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Inhibition of ATP-sensitive K^+ channels of mouse skeletal muscle by disopyramide

Christa Moser, Solveig Hehl [1], Berthold Neumcke *

I. Physiologisches Institut, Universität des Saarlandes, D-66421 Homburg, Germany

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Abstract

Single ATP-sensitive K^+ channels (K_{ATP} channels) were studied in inside-out membrane patches excised from mouse skeletal muscle. The class Ia antiarrhythmic, disopyramide (5–100 μM), applied to the cytoplasmic membrane surface inhibited K_{ATP} channels at −40 and +40 mV. Channel inhibition by disopyramide started slowly and reached an almost stationary level within 1 min. Recovery from channel inhibition by disopyramide was incomplete. At pH 7.4, the disopyramide concentrations producing 50% channel inhibition were 8.1 μM at −40 mV and 7.1 μM at +40 mV. The Hill coefficients of the concentration-response curves were close to unity at both potentials. Raising the internal pH from 7.4 to 8.0 had no significant effect on the actions of disopyramide, but lowering the pH to 6.5 greatly potentiated the inhibition of K_{ATP} channels by the antiarrhythmic. Thus the open probabilities of K_{ATP} channels at −40 mV and in the presence of disopyramide (20 μM) were smaller by a factor of 18 at pH 6.5 than at pH 7.4. The results suggest that disopyramide interacts with K_{ATP} channels through the lipid phase of the membrane and that lowering the intracellular pH increases the affinity of K_{ATP} channels to disopyramide. Thus disopyramide at therapeutic concentrations (6–15 μM) affects muscular K_{ATP} channels, in particular at reduced intracellular pH values that occur under ischaemic conditions and during fatiguing exercise.

Keywords: Patch clamp; Antiarrhythmic, class Ia; Disopyramide; Skeletal muscle; K_{ATP} channel; Channel inhibition, pH dependent

1. Introduction

Disopyramide is a Vaughan-Williams class Ia antiarrhythmic agent and is used in the therapy of atrial and ventricular tachyarrhythmias (for a review see Zipes and Troup, 1978). The major action of agents of this class is a fast and use-dependent block of cardiac Na^+ channels. In addition, class Ia antiarrhythmic agents affect other types of ion channels, e.g. disopyramide inhibits ATP-sensitive K^+ channels (K_{ATP} channels) in ventricular myocytes and pancreatic B-cells (Horie et al., 1992; Wu et al., 1992; Hayashi et al., 1993).

This study showed that disopyramide exerts similar inhibitory effects on K_{ATP} channels in mammalian skeletal muscle. To obtain information on the mode of action of the agent, we studied the starting time and the concentration dependence of channel inhibition by disopyramide, the recovery after removal of the agent and a possible voltage dependence of the effects of disopyramide. A major finding of the present investigation was a pronounced pH dependence of the inhibition of K_{ATP} channels by disopyramide, and we discuss the clinical relevance of this new result.

Some of the results have been published in abstract form (Moser et al., 1994).

2. Materials and methods

2.1. Preparation of single skeletal muscle fibres

Flexor digitorum brevis muscles of adult female mice (killed by cervical dislocation) were dissociated into single fibres as described previously (Woll et al., 1989). Briefly, the muscles were immersed in a Na^+-rich

* Corresponding author. Tel. +49-6841-166486, fax +49-6841-166468.

[1] Present address: Department of Physiology and Biophysics, University of Washington School of Medicine, SJ-40, Seattle, WA 98195, USA.

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solution containing 2 mM $CaCl_2$ and collagenase (Sigma type I) at a concentration of 2.7 mg/ml, and the preparation was slowly stirred at 37°C for 90 min. Afterwards, the muscle bundles were transferred into a Ca^{2+}-free, Na^+-rich solution, and single fibres were separated by gentle trituration at room temperature.

2.2. Experimental techniques

Patch pipettes were fire-polished, filled with a K^+-rich solution and had resistances between 7 and 8 MΩ. After a GΩ seal was formed on the surface of a muscle fibre, a membrane patch of the inside-out configuration (Hamill et al., 1981) was excised in a Ca^{2+}-free, Na^+-rich solution. Immediately afterwards, the pipette was transferred to a small chamber in which the Na^+-rich solution was exchanged for K^+-rich solutions without or with added disopyramide. Currents were recorded at membrane potentials (potential differences between the intracellular and extracellular sides of the membrane patch) of −40 and +40 mV using an L/M-EPC-7 amplifier (List Electronics, Darmstadt, Germany). After appearance of openings of single K_{ATP} channels in the K^+-rich solution, channel activity declined spontaneously within 1–2 min to an almost stationary level (see Fig. 1). Subsequently, currents through single K_{ATP} channels were recorded in the K^+-rich solution (control), after application of disopyramide and after removal of the agent (wash-out). All experiments were performed at room temperature (20–23°C).

2.3. Solutions

The pipettes were filled with a K^+-rich solution which bathed the extracellular membrane surface. The external solution contained (mM): 155 KCl, 3 $MgCl_2$, 0.5 EGTA (ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid), 10 Hepes (4-(2-hydroxyethyl)-1-piperazine ethane-sulfonic acid) and was titrated to pH 7.4 with 1 N KOH. The muscle fibres were prepared and stored in a Na^+-rich solution containing (mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Hepes-NaOH, pH 7.4. Seals were formed and membrane patches excised in a Ca^{2+}-free, Na^+-rich solution containing (mM) 150 NaCl, 5 KCl, 1 $MgCl_2$, 0.5 EGTA, 10 Hepes-NaOH, pH 7.4. The effects of disopyramide were studied in K^+-rich solutions which bathed the intracellular membrane surface and at pH values of 7.4, 8.0 and 6.5. The internal solutions were composed of (mM) 160 KCl, 1 $MgCl_2$, 1 EGTA, 10 Hepes-KOH, pH 7.4 or pH 8.0 and 160 KCl, 1 $MgCl_2$, 1 EGTA, 10 Pipes (1,4-piperazinediethane sulfonic acid)-KOH, pH 6.5. After addition of disopyramide, the pH value of the solutions was readjusted by addition of 1 N HCl.

2.4. Data collection and analysis

Membrane currents were filtered by a four-pole low-pass filter with 1 kHz corner frequency, digitized at 0.1-ms intervals and stored on computer files (Hehl et

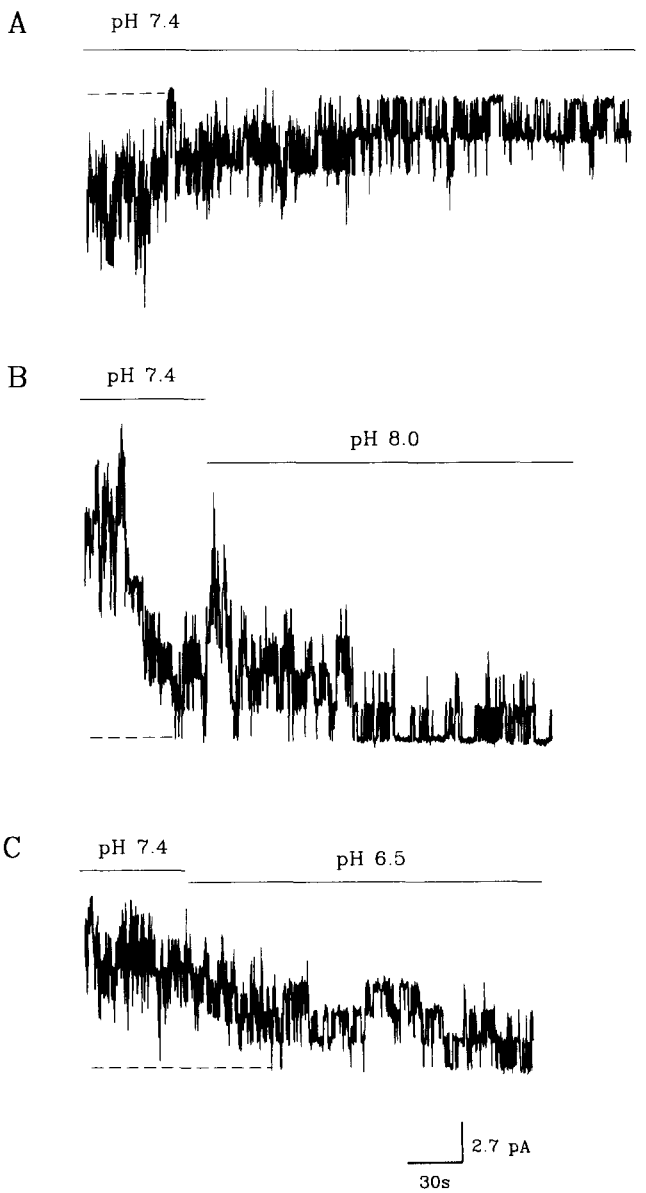

Fig. 1. Spontaneous decline in K_{ATP} channel activity (run-down) at various pH values of the K^+-rich internal solution. (A) Inward currents through K_{ATP} channels at −40 mV, pH 7.4. (B) Outward currents at +40 mV in solutions with pH 7.4 and 8.0 as indicated by the solid horizontal lines. (C) Outward currents at +40 mV in solutions of pH 7.4 and 6.5 as indicated by the solid horizontal lines. All records begin at the time of appearance of K_{ATP} channel activity in the K^+-rich internal solution, pH 7.4. The broken horizontal lines mark the current levels during closure of all channels at the end of the records. The vertical scaling (2.7 pA) gives the amplitude of the single-channel currents at −40 mV in the pH 7.4 solution. Records in A, B and C are from three different patches.

al., 1994). Point amplitude histograms were constructed from 30-s periods of current recordings and fitted by Gaussian functions. The current i through one open channel was obtained as the difference between the peak positions of the distributions for closed channels and one open channel. The total area under the histograms was normalised to unity, and the open probability p_o of a K_{ATP} channel was calculated from the relation

$$p_o = \frac{1}{N} \cdot \sum_{i=1}^{N} i A_i \quad (1)$$

In this equation, N denotes the number of active channels (taken as the maximum number of simultaneously open channels during the experiment) and A_0, A_1, ..., A_N the areas under the Gaussian curves for closed channels and for 1, ... N open channels.

Relative open probabilities (rel p_o) are given as percentages. Hill coefficients h for channel inhibition by disopyramide were obtained by fitting the relation

$$\text{rel } p_o = 100\left[\frac{1}{1+(c/K_D)^h}\right] \quad (2)$$

to rel p_o values with c denoting the disopyramide concentration and K_D the concentration producing 50% channel inhibition.

3. Results

3.1. Spontaneous run-down of K_{ATP} channel activity

Immediately after excision of membrane patches from the surface of mammalian skeletal muscle fibres in a Ca^{2+}-free solution, the activity of K_{ATP} channels is very high but subsequently declines slowly (Hehl et al., 1994; Hussain and Wareham, 1994). Fig. 1 shows this spontaneous 'run-down' of K_{ATP} channel activity at pH values of 7.4, 8.0 and 6.5 of the K^+-rich, Ca^{2+}-free internal solution. The figure illustrates that inward currents through K_{ATP} channels at −40 mV and outward currents at +40 mV reached an almost stationary level 1–2 min after the appearance of channel activity. In all subsequent experiments, such steady-state conditions were established before the inhibitory actions of disopyramide on K_{ATP} channels were studied. Replacement of Cl^- in the internal solution by certain organic anions, e.g. gluconate, strongly delays the run-down of K_{ATP} channels, and a stationary level is hardly reached even after 10 min (McKillen et al., 1994). Therefore, our experiments were performed in KCl internal solutions to allow a distinction between run-down of K_{ATP} channels and channel inhibition by disopyramide.

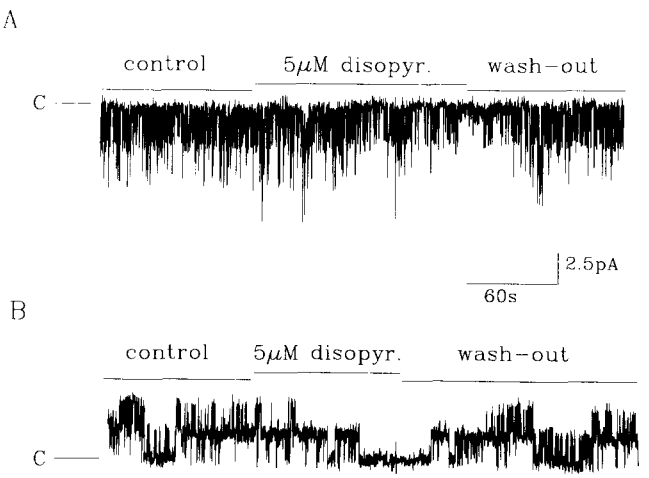

Fig. 2. Continuous recordings of currents through K_{ATP} channels at pH 7.4 and at −40 mV (A) and +40 mV (B) before (control), in the presence of 5 μM disopyramide and after removal of the agent (wash-out). The control records begin approximately 1.5 min after transfer of the patch to the K^+-rich solution. C denotes the closed channel state at the beginning of the records. At +40 mV (B) the current baseline declined by 0.8 pA during the 350 s recording period. Records in A and B are from two different patches.

3.2. Slow start of disopyramide action and incomplete recovery of channel inhibition

Fig. 2 illustrates the effects of application of a low concentration of disopyramide (5 μM) to the intracellular membrane surface on currents through K_{ATP} channels at −40 mV (Fig. 2A) and +40 mV (Fig. 2B). Though the exchange of the solutions was complete within 5–10 s, the inhibitory actions of disopyramide (decline of K_{ATP} channel activity) started with a delay of approximately 1 min and continued for almost 30 s after removal of the agent from the internal solution. At higher concentrations of disopyramide (10–100 μM), the inhibitory actions of this agent reached an almost stationary level after about 1 min. Upon washout for several minutes, inhibition of K_{ATP} channels was only poorly reversible (Fig. 3 and Fig. 5). Many membrane patches were lost during or after application of disopyramide. Because of these complications, open probabilities p_o of K_{ATP} channels were determined from currents recorded between 60 and 90 s after the application of disopyramide and were referred to the initial control p_o values measured in the same patch, at the same membrane potential.

3.3. Channel inhibition by disopyramide at pH 7.4

The actions of internally applied disopyramide on K_{ATP} channels at pH 7.4 were studied at various concentrations (5–100 μM) of the agent and at membrane potentials of −40 and +40 mV. In no case did disopyramide significantly affect the current i through a

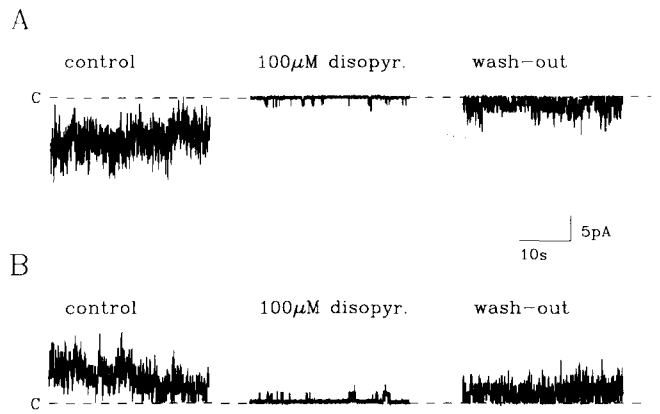

Fig. 3. Segments of current recordings from K_{ATP} channels at pH 7.4 and at −40 mV (A) and +40 mV (B) before (control), in the presence of 100 μM disopyramide and after removal of the agent (wash-out). The records begin approximately 1.5 min after transfer of the patch to the K^+-rich solution (control), 1 min after application of disopyramide (100 μM disopyramide) and 1 min after removal of the agent (wash-out). C denotes the closed channel state. Records A and B are from one patch.

single K_{ATP} channel (see Fig. 2 and Fig. 3). For example, the currents i at −40 mV were −2.66 ± 0.03 pA (mean ± S.E.M., $n = 19$) in the absence and −2.66 ± 0.04 pA ($n = 10$) in the presence of 20 μM disopyramide. The corresponding i values at +40 mV were 2.09 ± 0.04 pA ($n = 17$, control) and 2.05 ± 0.01 pA ($n = 9$, 20 μM disopyramide). Instead, disopyramide inhibited K_{ATP} channels by reducing their open probability p_o. Fig. 4 shows the concentration dependence of the relative open probabilities (rel p_o) at −40 mV (Fig. 4A) and +40 mV (Fig. 4B). The concentration-response curves through the mean values represent fits (see equation 2); the values of the fit parameters K_D and h are listed in the legend to Fig. 4. The results indicate that the disopyramide concentrations K_D, at which half-maximal channel inhibition was reached, were between 7 and 8 μM, that the Hill coefficients h of the curves were close to unity, and that both parameters K_D and h were hardly voltage-dependent.

3.4. Effects of pH upon channel inhibition by disopyramide

The inhibitory actions of disopyramide (20 μM) on K_{ATP} channels were studied in internal K^+-rich solutions with pH values of 6.5, 7.4 and 8.0. In the absence of disopyramide, lowering the pH value from 7.4 to 6.5 reduced the single-channel currents i at −40 and +40 mV by 0.2 to 0.3 pA, whereas raising the pH to 8.0 increased the i values by the same amount. These findings are in agreement with results reported for frog skeletal muscle (Davies, 1990). Disopyramide (20 μM) had no effect on single-channel currents i at any of the pH values studied. Thus, the currents i at −40 mV, pH 6.5 were −2.44 ± 0.08 pA (mean ± S.E.M., $n = 3$) in the absence and −2.48 ± 0.05 pA ($n = 3$) in the presence of 20 μM disopyramide. Also, there was no significant difference between the open probabilities p_o of K_{ATP} channels in the presence of disopyramide (20 μM) at pH 7.4 and 8.0. In contrast, lowering the pH to 6.5 produced a pronounced enhancement of channel inhibition by disopyramide. Fig. 5 shows currents recorded at pH 6.5 and illustrates the strong inhibitory effect of disopyramide (20 μM) at this pH value and the poor reversibility of its action even several minutes after removal of the agent. Fig. 6 contains a summary of rel p_o values in the presence of disopyramide (20 μM) determined at three pH values

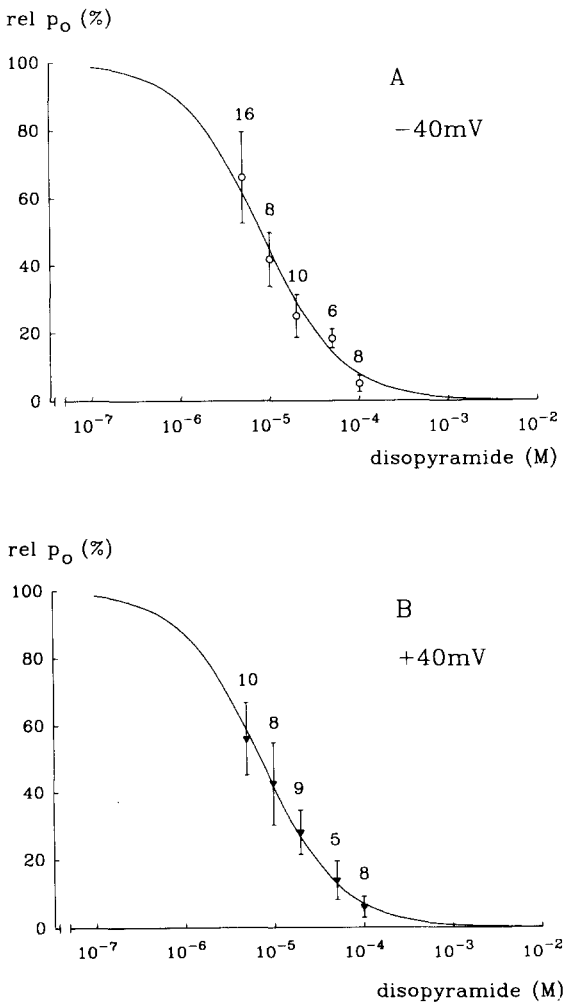

Fig. 4. Concentration-response curves of channel inhibition by disopyramide at pH 7.4 and at −40 mV (A) and +40 mV (B). The open probabilities p_o were normalized with respect to the p_o value before application of disopyramide determined in the same patch, at the same membrane potential. Numbers and bars denote the number of experiments and the S.E.M. values. The curves through the symbols represent fits of the mean relative p_o values by Eq. 2, the values of the parameters are (A): $K_D = 8.1$ μM, $h = 0.98$ and (B): $K_D = 7.1$ μM, $h = 0.99$.

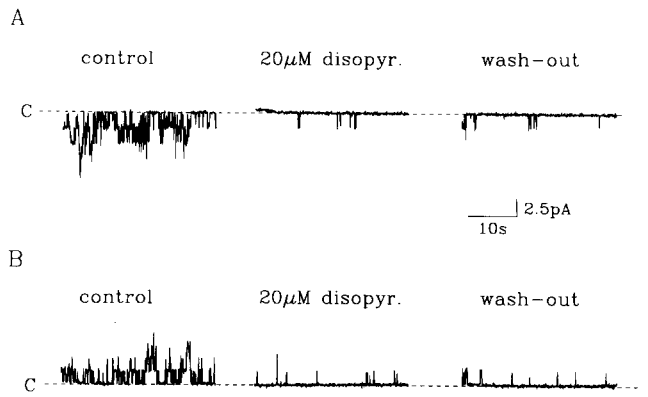

Fig. 5. Segments of current recordings from K_{ATP} channels at pH 6.5 and at −40 mV (A) and +40 mV (B) before (control), in the presence of 20 μM disopyramide and after removal of the agent (wash-out). The records begin approximately 1.5 min after transfer of the patch to the K^+-rich solution (control), 1 min after application of disopyramide (20 μM disopyramide) and 1 min after removal of the agent (wash-out). C denotes the closed channel state. Records A and B are from one patch.

(6.5, 7.4, 8.0) and two membrane potentials (−40, +40 mV). At −40 mV, the rel p_o values were 25.0 ± 6.3% (mean ± S.E.M., $n = 10$) at pH 7.4 and 1.4 ± 1.0% ($n = 5$) at pH 6.5. The potentiation of the disopyramide effects at lowered pH occurred at both potentials; there were no significant differences between the rel p_o values at −40 and +40 mV at any of the three pH values studied.

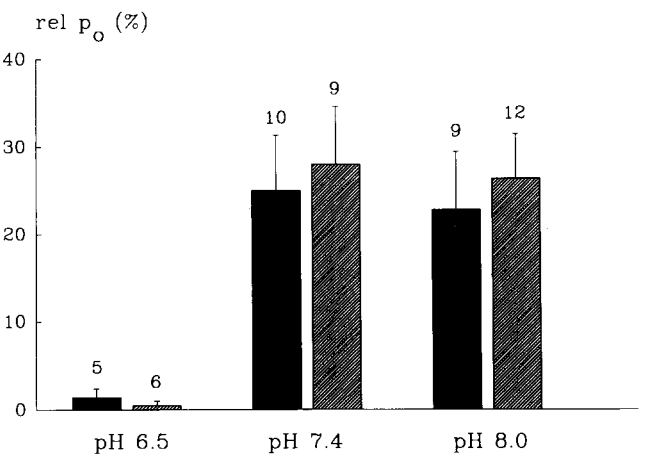

Fig. 6. Relative open probabilities (rel p_o) of K_{ATP} channels in the presence of 20 μM disopyramide at pH 6.5, 7.4, 8.0 and at −40 mV (filled columns) and +40 mV (hatched columns). The probabilities p_o are normalized with respect to measurements before application of disopyramide determined in the same patch, at the same membrane potential. Numbers and bars denote the number of experiments and the S.E.M. values.

4. Discussion

4.1. Mode of action of disopyramide on K_{ATP} channels

Published pK_a values for disopyramide are within the range 8.36–10.4 (for references see Grant et al., 1993). Hence, disopyramide in the internal solutions is almost entirely present in the positively charged form at pH 6.5 and 7.4, and even at pH 8.0 there are more protonated disopyramide cations than uncharged drug molecules.

The start of the inhibition of K_{ATP} channels by disopyramide (5 μM) is slow, and channel inhibition continues for approximately 30 s after removal of the agent (Fig. 2). Recovery of channel inhibition after treatment with higher concentrations of disopyramide (10–100 μM) is incomplete (Fig. 3 and Fig. 5). These observations suggest that disopyramide reaches K_{ATP} channels through the lipid phase of the membrane, and that it is still present in the lipid environment near the channels after removal from the internal solution. The slow starting and ending effects of disopyramide on K_{ATP} channels can then be explained by the small fraction of the neutral drug form capable of crossing the lipid phase and accumulating in this environment. A similar hydrophobic pathway has been proposed for the blockage of K_{ATP} channels by glibenclamide (Findlay, 1992). Despite this similarity between inhibition of K_{ATP} channels by disopyramide and glibenclamide, the channel receptors for the two agents in rat pancreatic B-cells are distinct from each other (Hayashi et al., 1993).

At none of the concentrations, membrane potentials or pH values studied did disopyramide diminish the current through a single K_{ATP} channel, but it did reduce the probability p_o of channel opening during our measuring period (30 s). Hence, inhibition of K_{ATP} channels by disopyramide occurs on a slow time scale (Hille, 1992), at which the on and off rates of channel inhibition are within the bandwidth of our recording system (1 kHz). At pH 7.4, the dependence of p_o on the concentration of disopyramide could be described with Hill coefficients h close to unity at −40 and +40 mV (Fig. 4A and B). This suggests a 1:1 stoichiometry of channel inhibition by disopyramide at both potentials.

The disopyramide concentrations K_D producing 50% channel inhibition at pH 7.4 were 8.1 μM at −40 mV and 7.1 μM at +40 mV in our experiments on inside-out membrane patches of mouse skeletal muscle (Fig. 4A and B). With the same patch configuration and disopyramide application to the cytoplasmic membrane surface, smaller K_D values and thus stronger inhibitions of K_{ATP} channels by disopyramide were reported for rat cardiac myocytes ($K_D = 1.8$ μM; Horie et al., 1992) and rat pancreatic B-cells ($K_D = 3.6$ μM;

Hayashi et al., 1993), whereas K_{ATP} channels in follicular cells of *Xenopus* oocytes have a lower disopyramide sensitivity ($K_D = 18$ μM; Sakuta et al., 1992). In the study by Hayashi et al. (1993), the potencies of disopyramide to inhibit pancreatic K_{ATP} channels were compared for different patch configurations and directions of applications. Compared to intracellular application on inside-out patches ($K_D = 3.6$ μM), lower disopyramide sensitivities were found with extracellular application to outside-out patches ($K_D = 11.0$ μM) and bath application to cell-attached patches ($K_D = 87.4$ μM). Thus, disopyramide reaches and interacts with the K_{ATP} channel more easily from the intracellular side, and diffusion of the agent is restricted along the membrane.

In contrast to our preliminary report (Moser et al., 1994) in which the inhibitory effects of disopyramide on K_{ATP} channels and the spontaneous run-down of channel activity were not clearly discriminated, we did not observe a significant voltage dependence of the effects of internally applied disopyramide in the present study (Fig. 4 and Fig. 6). Similarly, the class Ia antiarrhythmic cibenzoline inhibits single K_{ATP} channels of rat ventricular myocytes at +40 and −40 mV with the same efficiency (Horie et al., 1992). On the other hand, inhibition of whole-cell K_{ATP} currents in cat ventricular myocytes by externally applied disopyramide strongly increases with depolarization (De Lorenzi et al., 1995). The conflicting results from single-channel experiments and whole-cell measurements could be related to the different sites of drug application (intracellular versus extracellular), to the different states of K_{ATP} channels (spontaneous openings of single K_{ATP} channels in isolated membranes in the present study versus whole-cell K_{ATP} currents elicited by levcromakalim, pinacidil or by metabolic inhibition in the experiments of De Lorenzi et al. (1995)) or to the complications in discriminating various current components in whole-cell measurements.

Our experiments on mammalian skeletal muscle fibres revealed a pronounced pH dependence of the effects of disopyramide on K_{ATP} channels: compared to that at pH 7.4 and 8.0, channel inhibition by disopyramide is greatly potentiated at pH 6.5 for −40 and +40 mV (Fig. 6). This pH dependence cannot be explained by various dissociation degrees of disopyramide at the different pH values studied, because the agent is practically entirely in the positively charged form at pH 7.4 and 6.5 (see above). Hence, the pH dependence must be attributed to K_{ATP} channels, which we propose are transformed into a state of higher affinity for disopyramide at lowered pH values. Changes of the gating kinetics of K_{ATP} channels after a decrease in pH (Davies, 1990) support the concept of a pH-dependent modification of the channels.

4.2. Clinical relevance of inhibition of K_{ATP} channels by disopyramide

At therapeutic concentrations of disopyramide (6–15 μM), K_{ATP} channels are inhibited in rat pancreatic B-cells (Hayashi et al., 1993), in ventricular myocytes of rat (Horie et al., 1992) and guinea pig hearts (Wu et al., 1992) and in mouse skeletal muscle (this study). As a consequence of the channel inhibition in B-cells, insulin secretion is enhanced by disopyramide (Hayashi et al., 1993), and this can provoke fasting hypoglycaemia and even hypoglycaemic attacks (Goldberg et al., 1980).

The clinical relevance of K_{ATP} channel inhibition by disopyramide in cardiac and skeletal muscle is less clear. In papillary muscles of guinea pig heart, disopyramide (100 μM) does not affect the amplitude and duration of action potentials (Honerjäger et al., 1986), and in guinea pig ventricular cells, disopyramide (30 μM) even shortens the potentials (Wu et al., 1992), which is opposite to the prolongation of action potentials expected from the inhibition of a K^+ conductance. Thus inhibition of K_{ATP} channels by disopyramide does not seem to affect cardiac excitability or to interfere with its antiarrhythmic action under normoxic conditions. During maintained anoxia, K_{ATP} channels in ventricular cells of guinea pig heart first open and subsequently close within several minutes (Thierfelder et al., 1994). Disopyramide at therapeutic concentrations would then inhibit the transient activation of K_{ATP} channels and thereby reduce K^+ loss during the first phase of anoxia.

With the exception of channel block by glibenclamide, K_{ATP} channels in cardiac and skeletal muscle have similar pharmacological properties (Allard and Lazdunski, 1993). Hence, the actions of disopyramide on the excitability and K^+ loss of skeletal muscle fibres will be comparable to the effects described for cardiac muscle.

Under ischaemic conditions, the intracellular pH value (pH_i) of rat heart muscle is lowered by approximately one unit (Allen and Orchard, 1987). A comparable pH_i decline to 6.1 occurs in human skeletal muscle at the end of fatiguing exercise (Pan et al., 1988). According to our results (Fig. 6), the inhibitory actions of disopyramide on K_{ATP} channels are greatly potentiated under these conditions, and a similar pH_i dependence of K_{ATP} channel inhibition is also expected for other class Ia antiarrhythmics. Such an enhanced K_{ATP} channel inhibition by antiarrhythmic agents at reduced pH_i values has beneficial clinical consequences, because it tends to prevent K^+ loss and arrhythmias during acute myocardial ischaemia (Mortensen et al., 1993) and during fatiguing muscle exercise.

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References

Allard, B. and M. Lazdunski, 1993, Pharmacological properties of ATP-sensitive K^+ channels in mammalian skeletal muscle cells, Eur. J. Pharmacol. 236, 419.

Allen, D.G. and C.H. Orchard, 1987, Myocardial contractile function during ischemia and hypoxia, Circ. Res. 60, 153.

Davies, N.W., 1990, Modulation of ATP-sensitive K^+ channels in skeletal muscle by intracellular protons, Nature 343, 375.

De Lorenzi, F.G., T.R. Bridal and W. Spinelli, 1995, Voltage-dependent inhibition of the ATP-sensitive K^+ current by the class Ia agent disopyramide in cat ventricular myocytes, J. Pharmacol. Exp. Ther. 272, 714.

Findlay, I., 1992, Inhibition of ATP-sensitive K^+ channels in cardiac muscle by the sulphonylurea drug glibenclamide, J. Pharmacol. Exp. Ther. 261, 540.

Goldberg, I.J., L.K. Brown and E.J. Rayfield, 1980, Disopyramide (Norpace®)-induced hypoglycemia, Am. J. Med. 69, 463.

Grant, A.O., D.J. Wendt, Y. Zilberter and C.F. Starmer, 1993, Kinetics of interaction of disopyramide with the cardiac sodium channel: fast dissociation from open channels at normal rest potentials, J. Membr. Biol. 136, 199.

Hamill, O.P., A. Marty, E. Neher, B. Sakmann and F.J. Sigworth, 1981, Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches, Pflüg. Arch. 391, 85.

Hayashi, S., M. Horie, Y. Tsuura, H. Ishida, Y. Okada, Y. Seino and S. Sasayama, 1993, Disopyramide blocks pancreatic ATP-sensitive K^+ channels and enhances insulin release, Am. J. Physiol. 265 (Cell Physiol. 34), C337.

Hehl, S., Ch. Moser, R. Weik and B. Neumcke, 1994, Internal Ca^{2+} ions inactivate and modify ATP-sensitive potassium channels in adult mouse skeletal muscle, Biochim. Biophys. Acta 1190, 257.

Hille, B., 1992, Ionic Channels of Excitable Membranes, 2nd edn. (Sinauer Associates, Sunderland).

Honerjäger, P., E. Loibl, I. Steidl, G. Schönsteiner and K. Ulm, 1986, Negative inotropic effects of tetrodotoxin and seven class 1 antiarrhythmic drugs in relation to sodium channel blockade, Naunyn-Schmied. Arch. Pharmacol. 332, 184.

Horie, M., S. Hayashi, Y. Yuzuki and S. Sasayama, 1992, Comparative studies of ATP sensitive potassium channels in heart and pancreatic β cells using Vaughan-Williams class Ia antiarrhythmics, Cardiovasc. Res. 26, 1087.

Hussain, M. and A.C. Wareham, 1994, Rundown and reactivation of ATP-sensitive potassium channels (K_{ATP}) in mouse skeletal muscle, J. Membr. Biol. 141, 257.

McKillen, H.-C., N.W. Davies, P.R. Stanfield and N.B. Standen, 1994, The effect of intracellular anions on ATP-dependent potassium channels of rat skeletal muscle, J. Physiol. 479, 341.

Mortensen, E., T. Yang and H. Refsum, 1993, Potassium channel blockade as an antiarrhythmic principle, Cardiovasc. Drug Rev. 11, 370.

Moser, Ch., S. Hehl and B. Neumcke, 1994, Voltage-dependent inhibition of K_{ATP} channels in mouse skeletal muscle by disopyramide, Pflüg. Arch. 426 (Suppl.), R88.

Pan, J.W., J.R. Hamm, D.L. Rothman and R.G. Shulman, 1988, Intracellular pH in human skeletal muscle by ^1H NMR, Proc. Natl. Acad. Sci. USA 85, 7836.

Sakuta, H., K. Okamoto and Y. Watanabe, 1992, Blockade by antiarrhythmic drugs of glibenclamide-sensitive K^+ channels in *Xenopus* oocytes, Br. J. Pharmacol. 107, 1061.

Thierfelder, S., B. Doepner, C. Gebhardt, H. Hirche and K. Benndorf, 1994, ATP-sensitive K^+ channels in heart muscle cells first open and subsequently close at maintained anoxia, FEBS Lett. 351, 365.

Woll, K.H., U. Lönnendonker and B. Neumcke, 1989, ATP-sensitive potassium channels in adult mouse skeletal muscle: different modes of blockage by internal cations, ATP and tolbutamide, Pflüg. Arch. 414, 622.

Wu, B., T. Sato, T. Kiyosue and M. Arita, 1992, Blockade of 2,4-dinitrophenol induced ATP sensitive potassium current in guinea pig ventricular myocytes by class I antiarrhythmic drugs, Cardiovasc. Res. 26, 1095.

Zipes, D.P. and P.J. Troup, 1978, New antiarrhythmic agents: amiodarone, aprindine, disopyramide, ethmozin, mexiletine, tocainide, verapamil, Am. J. Cardiol. 41, 1005.