The effect of glibenclamide on frog skeletal muscle: evidence for K^+_{ATP} channel activation during fatigue

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1. The purpose of this study was to determine whether ATP-sensitive K^+ (K^+_{ATP}) channels are activated and contribute to the decrease in force during fatigue development in the sartorius muscle of the frog, *Rana pipiens*. Tetanic force (elicited by field stimulation), action potential and membrane conductance (using conventional microelectrodes), were measured in the presence and absence of glibenclamide, a K^+_{ATP} channel antagonist. Experiments were performed in bicarbonate-buffered solutions at pH 7·2.

2. In unfatigued muscle 100 μmol l^{-1} glibenclamide had no effect on the resting potential, the overshoot, the half-depolarization time or the maximum rate of depolarization of action potentials, while the mean half-repolarization time increased by 19 ± 4 % ($\pm$ S.E.M.) and the maximum rate of repolarization decreased by 17 ± 5 %.

3. Fatigue was elicited using 100 ms tetanic contractions every 1 s for 3 min. In the absence of glibenclamide the mean half-repolarization time increased from $0{\cdot}57 \pm 0{\cdot}05$ to $0{\cdot}89 \pm 0{\cdot}05$ ms during fatigue. The mean half-repolarization times after fatigue, when muscle fibres were exposed to 100 μmol l^{-1} glibenclamide either 60 min prior to fatigue or 60 s before the end of fatigue, were $1{\cdot}16 \pm 0{\cdot}08$ and $1{\cdot}17 \pm 0{\cdot}07$ ms respectively. Application of 100 μmol l^{-1} glibenclamide after 5 min of recovery did not increase the half-repolarization time, but decreased the rate of recovery compared to control values.

4. In unfatigued muscles, 100 μmol l^{-1} glibenclamide did not affect the tetanic contraction. In the absence of glibenclamide, the mean tetanic force after fatigue was $11{\cdot}0 \pm 0{\cdot}9$ % of pre-fatigue values. Application of 100 μmol l^{-1} glibenclamide 60 min before fatigue increased the rate of fatigue development as the mean tetanic force was $4{\cdot}8 \pm 0{\cdot}8$ % after 3 min of stimulation. The addition of 100 μmol l^{-1} glibenclamide 60 s before the end of fatigue had no effect on tetanic force during this time compared to control.

5. In the absence of glibenclamide, muscles recovered $90{\cdot}1 \pm 1{\cdot}6$ % of their tetanic force after 100 min. Addition of 100 μmol l^{-1} glibenclamide 60 min prior to fatigue significantly reduced the capacity of muscles to recover their tetanic force: after 100 min of recovery tetanic force was only $47{\cdot}3 \pm 9{\cdot}4$ % of the pre-fatigue value. Application of 100 μmol l^{-1} glibenclamide 60 s prior to the end of fatigue had a much smaller effect on the recovery as $79{\cdot}4 \pm 6{\cdot}2$ % of the tetanic force was recovered in 100 min. Addition of glibenclamide after 5 min of recovery had no effect.

6. The results from this study support the proposal that K^+_{ATP} channels are activated during fatigue and they contribute to the repolarization phase of the action potential. Although no evidence was found that activation of K^+_{ATP} channels during fatigue contributes to the force decrease during fatigue development, the impairment of force recovery following fatigue in the presence of glibenclamide supports the notion that K^+_{ATP} channels play an important protective role.

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Recent evidence suggests that intracellular ATP (ATP_i) modulates a class of K^+ channels known as K^+_{ATP} channels. These channels have been found in a variety of tissue types such as cardiac (Noma, 1983), skeletal (Spruce, Standen & Stanfield, 1985) and smooth muscle (Standen, Quayle, Davies, Brayden, Huang & Nelson, 1989), β-pancreatic cells (Cook & Hales, 1984) and neurones (Ashford, Sturgess, Trout, Gardner & Hales, 1988). Although the K^+_{ATP} channel has a high density in the sarcolemma of skeletal muscle (Spruce *et al.* 1985), the function of these channels is still unknown.

The difficulty in understanding K^+_{ATP} channel activation in skeletal muscle is that under patch clamp conditions the ATP_i required to half close the channels is in the micromolar range (Spruce, Standen & Stanfield, 1987), whereas ATP_i remains in the millimolar range even during intense exercise (Carlson & Siger, 1960). This paradox can be explained in part as ATP_i is not the only modulator of the channel. H^+, ADP, Mg-ATP and internal cations have been found to modulate the activity of K^+_{ATP} channels (Findlay, 1988*a*, *b*; Woll, Lonnendonker & Neumcke, 1989; Davies, 1990; Davies, Standen & Stanfield, 1991*a*, 1992). Among these modulators, it is thought that the decrease in intracellular pH during fatigue is an important activator of K^+_{ATP} channels (Davies *et al.* 1991*a*, 1992; Standen, Pettit, Davies & Stanfield, 1992).

There is a net and large potassium efflux during fatigue which is well documented (Lucier & Mainwood, 1972; Hnik *et al.* 1976; Hirsche, Schumaker & Hagemann, 1980; Sjøgaard, Adams & Saltin, 1985). The accumulation of K^+ at the surface and in T-tubules is believed to depolarize the sarcolemma and lead to a failure of excitation–contraction coupling resulting in an impairment of force production (Sjøgaard *et al.* 1985; Sjøgaard, 1990, 1991; Renaud & Light, 1992). Several investigators have proposed that K^+_{ATP} channels are largely inactive in resting skeletal muscle, but become active during fatigue as metabolic changes occur such as a decrease in ATP_i and intracellular pH. It is thought that K^+_{ATP} channels contribute to the K^+ efflux and the subsequent force decrease during fatigue, consequently the channels serve as a safety mechanism preventing deleterious depletion of energy stores and muscle damage (Ashcroft, 1988; Ashcroft & Ashcroft, 1990; Sjøgaard, 1990, 1991; Davies, Standen & Stanfield, 1991*b*).

The K^+_{ATP} channel antagonists, glibenclamide and tolbutamide, reduce the K^+ efflux from metabolically exhausted frog skeletal muscle (Castle & Haylett, 1987). Activation of K^+_{ATP} channels by the agonist SR 44866 reduces both the duration of the action potential and the twitch force in unfatigued frog skeletal muscle (Sauviat, Ecault, Faivre & Findlay, 1991). However, metabolic exhaustion and agonist-induced opening of K^+_{ATP} channels in unfatigued muscle do not represent a true physiological condition under which K^+_{ATP} channels may open; i.e. muscle fatigue. To date there have been no direct measurements of K^+_{ATP} channel activity in intact fatigued skeletal muscle.

It was the aim of this study to investigate the effects of glibenclamide on the contractility and the action potential of intact frog sartorius muscle, to determine if K^+_{ATP} channels are activated and whether they contribute to the force decrease during muscle fatigue.

METHODS

Animals and sartorius muscle preparation

Frogs, *Rana pipiens*, were purchased from Anilab, Québec, Canada and maintained as described by Renaud & Light, 1992. Briefly, frogs were fed meal-worms and kept at 20 °C in tanks containing water and dry land with a photoperiod of 12 h : 12 h (light : dark). Frogs were maintained according to the guidelines of the CCAC (Canadian Council of Animal Care).

Frogs were killed by double pithing, and sartorius muscles were dissected out with a piece of the pelvic bone and a thread tied around the distal tendon. A bundle of fibres (1·0–1·5 mm) was then dissected (normal width of the sartorius muscle is 3–4 mm). This preparation had two main advantages: (1) serial microelectrode penetrations cause artifacts in fatigued skeletal muscle (Renaud & Mainwood, 1985*a*, *b*), and a muscle bundle offers a larger population of muscle fibres for sampling than single fibre preparations; (2) the diffusion distance from the core to the surface of a bundle is smaller and more uniform than a whole sartorius muscle.

Solutions

The bundles were superfused with solutions at all times during an experiment. The standard solution, buffered at pH 7·2, contained (mmol l^{-1}): 120 Na^+, 3 K^+, 1·8 Ca^{2+}, 1 Mg^{2+}, 113·6 Cl^-, 1 SO_4^{2-}, 2 PO_4^{2-}, 7 HCO_3^-. All solutions were continuously gassed with 99 % O_2 and 1 % CO_2. The flow of fresh solution through the muscle chamber (total volume of 5 ml) was maintained at 10–15 ml min^{-1} at all times during an experiment. Experimental temperature was 20 °C.

Glibenclamide at 30 μmol l^{-1} has been shown to reduce maximally K^+ efflux from metabolically exhausted fibres (Castle & Haylett, 1987). In unfatigued frog muscle, glibenclamide has a $[K^+]_i$ of 3 μmol l^{-1} with 95 % inhibition of K^+_{ATP} channels at 50 μmol l^{-1} (Standen *et al.* 1992). A glibenclamide concentration of 100 μmol l^{-1} was used during this study in order to block maximally any K^+_{ATP} channel activity, in as short as time as possible. Furthermore, 100 μmol l^{-1} glibenclamide has no effect on the following parameters in resting unfatigued muscles: tetanic contraction, resting membrane potential, overshoot, half-depolarization time and maximum rate of depolarization of action potentials (see Results).

A concentration of 100 μmol l^{-1} glibenclamide (purchased from Sigma, USA, and a generous gift from The Upjohn Company, USA) was obtained by adding the proper volume of 2 mmol l^{-1} glibenclamide stock solution dissolved in 0·05 mol l^{-1} NaOH. Tolbutamide was not used in this study because it has several non-specific effects in frog sartorius muscle which may include an effect on Na^+ channels (Comtois, Light, Renaud & Kong, 1993).

Stimulation

The muscle bundles were stimulated by passing a current between parallel platinum wires placed above and below the muscle. Stimuli were given by a Grass S88 (USA) stimulator via an isolation unit (Grass SIU5, USA). Tetanic contractions were elicited by 200 ms trains of 0·5 ms rectangular pulses at a frequency of 140 Hz with a supramaximal strength of 6 V.

Measurement of tetanic force

The pelvic bone was held in place by a clamp in the muscle chamber, while the distal tendon was hooked to a lightweight wire attached to either a servo-mode force transducer (model 300 B; Cambridge Technology, Inc., USA) or a Kulite force transducer (model BG 100; Kulite, USA). Tetanic contractions were digitized at a rate of 5 kHz with a Metrabyte A–D board (model DAS50; Metrabyte, USA). The following parameters were later obtained by analysing the contractions on a microcomputer. Tetanic force, defined as the maximum force developed during a contraction, was calculated as the difference between the baseline and maximum force. Force-200, an appraisal of the ability of the muscle to maintain a constant force during the plateau phase, was measured at the end of the 200 ms train of stimuli and was expressed as a percentage of the maximum tetanic force. The first derivative of the tetanic contraction was calculated from the slope of every ten data points (time interval 2 ms). The maximum rate of relaxation was then defined as the difference between the baseline and the lowest peak during the relaxation phase.

Measurement of resting and action potentials

Membrane potentials were measured using standard microelectrodes in the usual manner. Briefly, reference electrodes and microelectrodes were filled with 3 mol l^{-1} KCl. Microelectrodes were made from 1·5 mm o.d. borosilicate glass (1B150F-4; World Precision Instruments, Inc., USA). Resistance ranged from 8 to 15 MΩ, tip potentials were less than 5 mV. Ag–AgCl wires were used as the reversible half-cells for both reference and microelectrodes. A silver sheet (1 × 1 cm) was used as a ground electrode.

Microelectrodes were connected to either a Frederick Haer & Co. (model 74–77–1; Brunswick, ME, USA) or a World Precision Instruments, Inc. (model S-7071A; Sarasota, FL, USA) amplifier system, both allowed for capacitance compensation. Action potentials (APs) were elicited with a single 0·5 ms rectangular pulse. The stimuli were applied only at the surface of the muscle bundle using fine platinum electrodes and stimulus strength was adjusted to stimulate a small number of fibres to minimize movement artifacts. APs were digitized as described for tetanic force, except the sampling rate was 200 kHz. The following variables were later analysed: resting potential, overshoot, half- depolarization time, half-repolarization time, maximum rate of depolarization and repolarization. The half-depolarization time was defined as the time interval from 50 % of the action potential amplitude to the overshoot. The half-repolarization time was defined as the time interval from the overshoot to 50 % of the amplitude. The maximum rates of depolarization and repolarization were obtained from the action potential first derivative calculated from the slope of a regression analysis of every ten data points (time interval 50 μs) and were defined as the difference between the baseline and the highest or lowest peak during the depolarization or repolarization phase respectively. AP measurements were measured in three to five muscle fibres chosen at random (all measurements were made within 5 min). The different parameters of the AP were then averaged for each bundle, and the mean values of different muscle bundles reported were calculated from these averages, and not from individual measurements.

Experimental protocol

Muscle length was first adjusted to give maximum tetanic force, the bundles were then allowed to equilibrate for 30 min whilst being stimulated at a rate of one tetanic contraction every 100 s. APs were measured after this 30 min equilibrium period. The bundle was discarded if (1) a 5 % decrease in force, (2) an AP overshoot below 15 mV, or (3) a resting membrane potential more positive than −80 mV was observed during the equilibrium period.

Following the equilibrium period, all bundles were stimulated for a further 60 min either in the presence or absence of 100 μmol l^{-1} glibenclamide (see below). Fatigue was then produced by stimulating the muscle bundle with one tetanic contraction per second for 3 min. During the fatigue period, the duration of the train of stimuli was reduced from 200 to 100 ms to minimize muscle damage (Renaud & Mainwood, 1985*a*), but returned to 200 ms once every 30 s for data acquisition purposes. Following fatigue, the muscles were allowed to recover, while being stimulated with a 200 ms train every 5 min for 100 min.

In order to evaluate whether and at what time K^+_{ATP} channels are opened during fatigue and recovery, the bundles were divided into four groups (Fig. 1). The first group was the control group (no glibenclamide). The second group (pre-fatigue treated) was treated with 100 μmol l^{-1} glibenclamide 60 min prior to fatigue development with glibenclamide present for the full duration of the experiment. In the third group (during fatigue treated), 100 μmol l^{-1} glibenclamide was added 60 s prior to the end of the fatigue run and was removed after 35 min recovery. For the fourth group (post-fatigue treated), 100 μmol l^{-1} glibenclamide was added after 5 min of recovery and removed after 35 min recovery. In the last two groups, glibenclamide was removed after 35 min recovery to test whether any observed effects were reversible.

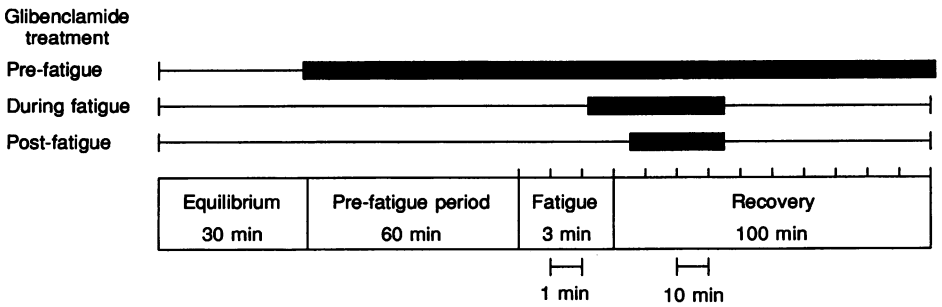

Figure 1. Experimental protocol for the glibenclamide treatment
The control group not exposed to glibenclamide is not shown. Horizontal filled bar represents the period of time muscles were exposed to 100 μmol l^{-1} glibenclamide for each of the three experimental groups.

Metabolic exhaustion

It has been shown previously that 100 μmol l^{-1} glibenclamide reduces K^+ efflux under metabolically exhausted conditions within 10 min (Castle & Haylett, 1987). To determine if this reflects the time required for glibenclamide to block the K^+_{ATP} channels, the time course of relative changes in membrane conductance in the absence and presence of 100 μmol l^{-1} glibenclamide was estimated; electrotonic potentials were measured with a conventional microelectrode 150–300 μm away from another microelectrode used to inject a depolarizing current as described elsewhere (Fink & Luttgau, 1976).

Electrotonic potentials were first measured while muscle fibres were immersed in a saline solution (pH 7·2) containing (mmol l^{-1}) 120 Na^+, 3 K^+, 1·8 Ca^{2+}, 1 Mg^{2+}, 113·6 Cl^-, 1 SO_4^{2-}, 2 PO_4^{2-}, 5 Hepes. Hepes was used instead of the bicarbonate buffer as solutions were not oxygenated. Muscle fibres were then metabolically exhausted by immersing them in a similar saline solution, but also containing 2 mmol l^{-1} NaCN and 1 mmol l^{-1} iodoacetate as described by Fink & Luttgau (1976). Bundles were exposed to this saline solution for 1 h and were then stimulated with a 2 ms rectangular pulse every 1 s for 2 min or until a contracture developed (as described by Castle & Haylett, 1987). Electrotonic potentials were again measured first in the absence and then in the presence of 100 μmol l^{-1} glibenclamide. In separate experiments electrotonic potentials in the absence and presence of glibenclamide were measured in resting, unfatigued muscle fibres.

Statistics

Analyses of variance (ANOVA) were done to test the significance of the different treatments: glibenclamide and time. A split-plot ANOVA design was used because measurements at different times were made from the same muscle. All calculations were made using the GLM procedures (General Linear Models procedures) of the SAS statistical software (SAS Institute Inc., Cary, NC, USA). When a main effect or an interaction was significant, a Duncan multiple comparison test (MCT) was used to locate the significant differences (Steel & Torrie, 1980). When necessary, data were transformed to obtain homoscedasticity. The word 'significant' refers only to a statistical difference ($P < 0·05$).

RESULTS

The effect of glibenclamide in resting and metabolically exhausted fibres

The application of 100 μmol l^{-1} glibenclamide had no effect on the electrotonic potential measured 150–300 μm from the site of current injection of three resting unfatigued fibres (measured from three different muscle bundles, results not shown). These data suggest that glibenclamide does not affect membrane conductance in resting unfatigued fibres.

Exposure for 1 h to NaCN and iodoacetate reduced the measured electrotonic potentials by 100-fold (results comparable to those of Fink & Luttgau, 1976). In one muscle fibre the application of 100 μmol l^{-1} glibenclamide caused a 2·95-fold increase in the electrotonic potential in 13 min (Fig. 2). The mean increase in electrotonic potential from three muscle fibres was 2·6 ($\pm$ 0·4)-fold (each fibre from a different muscle bundle) with a half-maximal effect varying between 3 and 6 min and a maximal effect in 10–13 min. This effect was also reversible as a decrease in electrotonic potential was always observed upon the removal of glibenclamide.

The effect of glibenclamide on the action potential

Membrane potential and action potential overshoot

In the absence of glibenclamide (control and post-fatigue treatment) the decrease in the mean resting potential during fatigue ranged between 8 and 16 mV, while the mean AP overshoot remained unchanged (Table 1). The effects of fatigue on both the resting potential and overshoot were not modified by 100 μmol l^{-1} glibenclamide whether it was applied 60 min prior to fatigue (pre-fatigue treatment) or 60 s before the end of fatigue (during fatigue treatment). When glibenclamide was added during or after the fatigue period, recovery of the resting potential to pre-fatigue level was completed within 10 min, a time similar to that of control. The recovery was slower than control when glibenclamide was added 60 min prior to fatigue (i.e. within 30 min).

The action potential repolarization phase

During fatigue development, the repolarization phase was prolonged to a much greater extent in the presence than in the absence of 100 μmol l^{-1} glibenclamide (Fig. 3*A* and *B*). To estimate the effect of glibenclamide on the repolarization phase we measured the half-repolarization time and the maximum rate of repolarization. In the absence of glibenclamide, the mean half-repolarization time changed very little before fatigue (Fig. 3*C*). However, the addition of 100 μmol l^{-1} glibenclamide increased the mean half-repolarization time of unfatigued muscle fibres within 30 min. The half-repolarization time increased significantly during fatigue and the increase was significantly greater in the presence of 100 μmol l^{-1} glibenclamide than in its absence. Furthermore, the mean values of the half-repolarization time after fatigue in the presence of glibenclamide was the same whether it was applied 60 min before fatigue or 60 s prior to the end of fatigue.

Following fatigue the half-repolarization time of control muscles decreased and was no longer significantly different from the pre-fatigue values after 20 min of recovery. In the presence of glibenclamide the recovery of the half-repolarization time was significantly delayed. The half-repolarization time of muscle fibres exposed to glibenclamide 60 min prior to fatigue remained significantly longer than both its pre-fatigue values and of control muscles for the first 60 min of recovery. The same was observed for the group of muscle fibres exposed to glibenclamide 60 s prior to the end of fatigue, with the removal of glibenclamide after 35 min of recovery allowing the half-repolarization time to return faster towards pre-fatigue values. Addition of glibenclamide after 5 min of recovery had no immediate effect as there was no difference from control after 10 min of

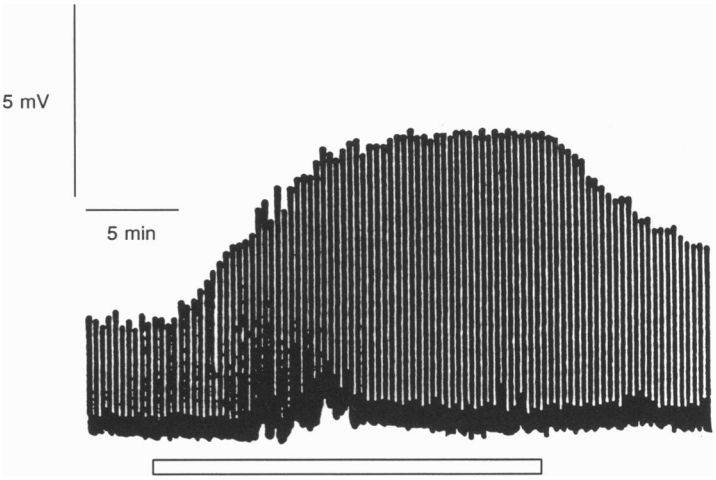

Figure 2. The effect of glibenclamide on the electrotonic potential from a metabolically exhausted muscle fibre
Prior to the measurements, the muscle fibre was exposed to 2 mmol l^{-1} NaCN and 1 mmol l^{-1} iodoacetate for 60 min and was then stimulated with one 2 ms twitch stimulation every 1 s for 2 min or until a contracture developed. Open bar corresponds to the application of 100 μmol l^{-1} glibenclamide. Current injected: +40 nA, 0·5 s rectangular pulse every 20 s.

recovery. After 20 and 30 min of recovery glibenclamide-exposed muscle fibres had longer half-repolarization times than control. However, they were similar to the values observed before fatigue in the presence of glibenclamide.

As expected from the half-repolarization time, the addition of 100 μmol l^{-1} glibenclamide decreased the maximum rate of repolarization of unfatigued muscle fibres from -136 ± 8 to -112 ± 8 V s^{-1} (results not shown). During fatigue the maximum rate of repolarization of control muscles decreased from -140 ± 9 to -97 ± 16 V s^{-1}, while for muscle fibres exposed to glibenclamide 60 min before fatigue the maximum rate of repolarization decreased from -112 ± 8 to -59 ± 5 V s^{-1}. The maximum rate of repolarization of muscle fibres exposed to glibenclamide 60 s before the end of fatigue was -63 ± 5 V s^{-1} immediately after fatigue, a value comparable to the value observed from the pre-fatigue- treated group. The recovery of the maximum rate of repolarization was also much slower in glibenclamide-exposed muscle fibres than in control fibres as expected from the results of the half-repolarization time.

The prolongation of the repolarization phase in the presence of glibenclamide can be attributed to an activation of K^+_{ATP} channels. For example, glibenclamide has previously been shown to prolong the AP duration in ischaemic and hypoxic cardiac muscle as it blocks K^+_{ATP} channels (Gasser & Vaughan-Jones, 1990; Deutch, Klitzner, Lamp & Weiss, 1991). However, one must assume that glibenclamide does not affect the K^+ delayed rectifier. Although glibenclamide does not affect the cardiac K^+ delayed rectifier (Escande, Thuringer, Le Guern, Courtieix, Laville & Cavero, 1989;

Table 1. The effect of 100 μmol l^{-1} glibenclamide on the resting potential and AP overshoot

Time (min)	Control	Pre-fatigue treatment	During fatigue treatment	Post-fatigue treatment
		Resting potential (mV)		
BF	−85·5 ± 2·4	−89·0 ± 2·7*	−89·1 ± 4·7	−90·0 ± 2·0
0	−77·9 ± 2·0	−74·3 ± 3·6*	−75·5 ± 5·6*	−74·9 ± 3·2
10	−81·2 ± 2·9	−78·6 ± 4·1*	−81·9 ± 5·7*	−80·5 ± 1·4*
20	−82·3 ± 2·0	−76·1 ± 3·2*	−85·3 ± 8·0*	−79·3 ± 2·1*
30	−80·9 ± 1·8	−84·3 ± 1·5*	−82·4 ± 4·8*	−81·4 ± 3·2*
		Overshoot (mV)		
BF	25·8 ± 3·0	24·1 ± 3·3*	26·9 ± 7·1	22·0 ± 6·5
0	23·8 ± 2·0	21·6 ± 4·4*	24·1 ± 5·4*	28·4 ± 3·4
10	23·0 ± 3·7	16·5 ± 3·5*	21·9 ± 6·8*	22·5 ± 4·7*
20	23·6 ± 4·0	21·4 ± 8·1*	23·7 ± 4·8*	19·6 ± 4·4*
30	22·8 ± 3·7	28·8 ± 4·1*	22·5 ± 8·0*	19·2 ± 6·1*

Fatigue was produced with 100 ms trains of pulses every 1 s for 3 min. Time represents the time during the recovery period when measurements of three APs in three different fibres started (measurements were completed within 5 min); BF, before fatigue. Experimental conditions: control muscles (number of bundles (n) = 7); pre-fatigue-treated muscles (n = 5) were exposed to glibenclamide 60 min prior to fatigue; during-fatigue-treated muscles (n = 4) were exposed to glibenclamide 60 s prior to the end of fatigue; post-fatigue-treated muscles (n = 5) were exposed to glibenclamide after 5 min of recovery. *Data obtained while muscles were exposed to glibenclamide. Data are presented as means ± S.E.M. No significant difference was observed between control and glibenclamide-exposed muscles (ANOVA, $P > 0.05$).

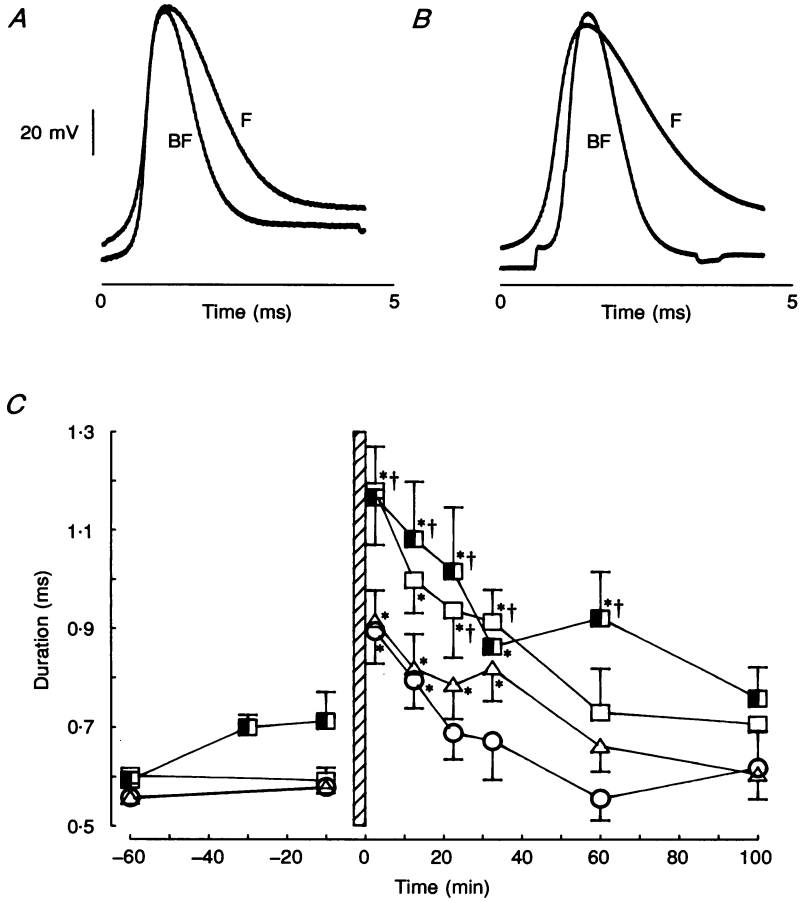

Figure 3. The effect of 100 μmol l^{-1} glibenclamide on the AP repolarization phase of unfatigued and fatigued sartorius muscle

Fatigue was produced with 100 ms trains of pulses every 1 s for 3 min. *A*, action potentials measured before fatigue (BF) and immediately after fatigue (F) in the absence of glibenclamide. *B*, action potentials measured before and after fatigue in the presence of 100 μmol l^{-1} glibenclamide. *C*, effect of 100 μmol l^{-1} glibenclamide on the recovery of the half-repolarization time (defined as the time interval from the overshoot to 50 % of the amplitude). Symbols: ○, control muscles (number of bundles, $n = 7$); ◧, pre-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide 60 min prior to fatigue and remained present until the end of the experiment; □, during-fatigue-treated fibres ($n = 4$) were exposed to glibenclamide 60 s prior to the end of fatigue until the 35th minute of recovery; △, post-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide after 5 min of recovery until the 35th minute. Data are presented as means ± S.E.M. * indicates that the mean value is significantly different from the pre-fatigue mean value of the same muscle group at time = −10 min (ANOVA and MCT, $P < 0{\cdot}05$). † indicates that the mean value is significantly different from the mean value of the control group at that time (ANOVA and MCT, $P < 0{\cdot}05$).

Gasser & Vaughan-Jones, 1990), it is still unknown whether or not 100 μmol l^{-1} glibenclamide affects these channels in skeletal muscle. P. E. Light & R. J. French (personal communication, University of Calgary, Medical Physiology, Calgary, Alberta, Canada) tested the effect of 10 and 100 μmol l^{-1} glibenclamide on rat skeletal muscle K^+_{ATP} channel and K^+ delayed rectifier incorporated into lipid bilayers. They found that at a membrane potential of +40 mV, 10 and 100 μmol l^{-1} glibenclamide reduced the open probability of the K^+_{ATP} channel by 25 and 95 % respectively, while it had no effect on the open probability and mean channel current of K^+ delayed rectifiers.

The action potential depolarization phase

Glibenclamide can also affect the repolarization phase if it slows down the depolarization phase. There is now evidence that tolbutamide, another sulphonylurea inhibitor of the K^+_{ATP} channel, affects the depolarization phase significantly, possibly by inhibiting the Na^+ channel (Comtois *et al.* 1993). The half-depolarization time and maximum rate of depolarization were calculated to determine if glibenclamide has any effect on Na^+ channels. In unfatigued muscle fibres, the mean half-depolarization time was not significantly affected by a 60 min exposure to 100 μmol l^{-1} glibenclamide

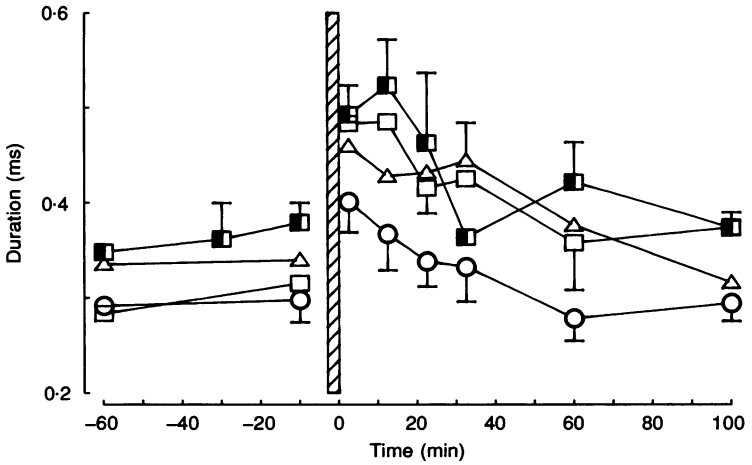

Figure 4. Effect of 100 μmol l^{-1} glibenclamide on the half-depolarization time
The half-depolarization time was defined as the time interval from 50 % of the action potential amplitude to the overshoot. Fatigue was produced with 100 ms trains of pulses every 1 s for 3 min. Symbols: ○, control muscles (number of bundles, $n = 7$); ◧, pre-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide 60 min prior to fatigue and remained present until the end of the experiment; □, during-fatigue-treated fibres ($n = 4$) were exposed to glibenclamide 60 s prior to the end of fatigue until the 35th minute of recovery; △, post-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide after 5 min of recovery until the 35th minute. Data are presented as means ± S.E.M. No significant differences were observed between control and glibenclamide-exposed muscles (ANOVA, $P > 0{\cdot}05$).

(Fig. 4). During fatigue the mean half-depolarization time increased by about 34 % in the absence of glibenclamide (control and post-treated group) and by 32 % in the presence of glibenclamide (pre-treated group). Following fatigue, the half-depolarization time returned to pre-fatigue values within 30 min in all groups of muscles tested.

Glibenclamide had no significant effect on the maximum rate of depolarization of unfatigued muscle fibres as the mean values were 387 ± 37 V s^{-1} before and 357 ± 24 V s^{-1} after 50 min of glibenclamide exposure (results not shown). During fatigue the maximum rate of depolarization of control muscle fibres decreased from 409 ± 28 to 256 ± 37 V s^{-1}. The addition of 100 μmol l^{-1} either 60 min before fatigue or 60 s prior to the end of fatigue caused a similar decrease in the maximum rate of depolarization as the values after fatigue were 201 ± 16 and 224 ± 18 V s^{-1} respectively. As with the half-depolarization time, glibenclamide did not affect the recovery of the maximum rate of depolarization which returned to pre-fatigue values within 30 min.

Table 2. The effect of 100 μmol l^{-1} glibenclamide on the tetanic contraction during fatigue development

	Tetanic force (%)		Force-200 (%)		Maximum rate of relaxation (%)	
Time (s)	Control	Pre-fatigue treatment	Control	Pre-fatigue treatment	Control	Pre-fatigue treatment
0	100	100	98·4 ± 0·3	98·4 ± 0·5	100	100
30	73·3 ± 2·1	71·2 ± 2·3	91·5 ± 4·8	96·9 ± 1·2	36·1 ± 2·5	35·8 ± 3·1
60	52·7 ± 1·5	49·6 ± 3·2	91·1 ± 2·2	98·4 ± 0·7	18·2 ± 1·6	17·1 ± 1·4
90	34·2 ± 1·7	28·5 ± 3·7	87·7 ± 2·3	98·4 ± 0·7	10·5 ± 0·9	11·8 ± 2·0
120	22·5 ± 1·4	15·4 ± 1·8	84·1 ± 3·2	95·2 ± 1·3	9·5 ± 2·0	7·6 ± 0·9
180	11·1 ± 1·0	4·9 ± 0·8	86·3 ± 2·4	91·2 ± 0·5	7·8 ± 2·1	6·2 ± 1·3

Fatigue was produced with 100 ms trains of pulses every 1 s for 3 min; every 30 s the train duration was increased to 200 ms to measure the kinetics of the tetanic contraction. Tetanic force (the maximum force generated during a contraction) and the maximum rate of relaxation are expressed as a percentage of the pre-fatigue value (time = 0). Force-200 (the force measured when the last pulse was applied at 200 ms) is expressed as a percentage of the maximum or tetanic force during that same contraction. Experimental conditions: control muscles ($n = 7$); pre-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide 60 min prior to fatigue. Data are presented as means ± S.E.M. No significant differences were observed between control and glibenclamide-exposed muscles (ANOVA, $P > 0{\cdot}05$).

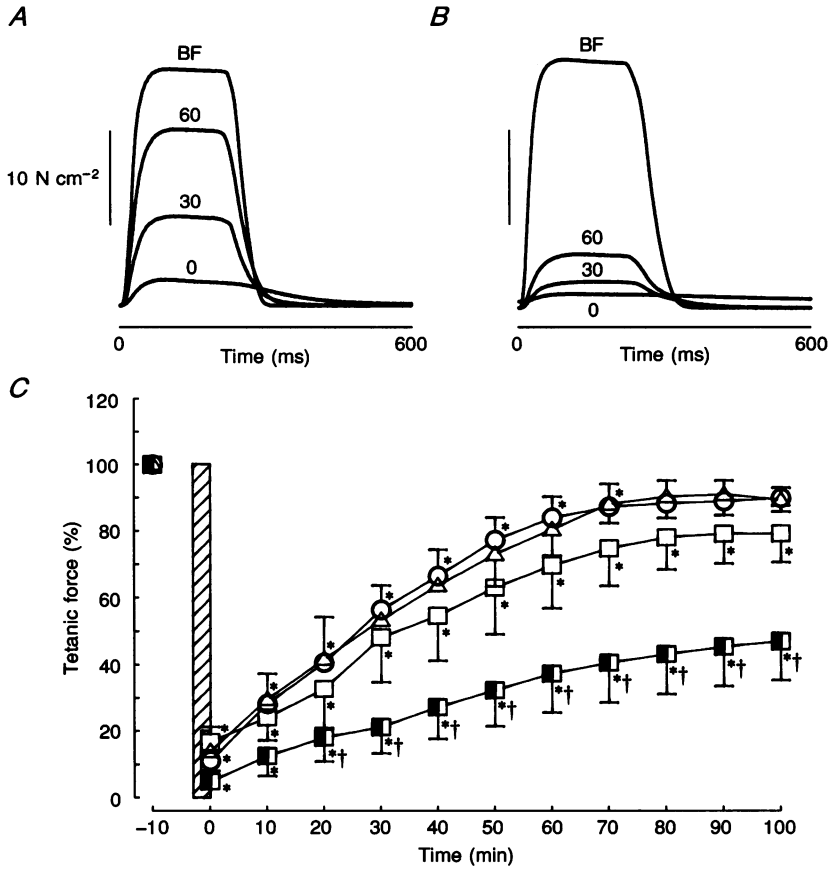

Figure 5. The effect of 100 μmol l^{-1} glibenclamide on the recovery of the tetanic force following fatigue

Fatigue was produced with 100 ms trains of pulses every 1 s for 3 min. *A*, recovery of the tetanic force in the absence of glibenclamide; BF, before fatigue; numbers represent the time during recovery. *B*, recovery of the tetanic force in the presence of 100 μmol l^{-1} glibenclamide applied 60 min prior to fatigue. *C*, tetanic force is expressed as a percentage of the pre-fatigue value (time = −10 min). Hatched bar represents the 3 min fatigue period. Symbols: ○, control muscles ($n = 7$); ◧, pre-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide 60 min prior to fatigue and remained present until the end of the experiment; □, during-fatigue-treated fibres ($n = 4$) were exposed to glibenclamide 60 s prior to the end of fatigue until the 35th minute of recovery; △, post-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide after 5 min of recovery until the 35th minute. Data are presented as means ± S.E.M. * indicates that the mean value is significantly different from the pre-fatigue mean value of the same muscle group at time = −10 min (ANOVA and MCT, $P < 0.05$). † indicates that the mean value is significantly different from the mean value of the control group at that time (ANOVA and MCT, $P < 0.05$).

The effect of glibenclamide on tetanic contraction

The tetanic force of unfatigued muscle bundles was not significantly affected by glibenclamide: during the 60 min preceding fatigue, the tetanic force of glibenclamide exposed muscles decreased by 5·1 ± 1·4 % which is comparable to a 1·8 ± 2·0 % decrease for control muscles.

Effects of glibenclamide during fatigue development

It has been proposed by several investigators that the activation of K^+_{ATP} channels during fatigue contributes to the K^+ efflux and to the decrease in force (Ashcroft, 1988; Davies, 1990). So in the presence of glibenclamide a slower decrease in tetanic force was expected during fatigue development. However, when muscle fibres were exposed to 100 μmol l^{-1} glibenclamide 60 min prior to fatigue, the decrease in tetanic force was always slightly greater than for control muscles (Table 2). When glibenclamide was applied 60 s prior to the end of fatigue the rate and magnitude of the mean tetanic force decrease during the last 60 s of fatigue was unaffected (results not shown).

Many muscle fibres failed to maintain a constant force during the 'plateau phase' of the tetanus following fatigue (Renaud, 1989). Force-200, an appraisal of the ability of the muscle to maintain a constant force during the plateau phase, was measured at the end of the 200 ms train of stimuli and was expressed as a percentage of the maximum

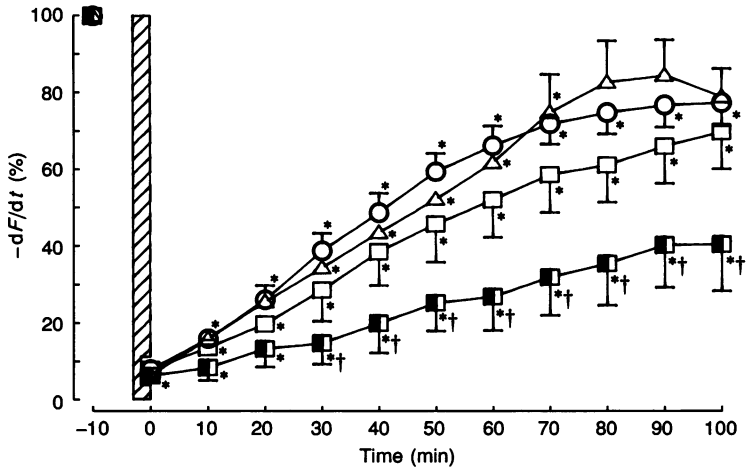

Figure 6. The effect of 100 μmol l^{-1} glibenclamide on the recovery of the maximum rate of relaxation following fatigue

Tetanic force is expressed as a percentage of the pre-fatigue value (time = −10 min). Hatched bar represents the 3 min fatigue period during which muscle fibres were stimulated every 1 s. Symbols: ○, control muscles ($n = 9$) are those not exposed to glibenclamide; ◧, pre-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide 60 min prior to fatigue and remained present until the end of the experiment; □, during-fatigue-treated fibres ($n = 4$) were exposed to glibenclamide 60 s prior to the end of fatigue until the 35th minute of recovery; △, post-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide after 5 min of recovery until the 35th minute. Data are presented as means ± S.E.M. * indicates that the mean value is significantly different from the pre-fatigue mean value of the same muscle group at time = −10 min (ANOVA and MCT, $P < 0.05$). † indicates that the mean value is significantly different from the mean value of the control group at that time (ANOVA and MCT, $P < 0.05$).

tetanic force. In the absence of glibenclamide, the decrease in force-200 was quite variable and non-significant. The addition of glibenclamide 60 min before fatigue did not affect the change in force-200 during fatigue development when compared to control muscle fibres.

During fatigue development, the decrease in the maximum rate of relaxation was much faster than for the tetanic force, and most of the decrease was observed during the first minute of stimulation. The decrease in the maximum rate of relaxation during fatigue in the presence of glibenclamide was similar to the decrease observed with control muscles.

The effects of glibenclamide during the recovery period

Following fatigue, the tetanic force in the control group increased and was not significantly different from the pre-fatigue value after 80 min (Fig. 5*A* and *C*). When 100 μmol l^{-1} glibenclamide was applied 60 min before fatigue, the recovery of tetanic force was largely impaired and became significantly different from control after 20 min (Fig. 5*B* and *C*). When glibenclamide was applied 60 s prior to the end of fatigue, the recovery of force was also less than control, but the effect of glibenclamide was much

Table 3. The effect of 100 μmol l^{-1} glibenclamide on the recovery of force-200

Time (min)	Control	Pre-fatigue treatment	During-fatigue treatment	Post-fatigue treatment
BF	98·4 ± 0·3	98·4 ± 0·5 *	98·3 ± 0·9	98·8 ± 0·3
0	86·3 ± 2·4	91·2 ± 0·5*	93·7 ± 1·2*	88·7 ± 4·4
10	90·7 ± 1·6	88·6 ± 3·1*	96·8 ± 1·5*	91·8 ± 2·5*
20	95·0 ± 1·0	94·8 ± 0·7*	96·6 ± 1·7*	96·6 ± 1·0*
30	96·2 ± 0·9	94·6 ± 1·2*	98·9 ± 0·4*	96·5 ± 1·1*

Fatigue was produced with 100 ms trains of pulses every 1 s for 3 min. Force-200 is expressed as a percentage of the maximum or tetanic force during that same contraction. BF: before fatigue. Experimental conditions: control muscles ($n = 7$); pre-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide 60 min prior to fatigue; during-fatigue-treated fibres ($n = 4$) were exposed to glibenclamide 60 s prior to the end of fatigue; post-fatigue-treated fibres ($n = 5$) were exposed to glibenclamide after 5 min of recovery. * Data obtained while muscles were exposed to glibenclamide. Data are presented as means ± S.E.M. No significant differences were observed between control and glibenclamide-exposed muscles (ANOVA, $P > 0.05$).

smaller (Fig. 5C). The application of glibenclamide after 5 min of recovery did not reduce the capacity of the muscle to recover force; as with the control group, the tetanic force was not significantly different from the pre-fatigue value after 80 min of recovery.

In the absence or presence of glibenclamide force-200 recovered rapidly following fatigue and was similar to pre-fatigue values within 20 min (Table 3). In the control group following fatigue the mean maximum rate of relaxation recovered to $77{\cdot}4 \pm 3{\cdot}9$ % of pre-fatigue values after 100 min (Fig. 6). When 100 μmol l^{-1} was applied 60 min before fatigue, the recovery of the maximum rate of relaxation was largely impaired and became significantly different to control after 30 min. When glibenclamide was applied 60 s prior to the end of fatigue, the recovery of the maximum rate of relaxation was also less than control. Addition of glibenclamide after 5 min of recovery did not significantly affect the recovery of the maximum rate of relaxation.

The slower recovery of the maximum rate of relaxation induced by the addition of glibenclamide can be related to two factors: (1) the relaxation process is not recovering, or (2) the mechanism of the relaxation phase recovers normally but the maximum rate of relaxation does not because of the associated lower tetanic force. When the maximum rate of relaxation was divided by the tetanic force the resulting ratio after 20 min recovery was the same for all four groups of muscles tested. This therefore suggests that the apparent slow recovery of the maximum rate of relaxation was due to a lower tetanic force from which to relax and not an effect of glibenclamide on the relaxation process.

DISCUSSION

In this study we have examined the effects of glibenclamide in frog sartorius muscle. Our results showed that (1) glibenclamide decreases the membrane conductance of metabolically exhausted fibres, (2) glibenclamide causes an increase in the half-repolarization time and a decrease in the maximum rate of repolarization of unfatigued sartorius muscle fibres, (3) this glibenclamide effect is also observed in fatigued muscle fibres, but to a greater extent, (4) addition of glibenclamide prior to or during fatigue has little effect on the magnitude and rate of force decrease during fatigue but impairs force recovery following fatigue, and (5) addition of glibenclamide during recovery slows down the recovery of the repolarization phase, but does not affect the recovery of the tetanic force.

The specificity and the time course of the glibenclamide effect

Tolbutamide and glibenclamide are two sulphonylurea compounds which are known to specifically block K^+_{ATP} channels (Sturgess, Ashford, Cook & Hales, 1985; Ashford *et al.* 1988; Gasser & Vaughan-Jones, 1990). However, in frog sartorius muscle tolbutamide was found to have several non-specific effects, including a reduction in tetanic force, a depolarization of the sarcolemma, a decrease in the maximum rate of depolarization and in some cases a complete inactivation of the sarcolemma (Comtois *et al.* 1993). This study shows that the effects of glibenclamide are more specific than those of tolbutamide in frog sartorius muscle. Firstly, in frog sartorius muscle the K^+ inward rectifiers play a major role in the membrane conductance and the maintenance of the resting potential. Since glibenclamide has no effect on either of these two parameters, it can be proposed that glibenclamide does not affect the K^+ inward rectifiers; this finding is in accord with previous studies in frog skeletal muscle (Standen *et al.* 1992) and mammalian cardiac muscle (Escande *et al.* 1989; Gasser & Vaughan-Jones, 1990). Secondly, glibenclamide has no effect on the maximum rate of depolarization and the half-depolarization time (Fig. 4) of action potentials in both unfatigued and fatigued muscle fibres. So, glibenclamide does not affect Na^+ channels (unlike tolbutamide). More importantly, any effect of glibenclamide on the repolarization phase is then expected to be an effect on K^+ channels and not a consequence of a slower depolarization phase.

In unfatigued muscle fibres, glibenclamide prolongs the repolarization phase as the half-repolarization time increases by 19 % (Fig. 3C) and the maximum rate of repolarization decreases by 17 %. An effect of glibenclamide on K^+ delayed rectifiers is unlikely because 100 μmol l^{-1} does not affect these channels in rat skeletal muscle (P. E. Light & R. J. French, personal communication). Furthermore, a similar prolongation of the repolarization phase in the presence of glibenclamide has also been reported in mammalian cardiac fibres (Faivre & Findlay, 1989), a tissue in which glibenclamide has no effect on K^+ delayed rectifiers (Escande, Thuringer, Le Guern, Courtieix, Laville & Cavero, 1989; Gasser & Vaughan-Jones, 1990). More importantly, the open probability of frog muscle K^+_{ATP} channels under patch clamp conditions is not zero at physiological concentrations of ATP_i (1–5 mmol l^{-1}) and increases as the membrane is depolarized (Spruce *et al.* 1985; Spruce, Standen & Stanfield, 1987; Vivaudou, Arnoult & Villaz, 1991). Considering the high density of K^+_{ATP} channels of this muscle preparation (Spruce *et al.* 1985), we therefore propose that in unfatigued muscle fibres a small fraction of K^+_{ATP} channels become active during an action potential and contribute to the repolarization phase.

Addition of 100 μmol l^{-1} glibenclamide 60 s prior to fatigue resulted in a similar effect on the repolarization phase to that produced by a 60 min pre-fatigue exposure (Fig. 3C). Since APs were measured 3 min after fatigue, it then appears that glibenclamide is capable of blocking K^+_{ATP} channels in less than 4 min. This is in agreement with previous studies using intact frog muscle fibres in which 1–10 μmol l^{-1} glibenclamide blocks the K^+_{ATP} current activated by cromakalin and SR44866 in less than 2 min (Sauviat *et al.* 1991; Benton & Haylett, 1992). The time course of the

glibenclamide effect in unfatigued and fatigued frog muscle fibres is, however, strikingly shorter than the effect observed in metabolically exhausted muscle fibres for which the change in membrane conductance (Fig. 2) and K^+ efflux (Castle & Haylett, 1987) takes 10 min before a new steady state is reached following the addition of 100 μmol l^{-1} glibenclamide. The ATP_i concentrations of metabolically exhausted muscle fibres are drastically reduced (Fink & Luttgau, 1976; Spruce *et al.* 1987) compared to unfatigued or fatigued muscle fibres (Mainwood, Worsley-Brown & Paterson, 1972). Recently, Vivaudou *et al.* (1991) showed that glibenclamide was less efficient in blocking K^+_{ATP} channels in the absence than in the presence of 3 mmol l^{-1} ATP_i. Also, if the effect of glibenclamide on the repolarization phase of fatigued muscle fibres (Fig. 3*C*) is compared with the effect on the membrane conductance of metabolically exhausted fibres (Fig. 2), it can be assumed that the latter fibres have a much greater proportion of activated K^+_{ATP} channels. Therefore, the slower effect of glibenclamide in metabolically exhausted muscle fibres is most probably due to these fibres possessing a much lower ATP_i concentration and a greater number of activated K^+_{ATP} channels than unfatigued and fatigued muscle fibres.

The activity and physiological role of K^+_{ATP} channels in fatigued muscle fibres

As discussed above we believe that a small number of K^+_{ATP} channels are active during the repolarization phase in unfatigued muscle fibres because the addition of glibenclamide increases the duration of the half-repolarization time and decreases the maximum rate of repolarization (Fig. 3*C*). Our results also suggest a further activation of K^+_{ATP} channels during fatigue because (1) the difference in the half-repolarization time between control and glibenclamide-exposed muscles after fatigue was 2-fold higher than the difference before fatigue, and (2) the maximum rate of repolarization of glibenclamide-exposed muscle measured immediately after fatigue was half the value of control muscles. Our results are also consistent with the study of Standen *et al.* (1992) who showed that the decrease in the intracellular pH in our fatigue model (reported earlier by Renaud, 1989) was large enough to activate K^+_{ATP} channels in unfatigued frog muscle.

The half-repolarization time and maximum rate of repolarization of muscle fibres exposed to glibenclamide during fatigue probably represents the repolarization phase of fatigued muscle fibres without the contribution of K^+_{ATP} channels; but more importantly without any side effect from having the K^+_{ATP} channels blocked at the onset of fatigue as the full effect of glibenclamide is observed in 1–2 min (Sauviat *et al.* 1991; Benton & Haylett, 1992). In fact, blocking the K^+_{ATP} channels at the onset of fatigue does not affect the repolarization phase any further because no differences in half-repolarization time and maximum rate of repolarization are observed between muscle fibres exposed to glibenclamide before or during fatigue. As reported earlier, the activation of K^+_{ATP} channels during fatigue is probably linked to the decrease in intracellular pH (Davies, 1990; Davies *et al.* 1991*a*, 1992; Standen *et al.* 1992) rather than the decrease in ATP_i concentration which is very small in this preparation (Mainwood *et al.* 1972).

When muscle fibres are fatigued in the presence of glibenclamide the half-repolarization times remained not only longer than control values during the recovery period, but they also returned to their pre-fatigue levels much later than control. The half-repolarization time in this study depends on the activity of K^+ channels located on the outer surface membrane (Hodgkin & Nakajima, 1972), mainly the K^+ delayed rectifiers and the K^+_{ATP} channels since K^+_{Ca} channels are exclusively located in the T-tubules (Spruce *et al.* 1987). Therefore, the half-repolarization time in the presence of glibenclamide should reflect the activity of the K^+ delayed rectifiers during recovery, whereas the difference in half-repolarization time between control and glibenclamide-exposed muscle should represent the contribution of K^+_{ATP} channels. On this basis, it appears as if the activity of the K^+ delayed rectifier has not returned to the pre-fatigue levels after 20 min of recovery, while the activity of the K^+_{ATP} channels is still above pre-fatigue levels, the net result being an apparent recovery of the half-repolarization time in control muscle.

If K^+_{ATP} channels are active for up to 20 min of recovery, then the addition of glibenclamide after 5 min of recovery should increase the half-repolarization time. However, there was no difference from control after 10 min of recovery, and after 20 min the half-repolarization time was similar to the value observed before fatigue in the presence of glibenclamide. So, contrary to our initial suggestion above, we propose that (1) after 20 min of recovery the activity of both K^+ delayed rectifier and K^+_{ATP} channels has returned to pre-fatigue levels, and (2) the slow recovery of the half-repolarization time in muscle fibres exposed to glibenclamide during fatigue is due to some other effect acting during the recovery period and not during fatigue. In fact, this slow recovery of the half-repolarization time is similar to the slow recovery of the tetanic force under these conditions (see below for further discussion).

As proposed in the Introduction, the activation of K^+_{ATP} channels during fatigue should contribute to the force decrease, providing a protective mechanism to prevent irreversible damage. Data from this study do not support the first part of this notion, as pre-treatment with glibenclamide did not affect significantly any of the force parameters (tetanic force, force-200 and maximum rate of relaxation) measured during fatigue. ATP_i levels are well buffered in skeletal muscle and often remain constant until force was decreased by up to 50 %, before starting to decline (Dawson, Gadian & Wilkie, 1978). An initial increase in intracellular pH is expected as phosphocreatine is hydrolysed at the onset of exercise (Mainwood & Renaud,

1985). Consequently, K^+_{ATP} channels would not be expected to contribute largely to the K^+ efflux and decrease in tetanic force during the first part of our fatigue protocol.

The impairment of force recovery from muscles fatigued in the presence of glibenclamide is, however, evidence of an important role for K^+_{ATP} channels. The delay in the recovery of both the K^+ inward rectifier and tetanic force suggests that following fatigue, in the presence of glibenclamide, muscles are not in a similar condition to those in control and some deleterious events are affecting the recovery process. Therefore our data support the idea that K^+_{ATP} channels do have a protective role as previously hypothesized. Furthermore, the difference in the impairment of force recovery between muscles exposed to glibenclamide 60 min prior to fatigue and those exposed for only the last 60 s of the fatigue suggest that K^+_{ATP} channels are indeed activated early in the fatigue stimulation and early blockage of the channel openings will cause more damage than will blocking the channels later in the fatigue run; that is, most of the protective mechanism occurs early in the fatigue protocol. The absence of any effect of glibenclamide in unfatigued muscle and on force recovery when glibenclamide is applied after fatigue also infers that the events which impair force recovery occur during fatigue itself and not either before fatigue or during the recovery period.

The nature of the protective mechanism or the exact cause of the slow recovery in glibenclamide-exposed muscles cannot be determined from the results obtained in this study. Several causes can be considered, including greater changes in metabolite concentrations, greater shifts in ion movement, or muscle damage. Further studies will be necessary to elucidate these events.

In conclusion, our results provide good evidence for the contribution of K^+_{ATP} channels to the repolarization phase of action potentials in unfatigued frog sartorius muscle fibres and a further activation of these channels during a 3 min fatigue. Although there was no apparent faster decrease in force during fatigue when K^+_{ATP} channels were blocked by glibenclamide, the slow recovery of tetanic force in glibenclamide-exposed muscle fibres strongly supports the notion that K^+_{ATP} channels play an important protective role and that this protection occurs early during the development of fatigue.

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