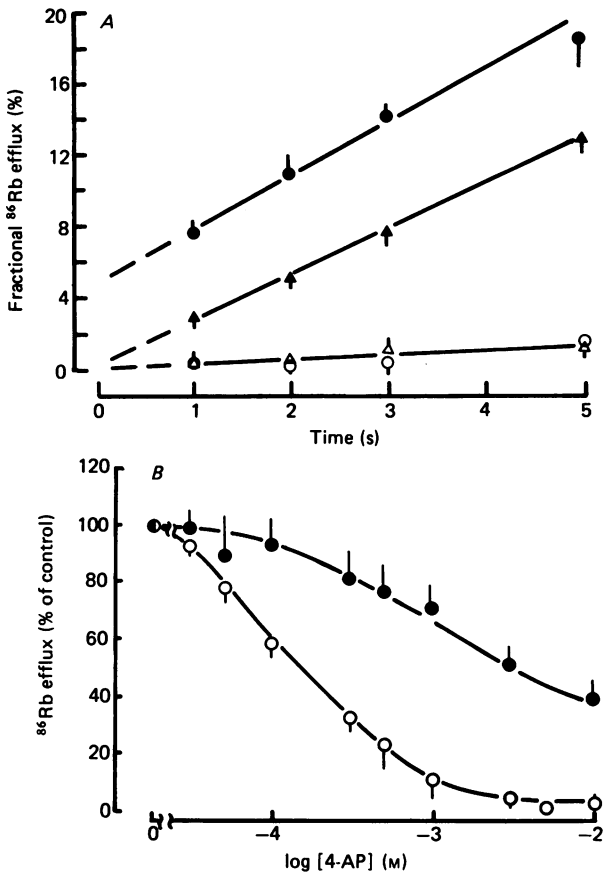

Fig. 10. Effect of 4-AP on ^{86}Rb efflux. *A*, a protocol similar to the experiment of Fig. 8*A* was used except where appropriate, 1 mM-4-AP was added to the wash and efflux solutions. 5 mM-K, 0 4-AP (○), 5 mM-K + 1 mM-4-AP (△), 100 mM-K, 0 4-AP (●), 100 mM-K + 1 mM-4-AP (▲). *B*, dose–response curve showing the percentage inhibition of component *T* (○) and component *S* (●) as a function of the 4-AP concentration. Each symbol indicates the mean of six determinations ± S.E. of mean.

and TEA on the fluorescence intensity of synaptosome suspensions incubated with a voltage-sensitive fluorescent dye that can be used to monitor changes in synaptosome membrane potentials (Blaustein & Goldring, 1975). Neither 2 mM-4-AP nor 10 mM-TEA significantly affected the increment in fluorescence intensity associated with an increase in $[K]_o$ from 5 to 75 mM. This implies that, even in the presence of these K channel blockers, the membrane potential was close to that predicted from the equilibrium potential for K (cf. Blaustein & Goldring, 1975). This may also indicate that the 'resting' K permeability is relatively insensitive to TEA and 4-AP.

DISCUSSION

The properties of K channels can be studied in synaptosomes

The results described in this report support the conclusion that the isolated nerve terminals have functional K channels. We show that the efflux of Rb from ^{86}Rb-loaded

synaptosomes is increased when synaptosomes are incubated under depolarizing conditions, and this stimulated efflux is blocked by several K channel-selective blockers. ^{86}Rb was employed in the majority of these experiments because most cells transport K and Rb in a similar fashion, and ^{86}Rb is a much more convenient isotope to use than ^{42}K. Most important for our purposes is the fact that K channels in several types of neurones are nearly as permeable to Rb as to K; the Rb:K selectivity ratios for various types of K channels, in various neurones, is between 0·7 and 0·95 (e.g. Hille, 1975; Reuter & Stevens, 1980; Gorman *et al.* 1982). Our experiments in which both ^{42}K and ^{86}Rb efflux were examined indicate that K and Rb are conducted in a qualitatively similar manner, and that Rb:K selectivity ratios are probably between 0·6 and 0·8 (but see Table 1).

Conventional electrophysiological methods cannot be employed to study ionic channels in these mammalian presynaptic terminals. However, this shortcoming is compensated by the fact that we can measure the unidirectional fluxes of single ionic species, rather than net ionic currents. With the techniques employed here, it is possible to determine the relative permeability to the specific ionic species of interest. This may simplify the study of some of the physiological and pharmacological properties of ionic channels. Furthermore, new types of information about ion channel selectivity and permeability may be obtained that are not available from conventional electrophysiological studies (cf. Nachshen & Blaustein, 1980, 1982).

It is becoming increasingly clear that there are a large variety of Ca and K conductance mechanisms with varying physiological and pharmacological properties. Different cells may, for example, have different K conductance mechanisms, and some cells may have more than one type of K channel. Moreover, certain channels may be confined to the plasma membrane in a limited portion of the cell; an example is the localization of Ca channels close to the terminal in the presynaptic neurone of the squid giant synapse (Katz & Miledi, 1969; and see Grinvald & Farber, 1981). Thus, the electrophysiological properties of cells (or parts of cells) *must be studied in the specific preparations of interest* if we hope to learn how these specific cells function. As discussed below, information about the ion conductance mechanisms in the presynaptic terminals may help us to understand synaptic plasticity, synaptic pharmacology, and perhaps even behavioural physiology and pathophysiology.

Synaptosome preparations also have several drawbacks that must be kept in mind. (i) The synaptosomes obtained from rat forebrain (as in the present study) are very heterogeneous; they include cholinergic, dopaminergic, GABAergic, etc. terminals. (ii) Some of the nerve terminals may be non-functional (cf. Fried & Blaustein, 1978); however, as discussed above, only metabolically active terminals appear to contribute to depolarizatoion-evoked ^{86}Rb efflux. (iii) All properties may not be exactly 'normal'; for example, many of the terminals may be slightly depolarized, so that the TTX-sensitive Na conductance mechanism is inactivated (Krueger & Blaustein, 1980). (Recently, B. K. Krueger (personal communication) observed that, in some preparations, the K-stimulated, TTX-sensitive Na influx curve could be shifted along the $[K]_o$ axis with high $[Mg]_o$ (see Fig. 7 of this report). This is presumably, a reflexion of an apparent shift in the voltage dependence of the Na conductance.) (iv) Finally, the slow time scale on which our experiments are performed (seconds rather than milliseconds) must be reckoned with. However, quench-flow methods have been employed with synaptosome preparations to make Ca influx measurements on a time

scale of tens of milliseconds (Nachshen, 1982); it may be possible to adapt similar methods for efflux studies such as those of ^{86}Rb efflux.

Synaptosomes have at least four classes of functional K channels or permeability mechanisms

The ^{86}Rb efflux from ^{86}Rb-loaded synaptosomes can be separated into at least four physiologically and pharmacologically distinct components. (i) There is a slow, sustained efflux of ^{86}Rb from synaptosomes incubated in standard PSS containing 5 mM-K (component '*R*'). (ii) Under depolarizing conditions in Ca-free media, two voltage-regulated components of ^{86}Rb efflux are observed: one appears to inactivate within 1 s ('*T*'), whilst the other inactivates slowly, if at all ('*S*'). (iii) There is also a component of ^{86}Rb efflux that appears to be activated by Ca entry (component '*C*') mediated either by depolarization-regulated Ca channels or by the introduction of the Ca-selective ionophore, A23187 (Bartschat & Blaustein, 1985). As discussed below, each of these components of ^{86}Rb efflux may be the expression of a particular class of K channels or permeability mechanisms known to be present in various types of neurones.

Resting K permeability. Virtually all nerve cells in higher animals have resting membrane potentials that, to a first approximation, behave like K diffusion potentials, and this type of behaviour has been demonstrated in rat brain synaptosomes (Blaustein & Goldring, 1975; Ramos *et al.* 1979). Therefore, it is logical to expect that, under normal 'resting' (i.e. non-depolarizing) conditions, with $[K]_o = 5$ mM, synaptosomes should demonstrate a significant permeability to K (or Rb: cf. Blaustein & Goldring, 1975). Consequently, it seems reasonable to conclude that the slow, steady ^{86}Rb efflux observed when ^{86}Rb-loaded synaptosomes are incubated in 5 mM-K PSS (component *R*) can be attributed largely to Rb exit through the mechanism(s) that are responsible for the resting K permeability of the terminals.

Many types of neurones can be depolarized when the 'resting' K conductance is blocked; for example, by high concentrations of TEA (see Fig. 1 in Hermann & Gorman, 1981*b*). The evidence that the pharmacology of component *R* differs from that of components *T*, *S* and *C* (this article, Bartschat & Blaustein, 1984, 1985, and D. K. Bartschat & M. P. Blaustein, unpublished data), is consistent with the view that ^{86}Rb efflux component *R* corresponds to a specific K conductance permeability mechanism.

Voltage-regulated K channels. When ^{86}Rb-loaded synaptosomes are depolarized by raising $[K]_o$ in nominally Ca-free media, the ^{86}Rb efflux increases, and the magnitude of this efflux is graded with $[K]_o$. Experiments in which $[Na]_o$ was either held constant or varied indicate that reduction of $[Na]_o$ is not responsible for the K-stimulated ^{86}Rb efflux. Critical evidence that this stimulated efflux is a consequence of depolarization, and not simply due to a rise in $[K]_o$ *per se*, comes from experiments in which veratridine was used to depolarize the synaptosomes (Fig. 4 and related text). This view is further supported by the experiments which suggest that the apparent voltage (or $[K]_o$) dependence of the ^{86}Rb efflux can be altered by screening membrane surface charges with elevated $[Mg]_o$ (Fig. 7 and related text).

Many types of neurones have voltage-regulated K channels that are opened by depolarization and selectively blocked by agents such as tetraalkylamines and

aminopyridines (e.g. Hille, 1975). Our observations on the depolarization-stimulated ^{86}Rb efflux in synaptosomes can be readily explained by the presence of such voltage-regulated K channels in the plasmalemma of the nerve terminals. However, this simple interpretation is complicated by the complex time course of K-stimulated ^{86}Rb efflux (Fig. 2): the efflux during the initial 1 s was greater than during the subsequent 4 s. This implies there may be two (or more) voltage-regulated K channels in synaptosomes, one of which rapidly inactivates and is sensitive to 4-AP (component *T*) and one of which inactivates slowly, or not at all, and is much less sensitive to 4-AP (component *S*).

(*a*) *Inactivating K channels.* Many types of neurones have K channels that rapidly activate in response to depolarization and that subsequently display time-dependent inactivation: for example, frog sciatic nerve node of Ranvier (Schwarz & Vogel, 1971; Ilyin, Katina Lonskii, Makovsky & Polishchuck, 1980; and Dubois, 1983), and giant neurones in *Tritonia* (A current; see Thompson, 1977) and *Aplysia* (Hermann & Gorman, 1981*a*). Furthermore, the pharmacology of some types of fast, inactivating K currents (or A currents) compares favourably with component *T* in synaptosomes: 4-AP blocks the A current in frog node (apparent half-maximal inhibition, $K_I = 10^{-5}$ M; Dubois, 1983) and molluscan neurones ($K_I = 1{\cdot}5$ mM: Thompson, 1977; Hermann & Gorman, 1981*a*). However, the A current in bull-frog sympathetic neurones is insensitive to 1 mM-4-AP (Adams, Constanti & Brown, 1982). Also, while the A current in frog node is sensitive to low concentrations of TEA (Dubois, 1983), the A current in *Tritonia* (Thompson, 1977) is much less sensitive to TEA.

(*b*) *Non-inactivating* (*or slowly inactivating*) *K channels.* In addition to voltage-regulated inactivating K channels, many types of neurones possess voltage-regulated K channels that either do not inactivate or inactive very slowly with prolonged depolarization, as appears to be the case for component *S*. In the frog node, the non-inactivating channels are less sensitive than the inactivating channels to external TEA and 4-AP (Dubois, 1983). Conversely, in *Tritonia* neurones, the 'slow' K channels (K current) are selectively blocked by TEA (Thompson, 1977; but see Hermann & Gorman, 1981*b*, regarding *Aplysia* neurones).

Superficially, our data seem consistent with the data from frog node, in that component *S* was less sensitive to the aminopyridines and tetraalkylamines than was component *T*, even though most or all of *S* could be blocked by sufficiently high concentration of 4-AP or TEA. However, component *S* is more complex because, as noted above, the 'resting' K permeability as well as non-inactivating voltage-regulated K channels may contribute to this ^{86}Rb efflux component. Analysis of component *S* may be simplified by our recent observation (Bartschat & Blaustein, 1984) that the psychotomimetic agent phencyclidine (PCP) and related drugs selectively block part of component *S* in submicromolar to micromolar concentrations, whilst component *R* is unaffected by 100 μM-PCP. Thus PCP may be a useful tool for the characterization of the voltage-regulated, non-inactivating K channels that contribute to component *S*.

Ca-activated K channels. A large fraction of the depolarization-stimulated ^{86}Rb efflux in synaptosomes is dependent upon external Ca (Fig. 2). Here, too, there is a precedent: many types of neurones have K channels that are activated by a rise in intracellular Ca concentration ($[Ca]_i$) (cf. Meech, 1978). Synaptosomes have

depolarization-activated Ca channels (Blaustein, 1975; Nachshen & Blaustein, 1980). Thus a likely explanation for the external Ca-dependent K-stimulated ^{86}Rb efflux is that this Rb exits through channels that are activated by an increase in $[Ca]_i$ as a result of the depolarization-promoted entry of Ca. This is addressed in more detail in the accompanying article (Bartschat & Blaustein, 1985; and see Bartschat & Blaustein, 1983).

Conclusions

Several problems complicate studies of K channel blockers in synaptosomes: the sidedness of action, the use dependence of the block, and the voltage dependence of the block (e.g. see Hermann & Gorman, 1981*a*, *b*). These are all important aspects that may be difficult to examine in synaptosome preparations. Nevertheless, the synaptosome preparation appears to be very useful for elucidating many of the physiological and pharmacological properties of the various K permeabilities in presynaptic nerve terminals. The pharmacological 'fingerprints' of the various K channels in synaptosomes (see above and Batschat & Blaustein, 1985) may be advantageously applied to electrophysiological studies in mammalian brain, to obtain insight into the types of presynaptic K channels that are involved in synaptic transfer and integrated neuronal function. These pharmacological fingerprints may also be useful for identification of specific K channels in prospective biophysical and biochemical experiments.

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