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Effects of mexiletine on ATP sensitive K^+ channel of rat skeletal muscle fibres: a state dependent mechanism of action

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1 The effects of mexiletine were evaluated on the ATP-sensitive K^+ channel (K_{ATP}) of rat skeletal muscle fibres using patch clamp techniques. The effects of mexiletine were studied on macropatch currents 20 s (maximally activated), 8 min (early stage of rundown) and 15 min (late stage of rundown) after excision in the absence or in the presence of internal ADP (50–100 μM) or UDP (500 μM). In addition, the effects of mexiletine were tested on single channel.

2 In the absence of ADP and UDP, mexiletine inhibited the current through maximally activated channels with an IC_{50} of -5.58 ± 0.3 M. Nucleoside diphosphates shifted the current versus mexiletine concentration relationship to the right on the log concentration axis. UDP (500 μM) was more efficacious than ADP (50–100 μM) in this effect.

3 At the early stage of rundown, the sensitivity of the channel to mexiletine was reduced and nucleoside diphosphates, particularly UDP, antagonized the effect of mexiletine. At the late stage of rundown, mexiletine did not affect the currents.

4 At the single channel level, 1 μM mexiletine reduced the mean burst duration by 63% and prolonged the arithmetic mean closed time intervals between the bursts of openings without altering the open time and closed time distributions. Mexiletine did not affect the single channel conductance.

5 These results show that in skeletal muscle, mexiletine is a state-dependent K_{ATP} channel inhibitor which either acts through the nucleotide binding site or a site allosterically coupled to it.

Keywords: Mexiletine; ADP; UDP; ATP-sensitive K^+ channel; skeletal muscle fibres

Introduction

Mexiletine is a well known antiarrhythmic Na^+ channel blocker of the class Ib which is used in the treatment of muscular disorders linked to abnormal excitability of skeletal muscle fibres, such as hyperkalaemic periodic paralysis and myotonias (Jackson *et al*., 1994; Lehmann-Horn & Rudel, 1996; De Luca *et al*., 1997). The common view is that this drug exerts its therapeutic effects by selectively blocking the voltage-dependent Na^+ channel present in both cardiac and skeletal muscle fibres. This results in a reduction of the number of available Na^+ channels and in the shortening of the action potential duration in skeletal muscle (De Luca *et al*., 1995). However, there is evidence (Wu *et al*., 1992; Moser *et al*., 1995; Olschewski *et al*., 1996) that some antiarrhythmic drugs of the Ia and Ib classes also interact with other ion channels such as the ATP sensitive K^+ channels (K_{ATP}) which are present in high density in cardiac and skeletal muscle fibres (Terzic *et al*., 1995; Tricarico *et al*., 1997). For example, therapeutic concentrations (6–15 μM) of disopyramide inhibit the cardiac and skeletal muscle K_{ATP} channels and this effect is potentiated by lowering of the internal pH suggesting a beneficial role of the drug in preventing the characteristic K^+ loss and arrhythmias which occur during acute myocardial ischaemia and muscle fatigue (Mortensen *et al*., 1993; Moser *et al*., 1995). However, how mexiletine affects K_{ATP} channel is still controversial. In ventricular myocytes a high concentration of mexiletine did not significantly affect the K_{ATP} current induced by 2,4-dinitrophenol (Wu *et al*., 1992), while it reduced the K_{ATP} current recorded in Xenopus oocyte but with an IC_{50} of 789 μM (Yoneda *et al*., 1993). More recently, it has been shown that, after rundown, mexiletine stimulates cardiac K_{ATP} channels in the presence of uridine diphosphate (UDP; Sato *et al*., 1995). These observations suggest that the drug affects the K_{ATP} channel by a state-dependent mechanism.

To test this hypothesis, the effects of mexiletine were evaluated on skeletal muscle K_{ATP} channels maximally activated, or after the time-dependent decay of the current (rundown). The experiments were performed in the presence and in the absence of internal adenosine diphosphate (ADP) or uridine diphosphate, known physiological activators of the K_{ATP} channels in various tissues including skeletal muscle (Allard & Lazdunski, 1992).

Methods

Isolation of single fibres

Single fibres were prepared from flexor digitorum brevis muscles of male adult Wistar rats of 5–6 months of age by enzymatic treatment as previously described (Tricarico & Conte Camerino, 1994).

Electrophysiology

Experiments were performed on inside-out membrane patches using standard patch-clamp techniques. Recordings of K_{ATP} current (**I**) were performed on macropatches during voltage steps from 0 mV (holding potential) to -60 mV with 150 mM KCl on both sides of the membrane patches, at 20°C. The

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spontaneous time-dependent decay of the current was observed at a constant voltage of −60 mV. The currents, sampled at 5 kHz and filtered at 0.5 kHz, were recorded using a VCR recording system and played back later for computer analysis (Tricarico *et al*., 1998). Recordings of single channel currents were performed on micropatches at a constant voltage, at 20°C, with 150 mM KCl on both sides of the membrane, at 20 kHz sampling rate and filtered at 2 kHz. The single channel current was also measured at various potentials (from −70 mV to +70 mV). Both macropatch currents and single channel currents were recorded using Axon hardware and pClamp software (Tricarico & Conte Camerino, 1994). The effects of mexiletine on macropatch currents were evaluated in the absence or in the presence of internal ADP (50–100 μM) or UDP (500 μM). Effects on channels under similar conditions were determined at −60 mV about 20 s (maximally activated), 8±1.5 min ($n=21$) (early stage of rundown) and 15±3 min ($n=21$) (late stage of rundown) after excision and exposure to MgATP (500 μM) or in the absence of ATP. Pipettes were prepared as previously described (Tricarico & Conte Camerino, 1994). The tip opening areas of the pipettes were measured by scanning electron microscopy (Cambridge Instruments). Measurements of patch conductance and tip opening area were performed on the same pipettes according to the method of Sakmann & Neher (1983). A linear correlation between the pipette conductance and the tip opening area has been found in the range of conductance from 50 nS to 1600 nS. The slope of the straight line was 0.00698, the intercept was 0.302 and the coefficient of correlation was 0.778. Macropipettes having an average tip opening area of 4.9±0.1 μm^2 ($n=44$) were used to measure the current sustained by multiple channels (25–35 channels/patch area) and the pharmacological properties of the K_{ATP} channels. The single channel conductance and kinetics were measured using micropipettes having a tip opening area of 0.9±0.1 μm^2 ($n=58$). Using this type of pipette, no more than 2–3 open channels were observed in the patches. A few micropatches, 3 out of 58, contained only a single unit. This was tested by observing the single channel transitions for a period of times of 40–60 s with 50 μM MgADP in the bath, a condition which assured the maximal channel open probability (Allard & Lazdunski, 1992).

Analysis

The current (**I**) flowing through the macropatches was calculated by subtracting the baseline level of the current (defined as the closed state of the channels and measured in the presence of ATP) from the open channel level. The macropatches were exposed to mexiletine for 30–120 s. The concentration-response relationships could be fitted with the following equation:

$$\text{I drug/I control} = 1/(1 + ([\text{Drug}]/IC_{50})^{n})$$

were **I** drug/**I** control is the ratio between the current measured in presence and in absence of mexiletine; IC_{50} is the concentration of mexiletine needed to reduce the current by 50%; n is the Hill coefficient of the curves; [Drug] is the mexiletine concentration.

The single channel conductance was calculated as the slope of the current-voltage relationship of the channel in the range of potentials from −70 mV to −10 mV and in the range of potentials from +10 mV to +70 mV. No correction was made for the liquid junction potential which was estimated to be less than +1.9 mV under our experimental conditions. The overall open probability (P_{open}) was measured as the ratio between the time spent by the channel in the open state and the total time of recording. Kinetic analysis was performed within the bursts of openings when single channels were active in the patches. To evaluate the mean burst durations in the absence and in the presence of mexiletine, the closed time interval separating the bursts of openings was evaluated. This parameter was calculated by plotting the maximum number of closing events within the bursts of openings versus increasing durations of the closed time. The goal of this type of analysis is to find the minimum closed time at which the number of closing events are relatively insensitive to further increase of this parameter. In our experiments, we found that for short closed times (between 1 ms and 20 ms), the number of closures within the bursts was linearly correlated with the duration of the closed times. For durations longer than 20 ms, no significant change occurred in the number of closing events within the bursts of openings. Therefore, with a single active channel in the patches, we calculated a closed time interval between bursts of 20±2 ms ($n=3$). The open and closed time distributions could be fitted with the following equation:

$$f(t) = P_1(1/\tau_1)\exp(-t/\tau_1) + P_2(1/\tau_2)\exp(-t/\tau_2)$$

were f(t) is the number of counts at determined time intervals, P_1 and P_2 are the fractional contributions for the respective components to the area under the curves; τ_1 and τ_2 are the time constants of each component; t is the fractional time. The effects of the drug on the long closed time intervals were quantified by determining the arithmetic mean of the long closed time intervals, between the bursts of openings, in the absence and in the presence of the drug. The algorithms of the fitting procedures used were based on the Marquardt least-squares fitting routine. Significant differences between individual pairs of means were determined using a paired Student *t*-test. The data are expressed as mean±s.d. The reported IC_{50} values represent means of log IC_{50}±s.d. calculated from each experiment.

Drugs and solutions

The solutions had the following composition: Pipette, 150 mM KCl, 2 mM $CaCl_2$, 10 mM MOPS, pH=7.2; Bath, normal Ringer 145 mM NaCl, 5.5 mM KCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM glucose, 10 mM MOPS, pH=7.2; symmetrical K^+ 150 mM KCl, 0.5 mM EGTA, 10 mM MOPS, pH=7.2. Stock solutions (5 mM) of the nucleotide tested, adenosine triphosphate magnesium salt (MgATP), adenosine diphosphate magnesium salt (MgADP) and uridine diphosphate magnesium salt (MgUDP) were prepared by dissolving the chemicals in the symmetrical K^+ solution. Mexiletine chloride was first dissolved in symmetrical K^+ solution at concentrations of 0.28 M. The nucleotides and drug were diluted in the symmetrical K^+ solution as needed.

Results

Effects of mexiletine on K_{ATP} current

In our experiments three stages of the decay of the K_{ATP} current could be described (Figure 1). Stage 1: a maximal current of 180±18 pA ($n=21$) was recorded a few seconds after patch excision and started to decay after 3±1.7 min. At this stage ADP (50–100 μM) did not affect the current, whereas UDP (500 μM) tended to increase the current amplitude, although this effect was not significant. Stage 2: the current reached a new value of 23±2 pA ($n=21$)

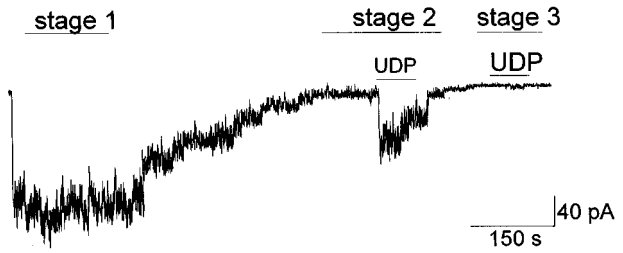

Figure 1 Time-dependent decay of the K_{ATP} current in an excised macropatch (pipette area = 4.8 μm^2) and effect of UDP. Sample trace of continuous recording of K_{ATP} current performed at −60 mV, at 20°C, on inside-out patch with 150 mM KCl on both sides of the membrane. The decay of the current could be described by three stages which were distinguished on the basis of their specific current amplitude. After excision, the current remained stable for 4 min (stage 1, time frames 20 s–4.5 min) then slowly decayed reaching a new level of lower amplitude (stage 2, time frames = 9–14 min). 14 min after excision the patch became silent (stage 3). In stage 2, the application of UDP (500 μM) to the inside-out macropatch induced a reversible stimulation of the channel current. In contrast, in stage 3, UDP at the same concentration failed to stimulate the channel currents.

(significantly different in respect to that measured in stage 1, $P<0.001$), 8 ± 1.5 min after patch excision and was sensitive to the stimulatory effect of the nucleoside diphosphates (Figure 1). Stage 3: 15 ± 3 min ($n=21$) after excision the patch became silent and neither ADP not UDP resuscitated the channels (Figure 1).

In the stage 1, the rapid perfusion of the macropatches with a bath solution containing mexiletine (1 nM – 1 mM) inhibited the K_{ATP} current (Figure 2a). Although the washout did not reverse the inhibitory effect of mexiletine (Figure 2a), the current was completely restored by application of 50 – 100 μM ADP (Figure 2a) or 500 μM UDP.

Mexiletine (1 nM – 1 mM) inhibited the K_{ATP} currents dose-dependently with a log IC_{50} of −5.58 ± 0.3 M (slope = 0.59) ($n=6$) (Figure 2d). Exposure of the macropatches to a solution containing mexiletine and ADP (50 μM) (Figure 2b) resulted in a less pronounced inhibitory effect of mexiletine on the K_{ATP} current. ADP (50 – 100 μM) induced a parallel rightward shift on the log dose axis of the concentration-response curves of the K_{ATP} current *versus* mexiletine concentrations (Figure 2d). The log IC_{50} values for mexiletine were −5.21 ± 0.3 M (slope = 0.55) ($n=5$) and −4.96 ± 0.2 M (slope = 0.53) ($n=4$) in the presence of 50 μM and 100 μM ADP, respectively, being significantly different ($P<0.001$) in respect to that calculated in the absence of the nucleotide. Under these experimental conditions, washout partially restored the current. The exposure of the macropatches to a solution containing UDP (500 μM) and mexiletine resulted in an almost complete loss of the inhibitory effect of the drug on the K_{ATP} current (Figure 2c). A certain inhibitory effect of the drug was observed exclusively at high concentrations (Figure 2e), whereas the increase in the current amplitude which was not significant but which was occasionally observed with low concentrations of mexiletine (1 μM) was due to the intrinsic stimulatory effect of UDP (500 μM) (Figure 2e).

In the stage 2, mexiletine (100 nM – 5 mM) weakly reduced the K_{ATP} currents with a log IC_{50} value of −3.56 ± 0.1 M (slope = 0.5) ($n=3$) (Figure 3a and d). In the presence of internal 50 μM and 100 μM concentrations of ADP, mexiletine was even less potent reducing the current with log IC_{50} values of −3.19 ± 0.4 M (slope = 0.77) ($n=4$) and −3.06 ± 0.3 M (slope = 0.71) ($n=5$) (Figure 3a and c), respectively, being

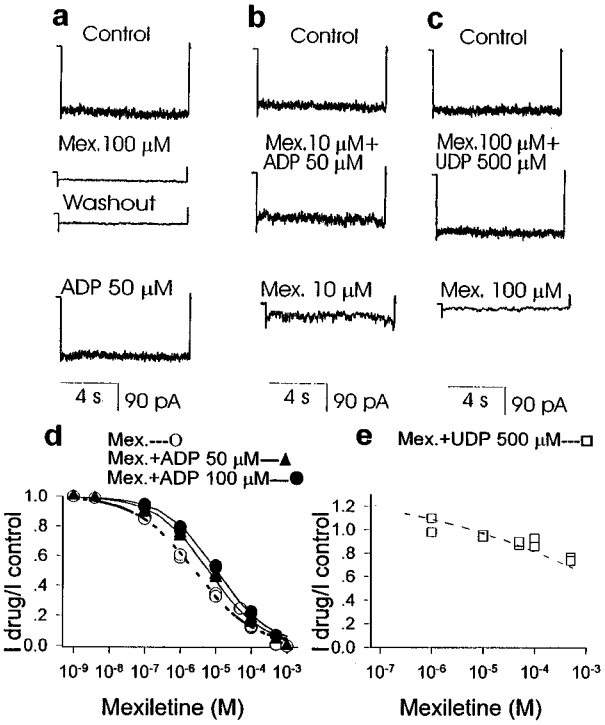

Figure 2 Effects of mexiletine on K_{ATP} currents of maximally-activated channels (stage 1) recorded in excised macropatches (pipette area = 4.9 – 5.1 μm^2) in the absence or in the presence of nucleoside diphosphates. The current recordings were performed during voltage steps going from 0 mV to −60 mV with 150 mM KCl on both sides of the membrane. Initial currents (Control) were recorded a few seconds after excision. (a) Mexiletine (Mex.) completely abolished the current and this effect was reversed only by application of internal ADP. (b,c) The nucleoside diphosphates antagonized the inhibitory effects of mexiletine on K_{ATP} currents. (d) Concentration-response relationships of relative K_{ATP} currents at −60 mV versus mexiletine concentrations were constructed (d) in the absence ($n=6$) or presence of 50 μM ($n=5$) or 100 μM ADP ($n=4$), or (e) in the presence of 500 μM UDP ($n=2$). The individual symbols represent the effects of the mexiletine alone or mexiletine with nucleoside diphosphates relative to controls from each patch. The nucleoside diphosphates antagonized the inhibitory effects of mexiletine on K_{ATP} currents.

significantly different ($P<0.001$) in respect to that calculated in the absence of the nucleotide. Also in this stage, in the presence of UDP (500 μM), low concentrations of mexiletine (0.1 μM – 1 μM) failed to inhibit the current ($n=2$) (Figure 3b), whereas a slight inhibitory effect of the drug was observed at high concentrations (Figure 3b). The increase in the current amplitude observed with the lowest concentration of mexiletine (0.1 – 1 μM) (Figure 3b) was due to UDP.

In the stage 3, mexiletine did not produce any stimulatory effect on the current either in the presence or in the absence of both nucleoside diphosphates.

Effects of mexiletine on single K_{ATP} channel

A few seconds after patch excision, the exposure of the patches to 1 μM mexiletine significantly reduced the overall open probability of the single K_{ATP} channel from 0.32 ± 0.02 ($n=3$) in the control to 0.12 ± 0.02 ($n=3$) in the presence of the drug ($P<0.001$). This effect was mediated by the reduction of the mean burst duration which was 48.66 ± 3.12 ms ($n=3$) in the control and 18.44 ± 2.10 ms ($n=3$) in the presence of mexiletine (Figure 4a) ($P<0.001$). The drug also prolonged the long closed time intervals between the bursts of openings.

This is demonstrated by the increase of the arithmetic mean of the long closed time intervals separating the bursts of opening which was 846.9±41.2 ms ($n=3$) in the control and 2259.1±261.2 ms ($n=3$) in the presence of mexiletine ($P<0.001$). The application of a higher concentration of mexiletine (10 μM) almost completely abolished channel openings (Figure 4a).

Kinetic analysis performed within the bursts of openings revealed that mexiletine did not alter the distribution of the channel open states. In the control condition the open dwell time distribution was well-fitted by the sum of two exponential functions with $\tau 1$ and $\tau 2$ values of 0.58±0.16 ms ($P=0.29\pm0.01$) ($n=3$) and 2.11±0.08 ms ($P=0.70\pm0.10$), respectively (Figure 4b). After mexiletine (1 μM) incubation $\tau 1$ and $\tau 2$ were 0.643±0.10 ms ($P=0.32\pm0.08$) ($n=3$) and 2.25±0.07 ms ($P=0.68\pm0.17$), respectively (Figure 4d). Also, no significant change in the closed dwell-time distribution was observed following mexiletine application. In the control condition the closed-dwell time distribution was well-fitted by the sum of two exponential functions showing $\tau 1c$ and $\tau 2c$ of 0.31±0.13 ms ($P=0.70\pm0.03$) ($n=3$) and 1.18±0.16 ms ($P=0.29\pm0.03$), respectively (Figure 4c). After mexiletine incubation the $\tau 1c$ and $\tau 2c$ values were 0.32±0.19 ms ($P=0.68\pm0.10$) ($n=3$) and 1.53±0.16 ms ($P=0.32\pm0.05$), respectively (Figure 4e).

Mexiletine did not affect the unitary conductance of the K_{ATP} channel. The slope conductance measured in the negative range of potentials was 69±6 pS in the control condition ($n=4$) and 70±5 pS in the presence of 1 μM concentration of the drug ($n=4$), whereas at the positive potentials it was 41±5 pS in the control condition and 39±6 pS in the presence of mexiletine (Figure 5a).

Unfortunately, we could not accurately measure the inhibitory effects of mexiletine on single channels after rundown; indeed, the large variability observed in the kinetic parameters after 15–20 min from excision led to ambiguous interpretation of the data. For example, the P_{open} ranged between 0.112 and 0.01 and the burst duration ranged between 5 ms and 21 ms. Also, it was often difficult to count the number of active channels present in the patches. However, we could measure the slope conductance of the K_{ATP} channel in the presence and in the absence of mexiletine. We found that, even after rundown, mexiletine did not affect the unitary conductance of the K_{ATP} channel. The slope conductance measured in the negative range of potentials was 67±5 pS in control conditions ($n=3$) and 68±7 pS ($n=3$) in the presence of 1 μM drug, while in the positive range of potentials it was 38±5 pS under control condition and 37±4 pS in the presence of the drug (Figure 5b).

Discussion

This is the first report which shows that mexiletine is a potent state-dependent K_{ATP} channel antagonist in skeletal muscle fibres. At least two mechanisms appear to explain the inhibitory effects of mexiletine on the K_{ATP} channel. Firstly, the fact that ADP and particularly UDP, a pure agonist of the K_{ATP} channel (Terzic *et al*., 1995), antagonized the inhibitory effects of mexiletine appears to be consistent with binding of

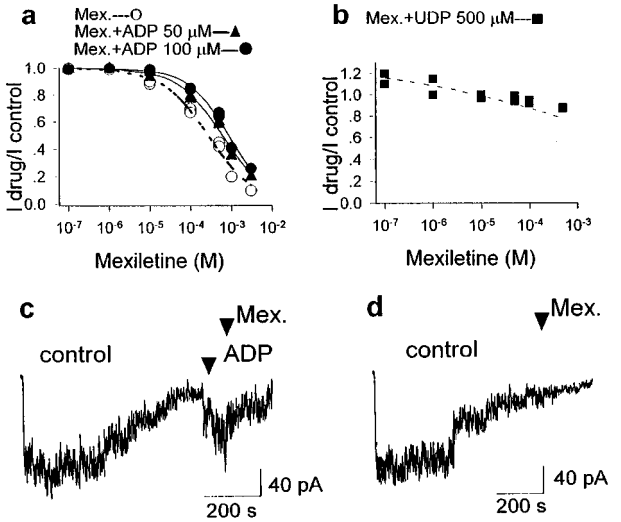

Figure 3 Effects of mexiletine on K_{ATP} currents during rundown (stage 2). Concentration-response relationships of relative K_{ATP} currents *versus* mexiletine concentrations were constructed (a) in the absence ($n=3$), or presence of 50 μM ($n=4$) or 100 μM ADP ($n=5$), or (b) in the presence of 500 μM UDP ($n=2$). The individual symbols represent the effects of the mexiletine alone or mexiletine with nucleoside diphosphates relative to controls from each patch. (c) Original trace showing stimulation of K_{ATP} current by 50 μM ADP. Subsequent application of 500 μM mexiletine (Mex.) only partially inhibited the current in the presence of the nucleoside diphosphate. (d) Application of 500 μM mexiletine alone reduced the residual current. All results were obtained from inside-out macropatches (pipette area = 4.8–5.2 μm^2), at −60 mV, with 150 mM on both sides of the membranes.

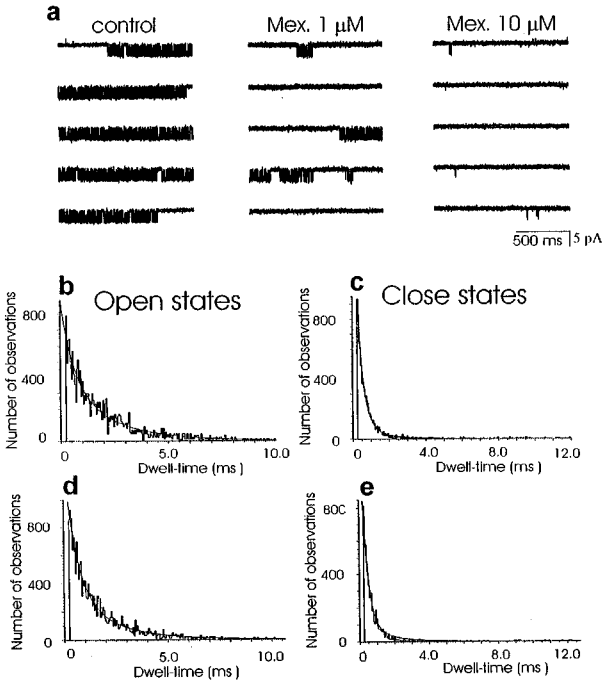

Figure 4 Effects of mexiletine on a single K_{ATP} channel. Downward deflections in the current record indicate inward current. (a) Continuous recording of channel activity at −60 mV with 150 mM KCl on both sides of the membrane in the absence (control) and in the presence of 1 μM and 10 μM mexiletine (Mex.). The drug decreased the mean burst duration and thus reduced the channel open probability. Kinetic analysis of the channel trace in (a), performed within the bursts of openings, indicated that under control conditions both the open (b, d) and closed-states (c, e) distributions could be fitted by the sum of two exponential functions. As shown in (d) and (e), respectively, the parameters of the fit were not modified by 1 μM mexiletine.

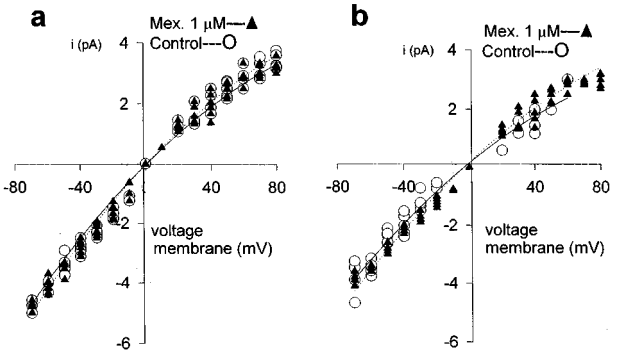

Figure 5 Effect of mexiletine on current-voltage relationships of K_{ATP} channels and effect of mexiletine. Current were measured in inside-out patches with 150 mM KCl on each sides of the membrane. Current-voltage relationships were constructed in the presence or absence of 1 μM mexiletine (a) a few seconds after patch excision ($n=4$) or (b) after channel rundown ($n=3$).

the drug on nucleoside diphosphate sites of the K_{ATP} channel or sites allosterically coupled to it. It has been shown that the K_{ATP} channels are heteromultimers of sulphonylurea receptors (SUR, the β subunit; Inagaki *et al*., 1995a; Nichols *et al*., 1996) and inward – rectifier K^+ channels of the Kir family (the α subunit; Inagaki *et al*., 1995b). A tetrameric architecture composed of 4 SUR and 4 Kir α subunits of the K_{ATP} channel (Clement *et al*., 1997) appears to explain the experimental data. The nucleoside diphosphate stimulatory sites are located on the SUR subunit. Several drugs are known to bind to this protein and to affect the channel gating without apparent modification of the single channel conductance and of the rectification properties (Terzic *et al*., 1995). In agreement with this notion, in our experiments mexiletine modified the channel gating without affecting the pore properties. Although our experiments support the view that mexiletine affects the K_{ATP} channel *via* nucleotide binding site/s of the K_{ATP} channel complex, we still cannot definitely exclude a possible interaction of the drug with the K_{ATP} α subunit, presumably Kir6.2 (Inagaki *et al*., 1995b). For example, Proks & Ashcroft (1997) have demonstrated that phentolamine binds the truncated form of the Kir6.2 subunit of the K_{ATP} channel complex when this is expressed independently from the sulphonylurea receptor in a cell line, producing a voltage-independent block of the channel without modification of the single channel conductance. Secondly, it seems that the binding of mexiletine to one site lowers the drug affinity of the neighbouring interacting channels. This is demonstrated by the fact that the inhibitory effect of the drug was more pronounced on the single channel current than on multichannel currents. For example, in the absence of rundown (stage 1), a 1 μM concentration of the drug reduced the macropatch currents by 45%, whereas on single channel, at the same concentration, mexiletine decreased the open probability by about 63%. The existence of a negative co-operativity (Hehl & Neumcke, 1993) between channels in our macropatches is also supported by the peculiar values for the Hill coefficient which we calculated from our experiments to be less than unity. It is interesting to note that this parameter increased when the channel underwent rundown possibly due to the reduced negative contribution of the residual interacting channels to the drug binding. Channel-channel interaction occurring in multi-channel preparations has already been described for several channel types (Neumcke & Stampfli, 1983) and accounts for the values of the Hill coefficient below unity for several channel blockers (Zunkler *et al*., 1988; Bodewei *et al*., 1992). Alternatively, it is possible that a subpopulation of K_{ATP} channels simultaneously present in our macropatches, could have been responsible for the low values of the Hill coefficient and for the discrepancies existing between the inhibitory effects exerted by mexiletine on multi-channel preparations and on single channel. However, this possibility seems to be rather unlikely, since from our experience only one K_{ATP} channel type is present in skeletal muscle fibres from adult rats (Tricarico *et al*., 1997).

Our observation that the inhibitory effects of mexiletine were less pronounced after channel rundown is consistent with a state-dependent mechanism of action. This is a common mechanism by which several K^+ channel modulators affect the K_{ATP} channel (Terzic *et al*., 1995). It has been reported that the effect of the sulphonylureas on K_{ATP} channel depends on the operative condition of the channel (Jovanovic *et al*., 1996; Brady *et al*., 1998), being strong inhibitors of the cardiac K_{ATP} channel at the beginning of ischaemia/poisoning and losing their potency during channel run-down (Findlay, 1992). The state-dependent action of mexiletine on the channel may help to explain the discrepancies existing between the inhibitory and the agonist effects of the drug respectively on the skeletal muscle and cardiac K_{ATP} channels after rundown (Sato *et al*., 1995). However in our experiments the effects of the drug were tested in the presence of 50–100 μM concentrations of ADP or 500 μM concentration of UDP which are in the range of the intracellular concentrations of nucleoside diphosphates measured in hypoxic cardiac and skeletal muscles (Nichols & Lederer, 1991; Allard & Lazdunski, 1992; Terzic *et al*., 1995). In contrast, the effect of mexiletine on cardiac K_{ATP} channels (Sato *et al*., 1995) was evaluated in the presence of a high concentration of UDP (3 mM) far exceeding the content of the nucleoside diphosphate measured in tissues (Hisanaga *et al*., 1986; Welsh & Lindinger, 1997). The use of low concentrations of UDP also offered the advantage of limiting the contribution of the intrinsic stimulatory effect of UDP on the channel; indeed the stimulatory effect of UDP on the K_{ATP} channel would mask the effects of the drug leading to an ambiguous interpretation of the data. Furthermore, although the cardiac and skeletal muscle K_{ATP} channels show a similar pharmacological profile, some differences appear to exist between the two channel forms. For example, in cardiac but not skeletal muscle the loss of K_{ATP} channel activity associated with rundown following patch excision can be recovered by internal application of MgATP (Furukawa *et al*., 1994; Barrett-Jolley *et al*., 1996).

The effects of mexiletine on the skeletal muscle K_{ATP} channel are of interest because this type of channel contributes significantly to the K^+ homeostasis in several pathophysiological conditions. For example, in working muscle or during ischaemia an over-activation of the channel favours a rapid repolarization of the fibres thus conserving the residual intracellular ATP (Davis *et al*., 1991). Conversely, abnormal closure of this type of channel appears to be linked to hypokalaemic states (Tricarico *et al*., 1997; Tricarico *et al*., 1998). Furthermore mexiletine, in addition to its role as an antiarrhythmic drug, is currently used in the treatment of muscular disorders related to the hyperexcitability of skeletal muscle fibres (Jackson *et al*., 1994; Lehmann-Horn *et al*., 1994; De Luca *et al*., 1997). In particular, mexiletine is a drug of choice in patients affected by hyperkalaemic periodic paralysis (Jackson *et al*., 1994; Lehmann-Horn *et al*., 1994). In this muscle disorder, which is linked to slowly inactivating Na^+ channels, the paralysis is precipitated by an excessive efflux of K^+ which accumulates in the T-tubules of the fibres during

trains of action potentials (Cannon, 1996). This is demonstrated by the fact that detubulation of the fibres reduces this ionic component preventing the characteristic after-discharge, the depolarization and paralysis (Cannon, 1996). It is possible that the combined inhibitory action of mexiletine on both Na^+ channels and K_{ATP} channels favours, respectively, the inactivation of abnormal Na^+ channels and the reduction of K^+ efflux from the muscle during trains of action potentials and explains the peculiar efficacy of mexiletine in the treatment of the hyperkalaemic periodic paralysis.

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