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EFFECT OF CHANNEL BLOCKERS ON POTASSIUM EFFLUX FROM METABOLICALLY EXHAUSTED FROG SKELETAL MUSCLE

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SUMMARY

1. ^{86}Rb and ^{42}K have been used to assess potassium exchange in frog skeletal muscle which had been metabolically exhausted by electrical stimulation (1 Hz) after treatment with 2 mM-cyanide and 1 mM-iodoacetate. These conditions led to the development of rigor.

2. Poisoning by itself induced a small but variable increase in tracer efflux. Complete mechanical exhaustion subsequent to electrical stimulation was, however, accompanied by a 5–6 fold increase in the rate coefficient for both ^{86}Rb and ^{42}K efflux. In the case of rubidium this was maintained for at least 20 min and often for up to 1 h.

3. The increase in tracer efflux induced by metabolic exhaustion was inhibited by barium (0·03–5 mM) in a reversible and concentration-dependent manner. Inhibition was also observed with glibenclamide (3–100 μM), tolbutamide (0·3–2 mM), TEA (5–100 mM) and the local anaesthetics lignocaine (1–3 mM) and tetracaine (1 mM). Quinine produced a dual response consisting of an inhibitory component which was most clearly seen at low concentrations (0·3 mM) and an enhancement of tracer efflux that became increasingly dominant at higher concentrations (1–10 mM).

4. Both apamin (30 and 100 nM) and Israeli scorpion (*Leiurus quinquestriatus*) venom (16 $\mu g\ ml^{-1}$) produced little or no block of the tracer efflux activated by metabolic exhaustion. Similarly 4-aminopyridine (3 mM) and decamethonium (0·3 mM) were without obvious effect.

5. It is concluded that metabolic exhaustion of frog skeletal muscle leads to an increased permeability to both ^{42}K and ^{86}Rb. Our results with channel blockers suggest that this K^+ permeability can be attributed neither to the delayed rectifier nor to an apamin- or charybdotoxin-sensitive calcium-activated K^+ permeability ($P_{K(Ca)}$) but may be predominantly due to activation of ATP-sensitive channels similar to those found in the β-cells of pancreatic islets.

INTRODUCTION

There are several types of membrane ion channel present in skeletal muscle which exhibit selectivity for potassium ions. In addition to the voltage-dependent delayed and inward rectifiers (Stanfield, 1983), skeletal muscle also possesses K^+ channels

whose opening is controlled by the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$) (Barrett, Barrett & Dribin, 1981) or adenosine triphosphate ($[ATP]_i$) (Spruce, Standen & Stanfield, 1985). The channels activated by Ca^{2+} are of at least two kinds, as indicated by the selective blocking actions of the bee venom peptide apamin and tetraethylammonium ions (TEA). Thus in cultured muscle from fetal or neonatal rats the calcium-activated K^+ permeability ($P_{K(Ca)}$) responsible for the hyperpolarization following an action potential is apamin sensitive but TEA insensitive, whereas a second $P_{K(Ca)}$ identified by single-channel recording shows the reverse sensitivity to the blockers (Romey & Lazdunski, 1984; see also Miller, Moczydlowski, Latorre & Phillips, 1985). Apamin-sensitive channels are more common in developing or denervated than in normal adult mammalian muscle (Schmid-Antomarchi, Renaud, Romey, Hugues, Schmid & Lazdunski, 1985) but may, however, be responsible for a component of slow outward K^+ current in adult frog muscle (Cognard, Traoré, Potreau & Raymond, 1984).

Channels which open in response to a decrease in $[ATP]_i$ have been identified in pancreatic β-cells (Cook & Hales, 1984), in heart muscle (Noma, 1983) and, more recently, in frog skeletal muscle (Spruce *et al.* 1985).

It is not yet established which, if any, of the channels described above are responsible for the well-documented K^+ conductance increase found in metabolically exhausted frog skeletal muscle (Fink & Lüttgau, 1976; Fink, Hase, Lüttgau & Wettwer, 1983). Poisoning will, however, produce a marked decrease in cellular ATP concentration (Fink *et al.* 1983) which might subsequently lead also to an increase in $[Ca^{2+}]_i$. Fink *et al.* (1983) did indeed obtain some evidence that an elevation of $[Ca^{2+}]_i$ was involved in the increase in potassium permeability (P_K).

The work described here examines the ability of various K^+ channel blockers to inhibit the increase in ^{86}Rb or ^{42}K efflux produced in skeletal muscle by metabolic exhaustion and so seeks to determine whether the channels involved can be directly identified with any of those already described for this tissue.

METHODS

Experiments were performed on the anterior branch of the semitendinosus muscle from adult frogs (*Rana temporaria*). The muscle was cleared of visible connective tissue, nerve and blood vessels and was then mounted vertically in a 2 ml bath under a resting tension of 1 g. Isometric tension was recorded throughout the experiment (Dynamometer UF1 transducer and MX4 chart recorder, both Devices, Welwyn Garden City, Hertfordshire). Semicircular stainless-steel electrodes, connected to a stimulator (S88 Grass, Quincy, MA, U.S.A.), were positioned around the upper and lower ends of the muscle. Throughout the experiment, except for the loading period, bathing solution flowed through the bath at a rate of 4–5 ml min^{-1}, entering the bath from the bottom and being drawn off to a fraction collector from the top. Oxygen was also bubbled continuously through the bath at a slow rate. All experiments were performed at room temperature. The frog Ringer solution contained (mM): NaCl, 115; KCl, 2·5; $CaCl_2$, 1·8; Na_2HPO_4, 2·15; NaH_2PO_4, 0·85. The pH was 7·2.

For experiments on denervated muscle, denervation was accomplished by removing a 5 mm section of sciatic nerve from the top of the right hind limb of frogs anaesthetized by immersion in water saturated with ether. The skin was treated with antiseptic both prior to and following the operation. The frogs were kept at room temperature for at least 21 days to allow nerve degeneration. Contralateral, non-denervated muscles were used as controls.

$^{86}Rb/^{42}K$ efflux studies

Muscles were incubated in Ringer solution containing ^{86}RbCl (2–3 μCi ml^{-1}) or ^{42}K (~ 30 μCi ml^{-1}) for 90 min, at which time perfusion with non-radioactive solution was started. The perfusate for the next 30 min, was discarded but thenceforward 1 min fractions were collected. Poisoning with NaCN (2 mM) and iodoacetate (1 mM) began 40 min after the commencement of wash-out and continued until the end of the experiment. The Ringer solution containing CN^- and iodoacetate was made up in the manner described by Fink & Lüttgau (1976). After 45 min of treatment with CN^- plus iodoacetate complete metabolic exhaustion was produced by stimulating the muscles with supramaximal square-wave pulses of 2 ms duration at 1 Hz until the twitch response disappeared and a contracture developed (2–3 min). At the end of the experiment the muscles were removed from the bath and digested with concentrated nitric acid to extract the remaining ^{86}Rb/^{42}K. Radioactivity in the efflux samples and muscle extract was determined by Cêrenkov counting in a liquid scintillation counter (LS 1801: Beckman, Irvine, CA, U.S.A.).

It should be noted that for experiments with radioactive potassium it was less expensive to use tracer which was supplied as a 1:1 mixture of ^{43}K and ^{42}K. As expected the decay of radioactivity over a six-day period conformed to a double exponential with the appropriate half-lives for the two isotopes. The contribution of ^{43}K to the count rate is initially quite low ($\simeq 25\%$) because of its weak β energy and for this reason these are described as '^{42}K experiments'. The actual corrections for decay were made by reference to the decay of a fixed amount of the original mixture.

Efflux rate coefficients (k) were calculated as the fractional loss per minute (tracer lost in each period divided by the duration of the period (usually 1 min) and by the mean tracer content of the muscle during this period).

Calculation of inhibition by blocking agents

Inhibition was calculated according to the following equation:

$$\text{Inhibition } (\%) = 100\left(1-\frac{k_i-k_s}{k_e-k_s}\right),$$

where k_s is the mean rate coefficient for tracer efflux during the 5 min prior to poisoning; k_e, the rate coefficient after metabolic exhaustion and k_i, the rate coefficient during maximal drug effect. In some experiments k_e showed a small change with time and this was allowed for by fitting a regression line to the seven values of k observed just before drug application and extrapolating into the drug period to give a value for k_e at the time of maximal drug effect. The value of k_i was, wherever possible, the mean of the peak value and those immediately on either side.

Materials

^{86}RbCl (6–7 Ci/g Rb) was obtained from the New England Nuclear Group, Boston, MA. ^{43}K (carrier free (see earlier section)) was purchased from the M.R.C. Cyclotron Unit, Hammersmith Hospital, London. Apamin was a generous gift from Dr P. N. Strong, Department of Pharmacology, University College London and the venom of the Israeli scorpion (*Leiurus quinquestriatus hebraeus*) was supplied by Latoxan, Rosans, France. Glibenclamide was obtained from Hoechst Pharmaceuticals and quinine HCl, tetracaine HCl, lignocaine HCl, tetraethylammonium chloride, 4-aminopyridine and tolbutamide from the Sigma Chemical Company, Poole, Dorset, while sodium cyanide and iodoacetic acid were purchased from BDH Chemicals Ltd, Poole, Dorset. Decamethonium iodide was obtained from Koch Light Laboratories Limited, Slough, Berkshire.

RESULTS

Effects of metabolic exhaustion on $^{86}Rb/^{42}K$ efflux and muscle tension

The resting rate coefficient for ^{86}Rb efflux in standard Ringer solution had settled to a relatively steady level of $2{\cdot}1 \pm 0{\cdot}1 \times 10^{-3}$ min^{-1} (mean ± S.E. of mean, twenty-six muscles) 30–40 min after removal of the muscles from the load solution but continued to fall slowly so that at 70 min it was $1{\cdot}6 \pm 0{\cdot}1 \times 10^{-3}$ min^{-1} ($n = 24$). Fewer experiments were carried out with ^{42}K and for this isotope k was significantly higher

than for ^{86}Rb. The equivalent value 35 min after removal from load was $4{\cdot}7 \pm 0{\cdot}3 \times 10^{-3}$ min^{-1} ($n = 8$). Fig. 1 illustrates the effect of treatment with CN^- plus iodoacetate on k. After 45 min k for ^{86}Rb had increased to $2{\cdot}5 \pm 0{\cdot}2 \times 10^{-3}$ min^{-1} ($n = 26$) and for ^{42}K to $6{\cdot}1 \pm 0{\cdot}5 \times 10^{-3}$ min^{-1} ($n = 8$). Approximately 10 % of poisoned

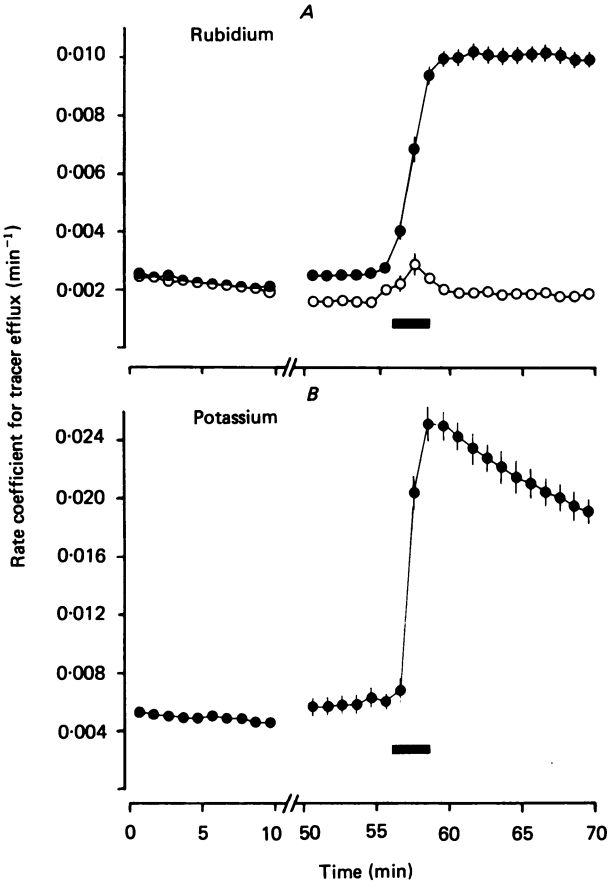

Fig. 1. The effect of CN^- plus iodoacetate poisoning and mechanical exhaustion on the efflux of (*A*) ^{86}Rb and (*B*) ^{42}K (●). Points are mean values and vertical lines indicate ± S.E. of mean where they exceed the size of the symbol. The number of observations, n, is 26 for (*A*) and 8 for (*B*). The time axes start 30 min after removal of muscle from load solution and poisoning commences following a further 10 min perfusion with standard Ringer solution. Electrical stimulation (1 Hz) is indicated by the filled bar. The effect of electrical stimulation on ^{86}Rb efflux in non-poisoned muscles is also shown (○, $n = 6$).

muscles failed to show any increase in k in response to poisoning alone and this could invariably be related to the observations made on tension. Muscles showing an increase in k on poisoning developed tension (see for example Fig. 2*B*) whereas the others did not. Muscles taken from the same animal usually showed similar tracer efflux and tension responses on poisoning.

Supramaximal electrical stimulation of poisoned muscles for 2–3 min at 1 Hz elicited twitches which began to decline in ampitude after about 1 min, eventually

disappearing whilst being replaced by a tonic contracture which continued to develop after the twitches had disappeared. Indeed, in several cases the tension was observed to increase even after stimulation was stopped. The contracture was usually maintained until the end of the experiment although the increase in tension had usually declined to between 20 and 70 % of the peak value. When poisoned muscles were removed from the bath at the end of the experiment they had become much less elastic indicating a state of rigor.

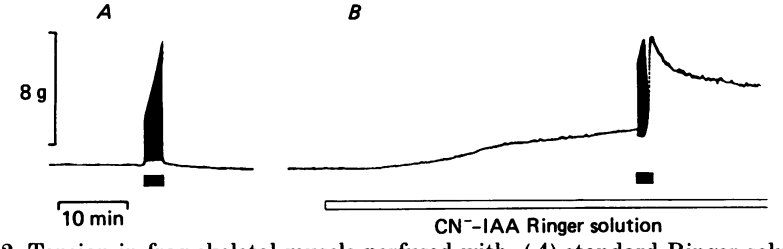

Fig. 2. Tension in frog skeletal muscle perfused with, (*A*) standard Ringer solution and (*B*) Ringer solution containing 2 mM-CN^- plus 1 mM-iodoacetate (IAA). All muscles were initially under a resting tension of 1 g. Electrical stimulation (1 Hz) is indicated by the filled bar.

As Fig. 1 also shows, electrical stimulation of the poisoned muscles produced a marked increase in $^{86}Rb/^{42}K$ efflux, in keeping with the findings of Fink & Lüttgau (1976). The rate coefficient for ^{86}Rb efflux increased to $10{\cdot}1 \pm 0{\cdot}3 \times 10^{-3}$ min^{-1} ($n = 26$) and for ^{42}K to $25{\cdot}5 \pm 1{\cdot}3 \times 10^{-3}$ min^{-1} ($n = 8$), 5–6 times the pre-poisoning level. In the case of ^{86}Rb, this increase was maintained for at least 20 min (Fig. 1*A*) and often for up to 1 h. For unknown reasons, in the experiments with ^{42}K (which were carried out some weeks after those with ^{86}Rb) the elevated efflux rate coefficients were less well maintained (Fig. 1*B*). On some occasions, perhaps due to the onset of membrane damage, k showed a progressive increase towards the end of the experiments (e.g. Fig. 3*B*).

Figs. 1*A* and 2*A* also show for comparison the effects of stimulation on ^{86}Rb efflux and tension in non-poisoned muscle. In these muscles the twitch amplitude increased rather than decreased during the stimulation period and ^{86}Rb efflux showed a relatively small increase during stimulation which largely reversed as soon as stimulation was stopped.

Effects of K^+-channel blockers

The increase in ^{86}Rb efflux produced by stimulation of poisoned muscles persisted at a sufficiently steady level (Figs. 1 and 3*B*) to allow various agents to be tested for their ability to block the underlying permeability increase. The calculation of the inhibition produced by the blockers is described in the Methods section, but it should be noted that since k declined during the experiment and because the blockers may also inhibit the resting K^+ permeability to some extent (see Results with Ba^{2+}), inhibitions calculated in this way could exceed 100 %.

None of the blockers tested had any obvious effect on muscle tension.

Tetraethylammonium ion (TEA). TEA in the range 1–100 mM is well known to block both the delayed and inward rectifiers of skeletal muscle (for a review see Stanfield,

1983). Concentrations of 5 and 10 mM applied to exhausted muscle produced inhibitions of 15 and 27 % respectively (Table 1). With 100 mM-TEA, however, the situation was more complicated. In all cases 100 mM-TEA, which replaced the molar equivalent of Na^+ in the Ringer solution, produced substantial block (Table 1) but this was superseded either during the second half of the exposure period or on wash-out by a large but transient increase in k (which reached 0·06–0·08 min^{-1} in all muscles tested). Despite this complication it appears that even at 100 mM it is not possible for TEA to completely inhibit the increase in ^{86}Rb efflux induced by exhaustion. This is in keeping with the results of Fink & Lüttgau (1976) and Fink & Wettwer (1978), who observed that the block of the increased membrane conductance was incomplete even with an external concentration of TEA as high as 115 mM.

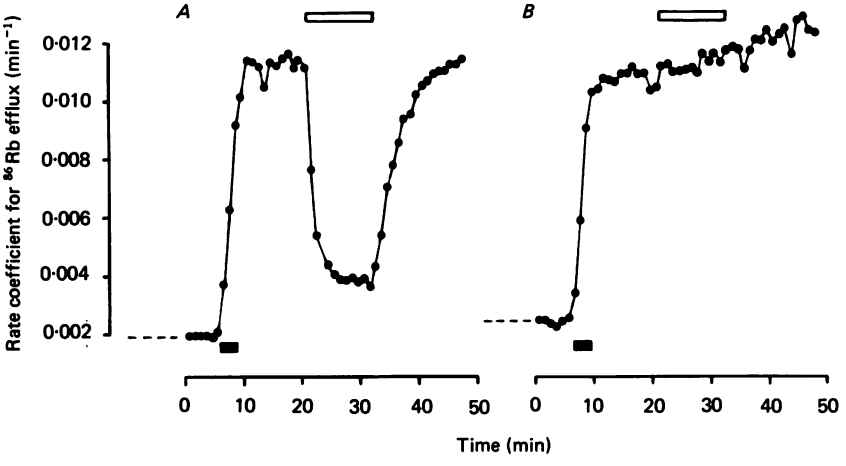

Fig. 3. Effect of (*A*) barium (0·3 mM) and (*B*) 4-AP (3 mM), present during open bar, on the increase in ^{86}Rb efflux induced by metabolic exhaustion. The rate coefficient of ^{86}Rb efflux prior to poisoning is indicated by the dashed line. Tracer efflux during the initial 40 min perfusion with CN^- plus iodoacetate is not shown. Electrical stimulation (1 Hz) is indicated by the filled bar.

Barium. Ba^{2+} is a moderately potent blocker of most K^+ channels. In exhausted muscle Ba^{2+} caused a rapid and reversible inhibition of both ^{86}Rb (Fig. 3*A*) and ^{42}K (not shown) efflux. The concentration dependence of this block is illustrated in Fig. 4. The effect of Ba^{2+} was also tested in non-poisoned muscle. In two experiments with ^{86}Rb and one with ^{42}K, 0·3 mM-Ba^{2+} was found to reduce resting efflux by approximately 70 %.

4-Aminopyridine (4–AP). 4-AP is a relatively selective and potent inhibitor of the delayed rectifier (Gillespie, 1977) and in keeping with this at 3 mM produced a 65 % inhibition of the 10-fold increase in ^{86}Rb efflux from non-poisoned muscles which was induced by stimulation for 2 min at 20 Hz (results of two experiments, not shown). As shown in Fig. 3*B* (see also Table 1), however, 4-AP at 3 mM for 10 min had no effect on the increase in k due to exhaustion.

Tolbutamide and glibenclamide. These sulphonylureas are used clinically to promote insulin release from pancreatic β-cells, an action which is attributed to a reduction

in the permeability of the cell membrane to potassium (Henquin, 1980). More recently they have been shown to act in particular on the ATP-sensitive K^+ channel, known to be present in β-cells (Sturgess, Ashford, Cook & Hales, 1985). Fig. 5*A* shows that glibenclamide produced a clear inhibition of ^{86}Rb efflux in metabolically exhausted muscle which reversed only slowly on removal of the drug. Fig. 5*B* presents a

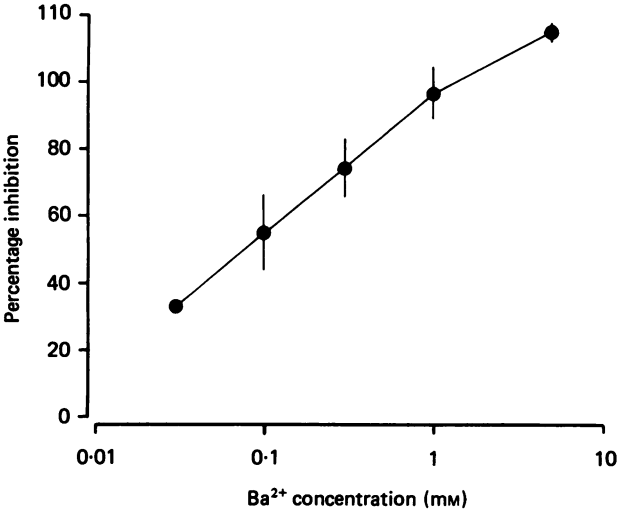

Fig. 4. Dose–response curve for inhibition by barium of the increase in ^{86}Rb efflux induced by metabolic exhaustion. Inhibition is expressed as a percentage reduction of the difference between the rate coefficients for tracer efflux observed before and after metabolic exhaustion. Each point is the mean ± S.E. of mean of values obtained with three separate muscles.

dose–response curve for the blocking action and shows that increasing the concentration of glibenclamide beyond 30 μM failed to reduce ^{86}Rb efflux by more than 50–60 %. A reduction in ^{86}Rb efflux was also seen with tolbutamide (Table 1) though it was approximately 100 times less potent than glibenclamide.

Quinine. This alkaloid which has an antiarrhythmic action attributable to Na^+ channel block, is also well known to block the delayed rectifier (Fishman & Spector, 1981), Ca^{2+}-activated (Armando-Hardy, Ellory, Ferreira, Flemming & Lew, 1975; Burgess, Claret & Jenkinson, 1981) and ATP-sensitive K^+ channels (Cook & Hales, 1984). The influence of quinine on ^{86}Rb efflux in exhausted muscle appeared to have two components. In each of five experiments, during a 10 min exposure to 0·3 mM-quinine the increase in k due to exhaustion was reduced by approximately 50 % (Fig. 6*A*) and on wash-out returned to the pre-drug value. In one of the five experiments, however, k continued to rise so that after 20 min it was approximately 4 times the pre-drug value (Fig. 6*B*). With progressively higher concentrations of quinine the inhibitory effect became increasingly masked by the earlier onset of the 'potentiating' phase, which now occurred in all muscles and for concentrations of 1 mM or more began prior to wash-out of the drug. Indeed with 10 mM-quinine an inhibitory component could not be detected, presumably because it was completely obscured by the second component which began without delay.

Local anaesthetics. As with quinine, local anaesthetics in addition to blocking Na^+ channels are also known to block K^+ channels (Almers, 1976). In exhausted muscle the local anaesthetics tetracaine and lignocaine reduced k by over 45 % when present at concentrations of 1 mM or more (Table 1). Unlike quinine, however, a secondary stimulatory response was not observed with either lignocaine or tetracaine.

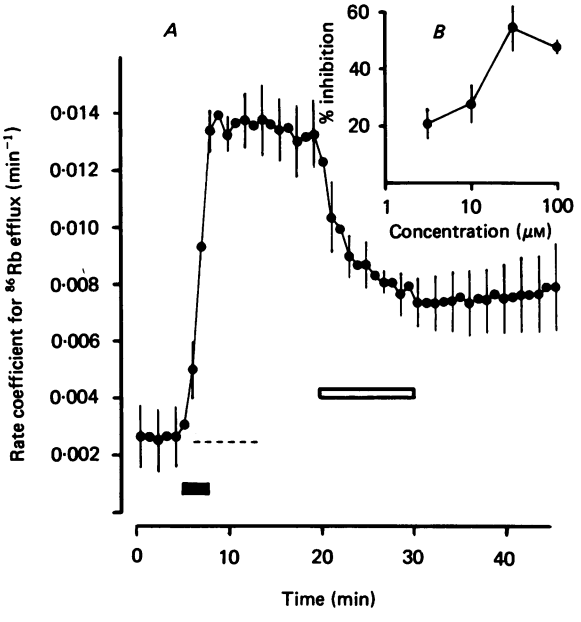

Fig. 5. *A*, effect of glibenclamide (100 μM), present during open bar, on the increase in ^{86}Rb efflux induced by metabolic exhaustion. Each point is the mean of three observations. Vertical lines indicate ± S.E. of mean and for clarity are shown for alternate points. The rate coefficient of ^{86}Rb efflux prior to poisoning is indicated by the dashed line. Tracer efflux during the initial 40 min perfusion with CN^- plus iodoacetate is not shown. Electrical stimulation (1 Hz) is indicated by the filled bar. *B*, dose–response relationship for inhibition by glibenclamide. Inhibition is calculated as detailed in Methods. Each point is the mean ± S.E. of mean of results from three separate muscles.

Apamin. Neither 30 nor 100 nM-apamin had any significant effect on ^{86}Rb efflux in exhausted muscle (Table 1). In three experiments 100 nM-apamin was also tested in muscles whose nerve had been cut at least 21 days previously. Apamin again had no effect although in two other denervated muscles 0·3 mM-quinine produced a block (50 %) which was comparable to that seen in innervated muscle. It may be noted that the denervated muscle responded to poisoning and exhaustion with similar increases in tension and ^{86}Rb efflux to those seen in normal muscle.

Leiurus quinquestriatus (L.q.) venom and decamethonium. Miller *et al.* (1985) have recently identified a component (charybdotoxin) of L.q. venom which is a potent blocker of the large conductance apamin-insensitive Ca^{2+}-activated K^+ channels found in mammalian skeletal muscle transverse (T) tubules. At a concentration (16 μg ml^{-1}) which has been reported to produce substantial block of T-tubule channels the venom had no significant effect on ^{86}Rb efflux from exhausted muscle (Table 1).

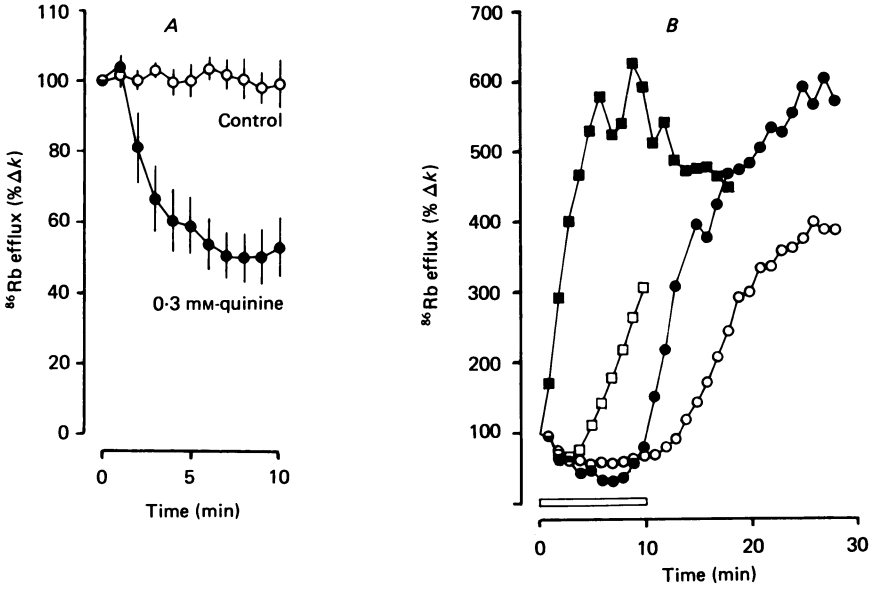

Fig. 6. *A*, ^{86}Rb efflux in metabolically exhausted muscle in the absence (○, $n = 4$), or during a 10 min application of 0·3 mM-quinine (●, $n = 5$). The effect of increasing the concentration of quinine (single experiments) is shown in *B*. (Concentrations (mM): ○, 0·3; ●, 1; □, 3; ■, 10). The period of drug application is indicated by the open bar. Δk is defined as the difference in ^{86}Rb efflux rate coefficients observed before and after metabolic exhaustion. Note the change in the vertical scale, made necessary to display the striking increase in efflux which followed the initial inhibition.

TABLE 1. Inhibition of ^{86}Rb efflux induced by metabolic exhaustion

Drug	Concentration	Percentage inhibition
TEA	5 mM	15·0±4·6
	10 mM	27·1±3·6
	100 mM	58·4±2·8
4-AP	3 mM	−3·7±3·0*
Tolbutamide	0·3 mM	18·5±4·2
	2 mM	41·2±5·7
Lignocaine	1 mM	45·5±2·6
	3 mM	68·3±5·4
Tetracaine	1 mM	61·6±7·1
Apamin	30 nM	−6·2±7·9*
	100 nM	−5·6±14·3*
Decamethonium	0·3 mM	−0·1±1·4*
Israeli scorpion venom	16 μg ml^{-1}	−6·8±3·5*

Percentage inhibition has been calculated as described in Methods. Values are given as means ± S.E. of mean of four observations obtained with separate muscles. Values which do not differ significantly from zero ($P > 0·05$) as determined by Student's *t* test are indicated by an asterisk.

Decamethonium blocks the large conductance K^+ channel of sarcoplasmic reticulum at micromolar concentrations (Coronado & Miller, 1980) and also the cation-selective, acetylcholine-activated, end-plate channel (Adams & Sakmann, 1978). In the present study, 300 μM-decamethonium was without effect on ^{86}Rb movements (Table 1).

Effects of hypotonic Ringer solution on ^{86}Rb efflux

Potassium channels that open in response to changes in cell volume have been identified in several cell types (Hamill, 1983). More recently Guharay & Sachs (1984) have reported a stretch-activated non-selective cation channel in embryonic chick muscle and in view of the findings of Fink, Grocki & Lüttgau (1980) that metabolic exhaustion produces an approximately 10% increase in muscle fibre volume it is relevant to consider the effect of swelling. (Note that since the channels described by Guharay & Sachs are not selective for potassium they would not be expected to contribute substantially to the conductance increase in poisoned muscle.) Non-poisoned muscles were exposed for 10 min to Ringer solution containing half the normal amount of NaCl (58 mM), a procedure which should produce substantial swelling (Blinks, 1965). Three of seven muscles so treated exhibited no change in ^{86}Rb efflux. The remaining four showed a variable increase of between 60 and 250%, which reversed quickly on return to normal Ringer solution. These results suggest that although swelling alone produces some increase in ^{86}Rb efflux it is probably incapable of causing the large changes in K^+ permeability observed in metabolically exhausted muscle.

DISCUSSION

The present results show that metabolic exhaustion of frog skeletal muscle leads to a substantial increase in both ^{42}K and ^{86}Rb efflux. This is in keeping with the increase in K^+ conductance observed by Fink & Lüttgau (1976). There is, however, a large discrepancy in the magnitudes of the flux and conductance changes. Fink & Lüttgau estimated K^+ conductance to increase by a factor of 100 or more, whereas the increase in tracer efflux is only 5- to 6-fold. Fink *et al.* (1980) have noted a similar inconsistency in regard to the net loss of K^+ from exhausted muscle. This apparent deviation from the Goldman–Hodgkin–Katz relationship (Goldman, 1943; Hodgkin & Katz, 1949) has not been examined further but may be at least partly explained as a consequence of K^+ (or in our case radiotracer) accumulation in the extracellular space, including the T-tubules.

We have used both ^{86}Rb and ^{42}K as tracers for K^+. Fig. 1 shows that although the absolute magnitudes of the resting and exhaustion-induced rate coefficients observed with the two isotopes differ by a factor of 2–3, the relative changes in k were similar. ^{86}Rb has been shown to be a qualitatively satisfactory marker for K^+ movements through Ca^{2+}-activated (DeWitt & Putney, 1983) and other K^+ channels (Bolton & Clapp, 1984). ^{86}Rb is probably also useful for monitoring K^+ movements through the ATP-sensitive channel in pancreatic β-cells although Rb^+ will carry a much smaller current than K^+ (Ashcroft, Kakei & Kelly, 1986). Gallacher, Maruyama & Petersen (1984) specifically addressed the problem of ^{86}Rb movement through K^+ channels of very low Rb^+ conductance and concluded that for tracer amounts of ^{86}Rb

a larger permeability is not unexpected. Our observations suggest that not only ^{42}K but also ^{86}Rb provide satisfactory probes for the channels activated by exhaustion in skeletal muscle.

The inhibition of ^{86}Rb efflux by the K^+ channel blockers, Ba^{2+} and TEA is consistent with the results of Fink & Lüttgau (1976) who showed that the increase in cation conductance in exhausted muscle was selective for K^+. It may be noted, in addition, that the block by Ba^{2+}, and indeed other agents, rules out the possibility that the increase in k in exhausted muscle could have been due to damage to the cell membrane.

We turn now to the question of the identity of the ion channels activated in exhausted muscle.

By poisoning muscles with CN^- alone Fink *et al.* (1983) were able to produce an 'intermediate' state of the fibres which now demonstrated a reversible increase in K^+ conductance on stimulation and in such fibres it was possible by injection of the Ca^{2+} chelator, EGTA or Ca–EGTA buffer to show that an elevation of $[Ca^{2+}]_i$ was a potentially important factor in the conductance increase. Since non-exhausted muscles do not exhibit an increase in K^+ permeability when $[Ca^{2+}]_i$ is elevated they further concluded that exhaustion enhanced the Ca^{2+}-sensitivity of the permeability mechanism. (Although such direct attempts were not made to alter $[Ca^{2+}]_i$ in iodoacetate–CN^- poisoned muscles, there is no reason to suppose that the mechanism responsible for the increase in P_K differs between CN^- and iodoacetate–CN^- poisoned muscles.) In our experiments neither apamin nor L.q. venom, which should have blocked the two kinds of Ca^{2+}-activated K^+ channels described in skeletal muscle, produced any clear inhibition of the ^{86}Rb efflux.

On the other hand, the marked inhibition produced by tolbutamide and glibenclamide, which block ATP-sensitive K^+ channels, suggests a major role for this channel type. The relative potencies of tolbutamide and glibenclamide found in our studies are in close agreement with observations made in an insulin-secreting cell line (Sturgess *et al.* 1985). The density of ATP-sensitive channels (Spruce *et al.* 1985) is probably sufficient to account for the large conductance increase seen in poisoned muscle. Spruce *et al.* (1985), however, found the ATP-sensitive channel to be insensitive to changes in Ca^{2+} concentration which would seem to be in conflict with the conclusions of Fink *et al.* (1983) if this channel were to make the major contribution to the conductance increase mediated by exhaustion. The two findings could perhaps be reconciled if the exhaustion procedure were to induce a Ca^{2+} dependence of the ATP-sensitive channel. An alternative possibility is that poisoning activates both Ca^{2+}-activated and ATP-sensitive channels. This might also serve to explain our observation that the maximal inhibition produced by glibenclamide fell far short of 100%. As noted above, however, any Ca^{2+}-activated channel present would need to be resistant to both apamin and L.q. venom.

The results with quinine are not conclusive. It would be expected to block both Ca^{2+}-activated (Armando-Hardy *et al.* 1975) and ATP-sensitive (Cook & Hales, 1984) K^+ channels (as well as any delayed rectifier channels (Fishman & Spector, 1981)). Interpretation of the present results was further complicated by the stimulatory effect on ^{86}Rb efflux of higher concentrations of quinine. If Ca^{2+}-activated channels were present it would be tempting to associate this stimulatory effect with the ability

of quinine to release Ca^{2+} from the sarcoplasmic reticulum (Isaacson & Sandow, 1967). A balance between activation and block would presumably then be established. Measurement of $[Ca^{2+}]_i$ during the action of quinine would be helpful.

Fink & Wettwer (1978) supposed that the conductance increase produced by exhaustion might be due to the generation of conditions which led to the permanent activation of the delayed rectifier. Both they and we, however, found 4-AP to be an ineffective blocker of the conductance so that unless, as they suggested, the channel's structure is modified in some way by exhaustion it is unlikely to be involved.

In summary, the present results suggest that a substantial part of the K^+ conductance activated in metabolically exhausted muscle may be attributed to ATP-sensitive channels. Further work is needed to explore the Ca^{2+} dependence of the response.

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