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Quinine Inhibits Mitochondrial ATP-regulated Potassium Channel from Bovine Heart

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Abstract. The mitochondrial ATP-regulated potassium (mitoK_{ATP}) channel has been suggested as trigger and effector in myocardial ischemic preconditioning. However, molecular and pharmacological properties of the mitoK_{ATP} channel remain unclear. In the present study, single-channel activity was measured after reconstitution of the inner mitochondrial membrane from bovine ventricular myocardium into bilayer lipid membrane. After incorporation, a potassium-selective current was recorded with mean conductance of 103 ± 9 pS in symmetrical 150 mM KCl. Single-channel activity of this reconstituted protein showed properties of the mitoK_{ATP} channel: it was blocked by 500 μM ATP/Mg, activated by the potassium-channel opener diazoxide at 30 μM, inhibited by 50 μM glibenclamide or 150 μM 5-hydroxydecanoic acid, and was not affected by the plasma membrane ATP-regulated potassium-channel blocker HMR1098 at 100 μM. We observed that the mitoK_{ATP} channel was blocked by quinine in the micromolar concentration range. The inhibition by quinine was additionally verified with the use of $^{86}Rb^+$ flux experiments and submitochondrial particles. Quinine inhibited binding of the sulfonylurea derivative [^{3}H]glibenclamide to the inner mitochondrial membrane. We conclude that quinine inhibits the cardiac mitoK_{ATP} channel by acting on the mitochondrial sulfonylurea receptor.

Key words: Heart mitochondrial — Potassium channel — Quinine — Glibenclamide — Potassium channel openers — Bilayer lipid membrane

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Introduction

In 1991, a mitochondrial ATP-regulated potassium channel (mitoK_{ATP} channel) was identified by patch-clamp single-channel recordings in the inner membrane of liver mitochondria (Inoue et al., 1991). Later, a similar channel was described in heart (Paucek et al., 1992), brain (Bajgar et al., 2001; Debska et al., 2001) and skeletal muscle mitochondria (Debska et al., 2002).

Similarly to the plasma membrane ATP-regulated potassium channel (K_{ATP} channel), the mitoK_{ATP} channel is inhibited by antidiabetic sulfonylureas and activated by potassium-channel openers (KCOs) (Szewczyk, 1998; Kicinska et al., 2000). Especially diazoxide is a potent activator of the mitoK_{ATP} channel (Garlid et al., 1996).

The molecular identity of the mitoK_{ATP} channel is unknown. Several observations on the pharmacological profile and immunoreactivity with specific antibodies suggest that the mitoK_{ATP} channel belongs to the inward rectifier K^+ channel family–Kir6.x (Suzuki et al., 1997; Zhou et al., 1999). Recently, it was hypothesized that succinate dehydrogenase forms part of a structure that constitutes the mitoK_{ATP} channel (Ardehali et al., 2004). Similarly, molecular properties of the mitochondrial sulfonylurea receptor (mitoSUR) are not clear. Use of the sulfonylurea derivative [^{125}I] glibenclamide leads to labeling of a 28 kDa protein in heart mitochondria (Szewczyk et al., 1997b, 1999). Recently, with the use of the fluorescent probe BODIPY-glibenclamide, a 64 kDa protein was labeled in brain mitochondria (Bajgar et al., 2001).

The primary function of the mitoK_{ATP} channel is to allow K^+ transport into the mitochondrial matrix. This phenomenon could be involved in mitochondrial volume homeostasis (Szewczyk et al., 1993, 1995;

Garlid, 2000). Additionally, the cardiac mitoK_{ATP} channel plays an important role in protecting cardiomyocytes during ischemia/reperfusion (for reviews, *see* Szewczyk & Marban, 1999; O'Rourke, 2000; Garlid, 2000; Szewczyk & Wojtczak, 2002; McCully & Levitsky, 2003).

Quinine is an anti-malarial drug previously known to influence potassium transport pathways in mitochondria (Nicolli, Redetti & Bernardi, 1991; Manon & Guerin, 1992; Castrejon, Pena & Uribe, 2002).

The molecular mechanism of its action is not fully understood. In the present work we analyzed the effect of quinine on the cardiac mitoK_{ATP} channel. We show that quinine interacts with the mitoSUR and thus inhibits [^{3}H]glibenclamide binding to the inner mitochondrial membrane. Quinine also inhibits electrogenic $^{86}Rb^+$ flux in cardiac submitochondrial particles. Further, we observed that quinine inhibited activity of the mitoK_{ATP} channel reconstituted into bilayer lipid membranes (BLM). Our data strongly suggest that the mitoK_{ATP} channel in cardiac mitochondria is blocked by quinine acting on the mitoSUR.

Materials and Methods

Materials

L-α-phosphatidyl-choline (asolectin), *n*-decane and protease (Subtilisin A, P5380) from *Bacillus licheniformis* were from Sigma-Aldrich, Germany. All other chemicals were of the highest purity available commercially.

Isolation of Mitochondria

Bovine heart mitochondria were isolated at 4°C as described earlier (Zhang et al., 2001). Briefly, a fragment of bovine heart muscle was minced in isolation buffer (in mM: 200 mannitol, 50 sucrose, 5 KH_2PO_4, 5 MOPS, 1 EGTA, 0.1% BSA, pH 7.15) and homogenized with 25 U protease/gram tissue using a teflon pestle. The homogenate was then centrifuged at 8,000 × *g*, for 10 min to remove protease. The pellet was resuspended in the isolation buffer and centrifuged again at 700 × *g*, for 10 min, to remove cellular debris The supernatant was centrifuged at 8,000 × *g*, for 10 min, at 4°C to pellet the mitochondria. The mitochondria were then washed and suspended in the isolation buffer without EGTA. The suspension was loaded on top of Percoll solution (30% Percoll, 0.25 M sucrose, 1 mM EDTA, 10 mM HEPES, pH 7.4) and centrifuged at 35,000 × *g*, for 30 min. The mitochondrial fraction was then collected and washed twice with the isolation buffer without EGTA and resuspended at 10–20 mg of protein/ml.

Preparation of Submitochondrial Particles (SMP)

Freshly prepared mitochondria were sonicated 8 × 15 s and centrifuged at 16,000 × *g*, for 15 min to pellet unbroken mitochondria. The supernatant was again centrifuged at 14,000 × *g*, for 35 min and SMP were resuspended in the isolation buffer without EGTA at 5 mg of protein/ml. The polarity of SMP vesicles was estimated to be from 40 to 50% outside-out, using cytochrome c oxidase activity measurements.

^{86}Rb Flux Measurements

The analysis of isotope flux through ion-conducting pathways was performed essentially as described previously (Garty, Rudy & Karlish, 1983; Garty & Karlish, 1989). In brief, for transport experiments with membrane preparations, external K^+ was removed by passing the vesicles through a cation-exchange column (Dowex 50-X8 Tris form). Aliquots of vesicle suspension at 5–10 mg of protein/ml were applied to small Dowex columns and eluted with 1.5 ml of 175 mM sucrose. This step exchanged external potassium cations for $Tris^+$ and diluted the suspension about 5-fold. At this point, quinine was added where indicated. The assay was initiated 30 s later by adding 25–50 μl of ^{86}RbCl (2–4 μCi). The vesicles were incubated with the isotope for the times given in figure legends. Subsequently, in order to separate the vesicles from the medium, 100 μl aliquots of the reaction mixture were applied to a 2–3 cm Dowex 50-X8 (Tris form) column in a Pasteur pipette. Vesicles were eluted directly into counting vials by addition of 1.5 ml of ice-cold 175 mM sucrose solution. Prior to use the columns were washed with 2 ml of 175 mM sucrose followed by 2 ml of 175 mM sucrose containing 25 mg/ml of bovine serum albumin and stored at 4°C. The amount of $^{86}Rb^+$ trapped within the vesicles was estimated by scintillation counting. The $^{86}Rb^+$ content was expressed as a percentage of the initial total radioactivity in the vesicle medium or as percentage of control (sample without added reagents).

Binding of [^{3}H] Glibenclamide to Mitochondrial Membranes

The binding of [^{3}H]glibenclamide to SMP membranes was performed as described previously (Szewczyk, Czyz & Nalecz, 1997a). For equilibrium binding assay, SMP (300–500 μg protein/ml) were incubated for 60 min at 4°C in 50 mM HEPES-NaOH pH 7.4, with required concentrations of [^{3}H]glibenclamide (usually 2–3 nM). Incubation was terminated by rapid filtration through Whatman GF/C filters under reduced pressure, followed by washing with 30 ml of 100 mM NaCl, 20 mM Tris-HCl pH 7.4, at 4°C. Prior to use, the filters were incubated for at least 30 min in a solution containing 0.5% polyethyleneimine, pH 7.4. After use, the filters were incubated for 24 h in 5 ml of scintillation cocktail Formula 989 (Du Pont NEN, Germany) and counted for radioactivity. Nonspecific binding was measured in the presence of 30 μM non-radioactive glibenclamide.

Bilayer Lipid Membrane (BLM) Measurements

BLMs were formed in a 250 μM diameter hole drilled in a Delrin cup (Warner Instrument, CT), which separated two chambers (cis and trans, each of 1 ml internal volume). The chambers contained 50/150 or 150/150 mM KCl (cis/trans) and 20 mM Tris-HCl, pH 7.2 solution. The outline of the aperture was coated with a lipid solution and N_2-dried prior to bilayer formation to improve membrane stability. BLMs were painted using asolectin in *n*-decane at a final concentration of 25 mg lipid/ml. Bovine heart SMP (5 mg of protein/ml, 1–5 μl) was added to the trans compartment. Incorporation of the mitoK_{ATP} channel into the BLM was usually observed within a few minutes. The orientation of the mitochondrial membrane in BLM was probably with the matrix side towards the cis side. All measurements were carried out at room temperature (25°C). Since we did not know exactly the orientation of the channels in BLM, the studied compounds were added to both the cis and trans compartments. Formation and thinning of the bilayer was monitored by capacitance measurements and optical observations.

The final accepted capacitance values ranged from 110 to 180 pF. Electrical connections were made by Ag/AgCl electrodes and agar salt bridges (3 M KCl) to minimize liquid junction potentials. Voltage was applied to the cis compartment of the chamber and the trans compartment was grounded. The current was measured using a Bilayer Membrane Amplifier (BLM-120, BioLogic).

Data Analysis

Single-channel data were filtered at 500 Hz. The current was digitized at a sampling rate of 100 kHz (A/D converter PowerLab 2/20, ADInstruments) and transferred to a PC or digital tape recorder (DTR-1204, BioLogic) for off-line analysis by Chart v4.1.2 (PowerLab ADInstruments) and pCLAMP8 (Axon Instruments). The pCLAMP8 software package was used for data processing. The channel recordings illustrated are representative of the most frequently observed conductances under given conditions. The conductance was calculated from the current-voltage relationship. Single-channel currents were recorded at different voltages in steps of 10 mV. The probability of a channel opening (P(open)) was calculated with automatic interval setting. The channel open (τ_{open}) and closed (τ_{closed}) lifetimes were calculated from the logarithmic binning mode using the Marquardt-LSQ fitting method, order one, without weighting. The parameter n denotes the number of experiments and N, the number of events. Values for γ, τ_{open}, τ_{closed}, P(open) were calculated from segments of continuous recordings lasting 60 s and with $N \geq 1000$ events. Data from the experiments are reported as mean value ± SE or SD (SE, standard error; and SD, standard deviation).

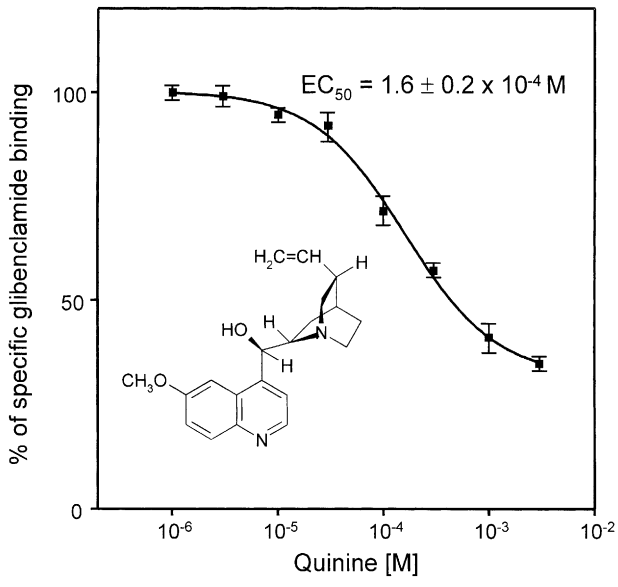

Fig. 1. Quinine inhibits [^{3}H]glibenclamide binding to bovine heart inner mitochondrial membrane. Percentage of specific glibenclamide binding to bovine heart inner mitochondrial membrane (SMP, submitochondrial particles) in the presence of various concentrations of quinine. Nonspecific glibenclamide binding was measured in the presence of 30 μM unlabeled glibenclamide. Binding experiments were performed as described under Materials and Methods. The results are representative of three independent experiments. The results are presented as mean ± SD. *Inset*: quinine chemical structure.

Results

Quinine Interacts with the Mitochondrial Sulfonylureas Receptor

The identification and characterization of the mitochondrial sulfonylurea receptor (mitoSUR) was performed previously with the use of [^{3}H]glibenclamide (Szewczyk et al., 1997b). In order to clarify whether quinine interacts with the mitoSUR, SMP were incubated with [^{3}H]glibenclamide (*see* Materials and Methods) and different concentrations of quinine. A decrease of [^{3}H]glibenclamide binding to SMP with increasing concentration of quinine was observed (Fig. 1). It was calculated that quinine inhibits [^{3}H]glibenclamide binding to mitochondrial inner membrane with an EC_{50} value of $(1.6 \pm 0.2) \times 10^{-4}$ M.

$^{86}Rb^+$ Flux Inhibition by Quinine in Cardiac Submitochondrial Particles

Moreover, the effect of quinine on K^+ transport was established with the use of ^{86}RbCl. Figure 2*A* presents the time course of $^{86}Rb^+$ uptake into beef heart SMP vesicles (expressed as a percentage of total radioactivity in the sample). In the absence of K^+ gradient (no diffusion potential was created) accumulation of $^{86}Rb^+$ was found to be low (Fig. 2*A*). This result suggests that K^+ transport operates by an electrogenic rather than electroneutral pathway. Figure 2*B* shows that the $^{86}Rb^+$ uptake into SMP vesicles is significantly inhibited by 400 μM quinine (72.97 ± 2.80% of the control value).

Single-Channel Properties of the mitoK$_{ATP}$ Channel

The inner mitochondrial membrane was reconstituted into BLM and current changes characteristic for single-ion-channel activity were observed (n = 35). The single-channel current traces were recorded at different voltages in the symmetrical 150/150 mM KCl or asymmetrical 50/150 mM (cis/trans) conditions (Fig. 3*A*, *B*).

Figure 3*C* shows current-voltage relationships for single-channel opening, at different voltages, under symmetrical (*continuous line*, ▲) and gradient (*dashed lines*, ■) conditions. The channel conductance was 103 ± 9 pS under symmetrical conditions. The reversal potential measured in the 50/150 mM KCl gradient was 26 mV and this indicates that the examined pore is cation-selective.

The distribution of closed and open dwell-times was also analyzed. Figure 4*A* shows histograms: open dwell-times at 50 mV and −50 mV with mean lifetimes of 3.79 ± 0.03 ms and 12.48 ± 0.04 ms, respectively; as well as closed dwell-times at 50 mV and

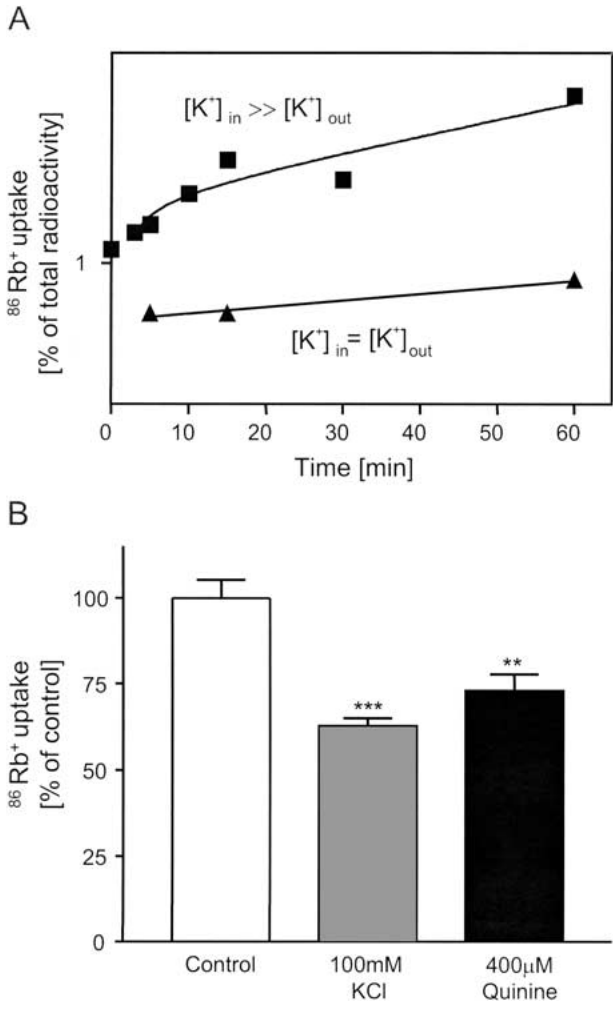

Fig. 2. $^{86}Rb^+$ uptake into bovine heart SMP. (*A*) Time course of $^{86}Rb^+$ uptake into bovine SMP. After addition of $^{86}RbCl$, accumulation of radioactivity (■) was measured as described under Materials and Methods. Accumulation of radioactivity without removal of external potassium ions is also shown (▲). Measurements were performed at 20°C. (*B*) Effect of quinine on $^{86}Rb^+$ uptake into bovine heart SMP vesicles. Columns indicate accumulated radioactivity after 10 min incubation (as percentage of control) in the presence of 100 mM KCl and 400 μM quinine. The results are presented as mean ± SD. *** $P < 0.001$ vs. control, ** $P < 0.01$ vs. control.

−50 mV with mean lifetimes of 19.10 ± 0.04 ms and 13.22 ± 0.04 ms, respectively. All measurements were performed in symmetric 150/150 mM KCl (cis/trans) solution. The voltage dependence of the mitoK_{ATP} channel open and closed mean times is shown in Fig. 4*B*. The mean open time decreases with increasing voltages and has a bell-shaped dependence. In Fig. 4*C* we show that the open probability *P*(open) of the mitoK_{ATP} channel in symmetric 150/150 mM KCl (cis/trans) solution is voltage dependent. *P*(open) decreases from 0.6 at −70 mV to 0.2 at 0 mV and remains at this value at positive voltages up to 70 mV.

Substances known to modulate the mitoK_{ATP} channel activity were also used to examine ion channel properties observed in our experiments. Figure 5*A* shows-single channel recordings in a 50/150 mM KCl (cis/trans) gradient at −50 mV before and after addition of 1 mM Mg^{2+} plus 500 μM ATP to both chambers. ATP/Mg inhibited the channel activity within 10 minutes. Figure 5*B* illustrates that this inhibitory effect was reversed by addition of 30 μM diazoxide (cis/trans) in the presence of ATP/Mg within 30 seconds. The effects are also seen from the amplitude histograms fitted with superimposed Gaussian curves calculated from the same experiment, as shown in Fig. 5*C*. We noticed that single-channel amplitude decreased after application of Mg/ATP, but we did not study this phenomenon in more detail.

The effect of various K_{ATP} channel inhibitors on single-channel activity was then examined. Both 150 μM 5-hydroxydecanoic acid (5-HD) and 50 μM glibenclamide inhibited the channel activity (Fig. 6*A* and *B*), as seen from the single-channel currents and evaluated amplitude histograms fitted with superimposed Gaussian curves. As shown in Fig. 6*C*, addition of 100 μM HMR 1098, a specific inhibitor of plasma membrane K_{ATP} channels (Gogelein et al., 2001; Liu et al., 2001), to both the cis and trans sides did not influence the channel activity. The amplitude histograms fitted with superimposed Gaussian curves under control conditions and after addition of 100 μM HMR1098 are shown below the corresponding single-channel recordings.

Quinine Inhibits the mitoK_{ATP} Channel from Bovine Heart Mitochondria

After characterization of mitoK_{ATP} single-channel properties we studied the effect of quinine on the channel. Quinine inhibited the channel, as seen from the single-channel recordings in a 50/150 mM KCl (cis/trans) gradient at −50 mV under control conditions and after addition of 10 μM and 100 μM quinine (cis/trans) (Fig. 7*A*). Amplitude histograms fitted with superimposed Gaussian curves showing the same effect quantitatively are presented in Fig. 7*B*. The probability of opening of the mitoK_{ATP} channel, *P*(open), decreases from 0.41 ± 0.09 at control conditions to 0.01 ± 0.01 in the presence of 100 μM quinine (Fig. 7*C*) in a dose-dependent manner. The mitoK_{ATP} channel was insensitive to diazoxide after quinine inhibition (*data not shown*).

Discussion

The mitoK_{ATP} channel was identified over ten years ago (Inoue et al., 1991). There is still insufficient information about the molecular identity of this protein and its single-channel properties. Moreover, our

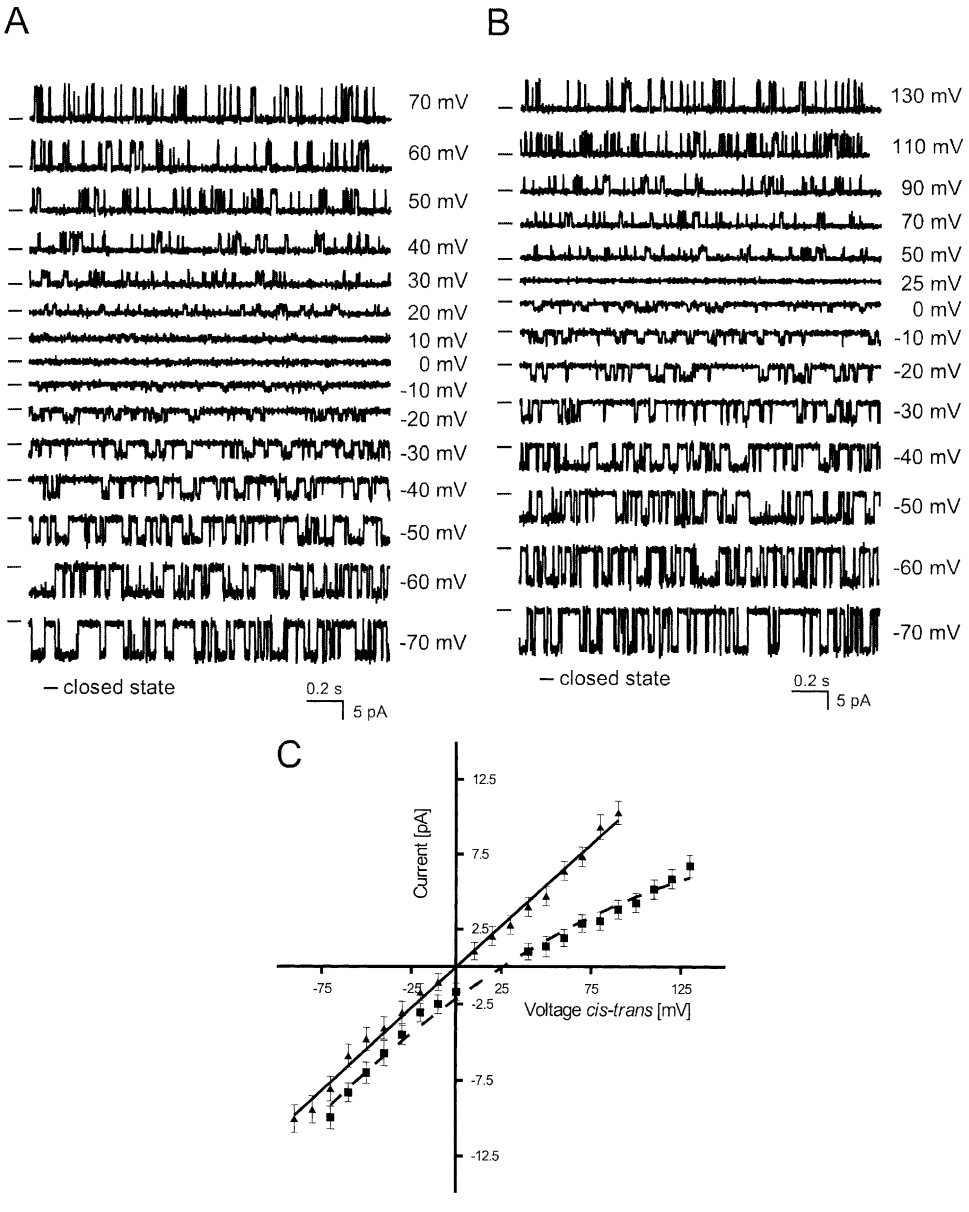

Fig. 3. Single-channel recordings of the bovine heart mitoK_{ATP} channel in BLM. (*A*) Single-channel recordings in symmetric 150/150 mM KCl (cis/trans) solution at different voltages. (*B*) Single-channel recordings in gradient 50/150 mM KCl (cis/trans) at different voltages. (*C*) Current-voltage characteristics of single-channel events in gradient 50/150 mM KCl (dashed lines, ■; $n = 3$), and in symmetric 150/150 mM KCl (solid line, ▲; $n = 3$) solution, "-" indicates current at the closed state of the channel. Recordings were low-pass filtered at 500 Hz. Reconstitution of the inner mitochondrial membrane into BLM was performed as described under Materials and Methods.

understanding of the pharmacological properties of this channel is based on studies that used isolated mitochondria and various biochemical assays, leading to confusing observations on the action of particular drugs (for a review *see* Szewczyk and Wojtczak, 2002). In this study we combined macro- and microscopic assays to study the interactions of the mitoK_{ATP} channel with quinine. Namely, [^{3}H]glibenclamide-binding studies and $^{86}Rb^+$ flux experiments were performed to characterize the interaction of quinine with the K^+ channel of the inner mitochondrial membrane. Further, reconstitution of the inner mitochondrial membrane into planar lipid bilayer allowed us to study single-channel properties of the K^+ channel located in this membrane. These approaches allowed us to show inhibition of the mitoK_{ATP} channel by quinine.

Quinine Binds to the mitoSUR

It is well known that similarly to the plasma membrane ATP-regulated potassium channel, the mitoK_{ATP} channel is inhibited by the antidiabetic sulfonylurea, glibenclamide (Inoue et al., 1991; Paucek et al., 1992). It was postulated that, similarly to the Kir6.x family, the mitochondrial channel contains a sulfonylurea receptor, i.e., the mitochondrial sulfonylurea receptor, mitoSUR. Indeed, using [^{3}H] glibenclamide, a specific interaction of this drug with the inner mitochondrial membrane was observed (Szewczyk et al., 1997b, 1999). The presence of a single class of low-affinity binding sites for glibenclamide in the inner mitochondrial membrane was found, with a K_D of 360 nM (Szewczyk et al., 1997b). In the present study we show that quinine is able to

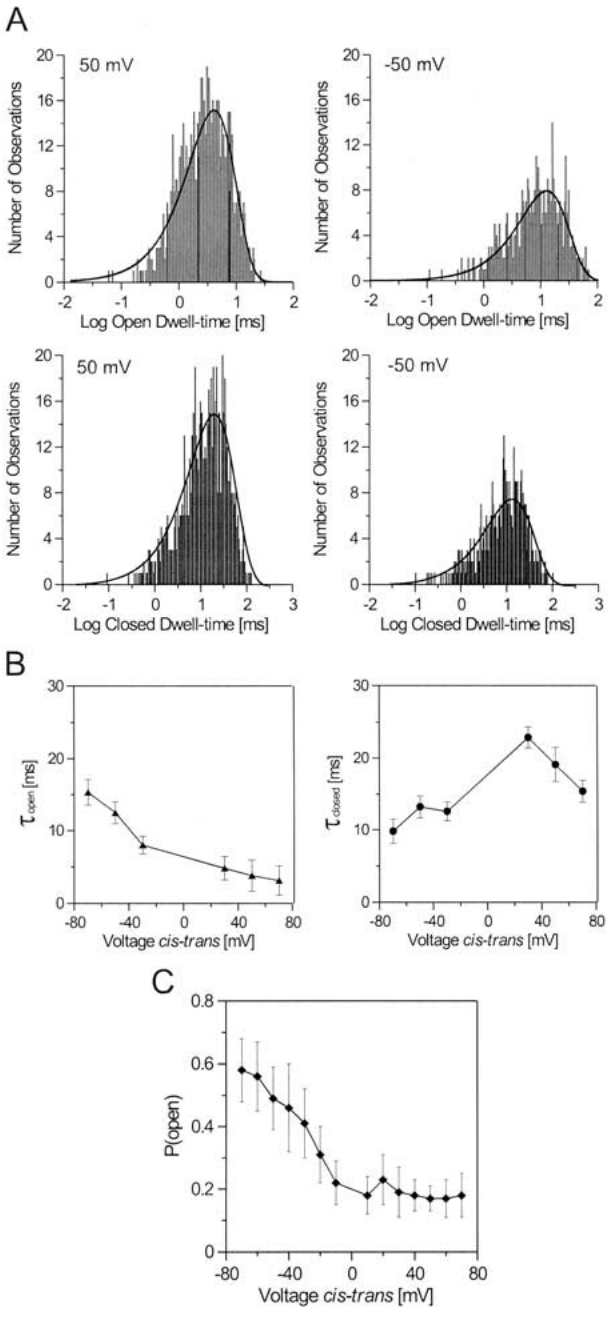

Fig. 4. Kinetic analysis of the mitoK_{ATP} channel activity recorded at different voltages in BLM. (*A*) Open and closed dwell-time distribution in symmetric 150/150 mM KCl (cis/trans) solution at 50 mV and −50 mV. (*B*) Voltage dependence of mean mitoK_{ATP} channel open and closed times in symmetric 150/150 mM KCl (cis/trans) solution. (*C*) Open probability (*P*(open)) of mitoK_{ATP} channel in symmetric 150/150 mM KCl (cis/trans) solution at different voltages.

displace [^{3}H]glibenclamide from the inner mitochondrial membrane, which strongly suggests that quinine interacts with mitoSUR. Interestingly, in the presence of 3 mM quinine, about 40% of [^{3}H]glibenclamide was still bound to the mitochondrial inner membrane compared with the amount bound in the absence of quinine. This observation suggests that cardiac inner mitochondrial membrane contains at least two classes of low-affinity binding sites for glibenclamide, one sensitive and the other insensitive to quinine.

Quinine Inhibits $^{86}Rb^+$ Flux

In order to confirm that quinine can affect electrogenic potassium transport in the inner mitochondrial membrane we used the $^{86}Rb^+$ flux assay. The assay was described earlier (Garty et al., 1983; Garty & Karlish, 1989; Szewczyk et al., 2001) and was successfully used to measure potassium- and sodium-channel activity. Briefly, SMP vesicles containing an inner concentration of 100 mM KCl were prepared. Shortly before assay, external K^+ was replaced with $Tris^+$. As a result of the K^+ gradient, an electrical diffusion potential was established in the vesicles containing active K^+ channels. The addition of $^{86}Rb^+$ isotope, a K^+ analogue, to external solution, led to the uptake of $^{86}Rb^+$ due to its equilibration with the membrane potential, but did not affect the level of the potential itself. Quinine significantly affected the $^{86}Rb^+$ uptake in our experiments. Although ion flux measurements have some disadvantages, they are an important macroscopic confirmation of the presence of a specific transport activity within specific intracellular compartments. These experiments were followed by single-channel measurements in BLM.

Quinine Inhibits the mitoK_{ATP} Channel

In order to prove that quinine inhibits the mitoK_{ATP} channel we reconstituted a highly purified preparation of inner mitochondrial membranes from bovine ventricular myocardium into BLM. Recently, such a procedure was successfully applied to study single-channel properties of the mitoK_{ATP} channel (Zhang et al., 2001; Nakae et al., 2003). We observed potassium-selective single channels using activity-modulating substances, i.e., inhibitors and potassium-channel openers. In order to claim that the observed K^+ channel activity is in fact the mitoK_{ATP} channel the following properties should be observed:

1. The channel has to be blocked by ATP/Mg (Inoue et al., 1991; Paucek et al., 1992; Jaburek et al., 1998; Zhang et al., 2001),
2. The ATP/Mg-inhibited channel should be activated by the potassium-channel opener diazoxide (Garlid et al., 1996; Jaburek et al., 1998),
3. The channel should be blocked by 5-hydroxydecanoic acid (5-HD), a substance known to be a blocker of the mitochondrial channel (Jaburek et al., 1998; Zhang et al., 2001; Nakae et al., 2003),

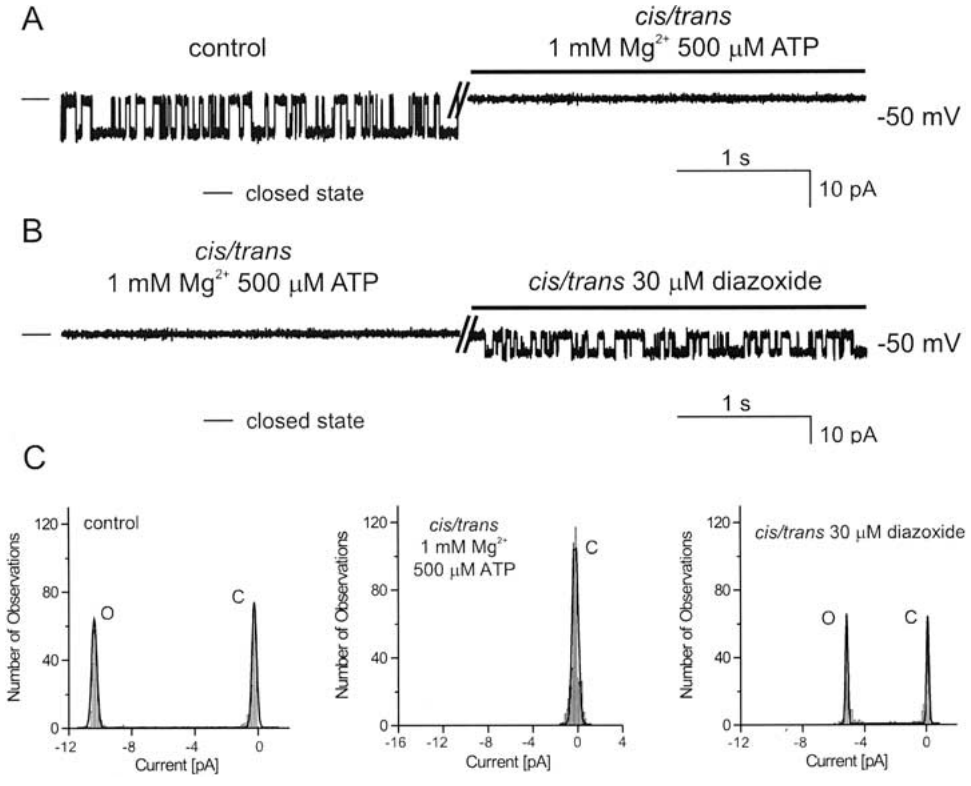

Fig. 5. ATP and diazoxide affect the activity of the mitoK_{ATP} channel. Single-channel recordings in gradient 50/150 mM KCl (cis/trans) solution at −50 mV. (*A*) Under control conditions and after addition of 500 μM ATP and 1 mM Mg^{2+} (cis/trans), channel inhibition was observed 2 to 10 minutes upon application of ATP/Mg. (*B*) In the presence of 500 μM ATP and 1 mM Mg^{2+}, (cis/trans) and after addition of 30 μM diazoxide (cis/trans), channel activation was observed after 2 minutes upon application of diazoxide. "—" indicates the closed state of the channel. (*C*) Amplitude histograms fitted with superimposed Gaussian curves. *O*, open state; *C*, closed state. Recordings were low-pass filtered at 500 Hz.

4. The channel should be blocked by glibenclamide (Inoue et al., 1991; Paucek et al., 1992; Jaburek et al., 1998),
5. The plasma membrane cardiac K_{ATP}-channel blocker HMR1098 should be without effect on the channel activity (Sato et al., 2000; Zhang et al., 2001).

Some of the substances acting on mitoK_{ATP} have additional ways of action on isolated mitochondria. Diazoxide is known to inhibit mitochondrial succinate dehydrogenase (Grimmsmann & Rustenbeck, 1998) and induce uncoupling of mitochondria (Kowaltowski et al., 2001). Glibenclamide is also able to uncouple mitochondria at a high concentration (Szewczyk et al., 1997a). Recently, it was shown that 5-HD is rapidly converted to 5-HD-CoA by mitochondrial fatty acyl CoA synthetase and acts as a weak substrate or inhibitor of respiration depending on the conditions employed (Lim et al., 2002). Despite the action of these substances on isolated mitochondria, they should modulate single-channel activity as previously described, in order for us to claim that we measure mitoK_{ATP} channel activity.

In fact, we did observe inhibition of the potassium channel by 500 μM ATP in the presence of magnesium cations followed by activation by 30 μM diazoxide. In addition, the channel was blocked by 150 μM 5-HD and 50 μM glibenclamide. No effect of 100 μM HMR1098 was observed on channel activity. All those observations proved that we measured the activity of the cardiac mitoK_{ATP} channel in lipid bilayer.

Interestingly, we observed changes of channel open probability with changing holding potential, i.e., the open probability increased at negative holding potential. This observation suggests that potassium transport in mitochondria via the mitoK_{ATP} channel can depend on mitochondrial potential. Because we were unable to establish firmly the polarity of the mitoK_{ATP} channel after reconstitution into BLM, it is not possible now to suggest how changes of the mitochondrial potential affect the channel activity in vivo.

Quinine is a well known inhibitor of potassium channels. These include, for example, the two-pore domain K^+ channel (Sano et al., 2003), G-protein-gated inwardly rectifying K^+ channel (Jeong et al., 2001), large-conductance calcium-activated potassium channels (Franciolini et al., 2001) and ATP-regulated potassium channel, Kir6.2 (Sakura et al., 1995).

For many years quinine has been used to study potassium transport in mitochondria. It has been shown that quinine can inhibit both the K^+/H^+ antiporter (Nakashima and Garlid, 1982; Garlid et al., 1986; Brierley et al., 1984; Jung et al., 1984) and the K^+ uniporter (Jung & Brierley, 1984; Diwan, 1986) located in the inner mitochondrial membrane. Quinine was also shown to block cation-selective

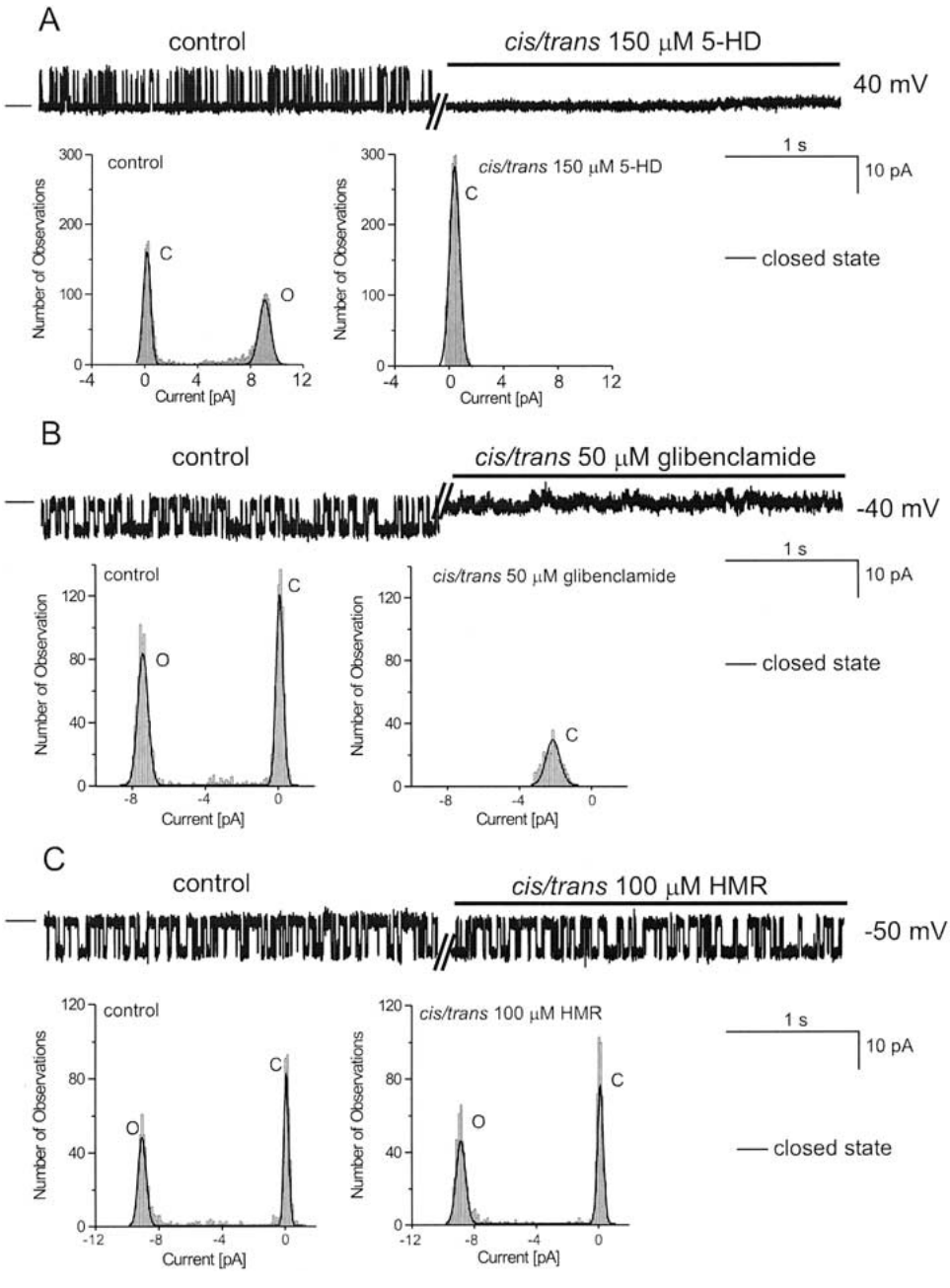

Fig. 6. Effect of 5-HD, glibenclamide and HMR1098 on the activity of the mitoK_{ATP} channel. (*A*) Single-channel recording in symmetric 150/150 mM KCl (cis/trans) solution at 40 mV with amplitude histograms fitted with superimposed Gaussian curves under control conditions and after addition of 150 μM 5-HD (cis/trans). (*B*) Single-channel recording in gradient 50/150 mM KCl (cis/trans) solution at −40 mV with amplitude histograms fitted with superimposed Gaussian curves under control conditions and after addition of 50 μM glibenclamide. (*C*) Single-channel recording in gradient 50/150 mM KCl (cis/trans) solution at a −50 mV with amplitude histograms fitted with superimposed Gaussian curves under control conditions and after addition of 100 μM HMR1098. *O*, open state; *C*, closed state; "–" Indicates the closed state of the channel. Channel inhibition by 5-HD and glibenclamide was observed after 2 minutes upon drug application. HMR1098 was without effect on channel activity up to 15 minutes of incubation. Recordings were low-pass filtered at 500 Hz.

current in mouse liver mitoplasts (Antonenko et al., 1991). A 53 kDa protein, able to increase potassium transport in liposomes, was purified from Triton X-100 extract of mitochondrial membranes by affinity chromatography on immobilized quinine (Diwan, Haley & Sanadi, 1988). Recently, a quinine-sensitive K^+ uptake pathway was described in yeast mitochondria (Castrejon et al., 2002). Interestingly, an inhibition of the mitochondrial permeability transition pore is promoted by quinine (Catisti & Vercesi, 1999).

Previous observations on quinine and glibenclamide binding to the inner mitochondrial membrane and inhibition of $^{86}Rb^+$ flux into submitochondrial particles suggested that quinine can inhibit the mitoK_{ATP} channel. We confirm this also by observation that quinine inhibits the mitochondrial channel in a dose-dependent manner, starting at 10 μM. At 100 μM quinine was able to block fully the activity of cardiac mitoK_{ATP} channel.

In summary, we have shown that quinine blocks the cardiac mitoK_{ATP} channel, probably by interaction with the mitochondrial sulfonylurea receptor.

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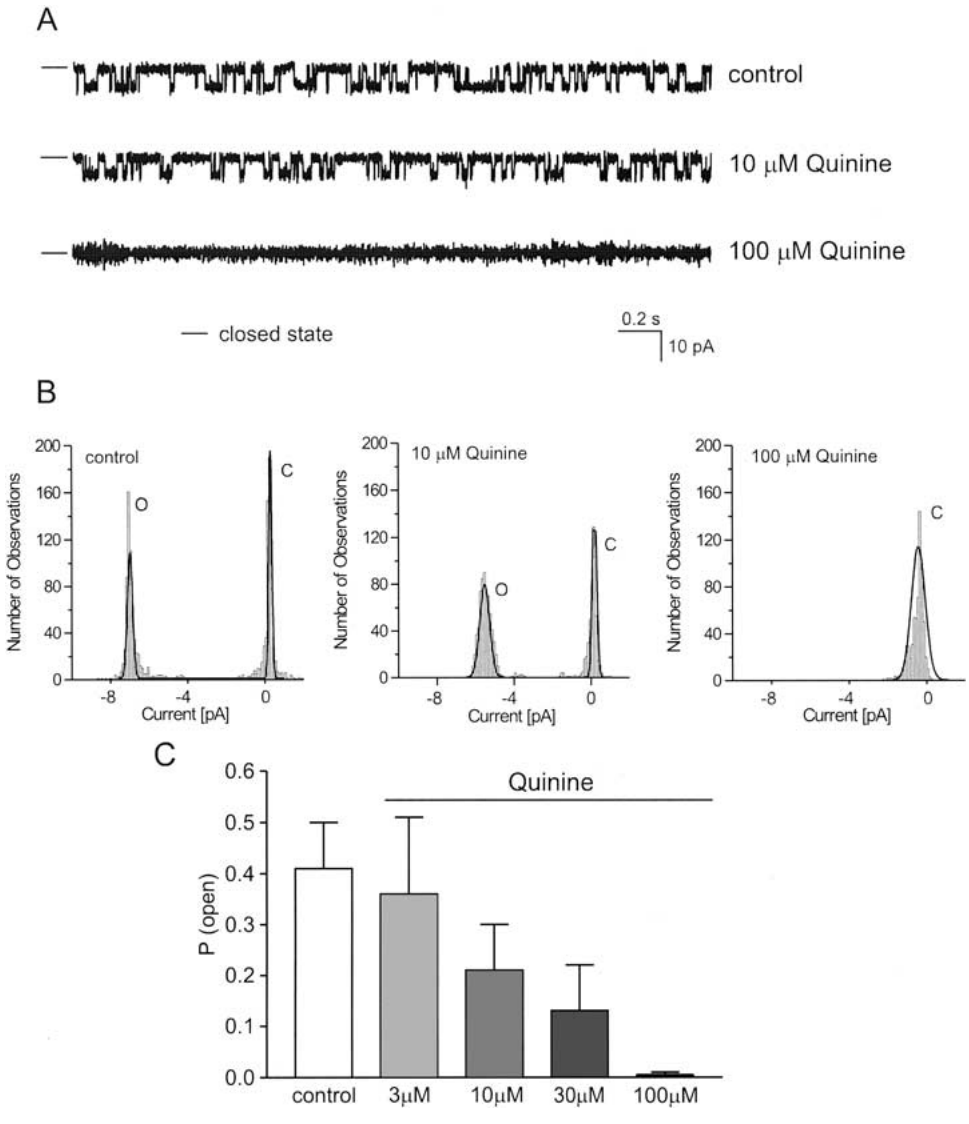

Fig. 7. Effect of quinine on the activity of the mitoK_{ATP} channel. (*A*) Single-channel recording in gradient 50/150 mM KCl (cis/trans) solution at −50 mV under control conditions and after addition of 10 μM or 100 μM quinine. (*B*) Amplitude histograms fitted with superimposed Gaussian curves under control conditions and in the presence of 10 μM or 100 μM quinine. (*C*) Probability of opening of mitoK_{ATP} channel (*P*(open)) under control conditions and in the presence of 3 μM, 10 μM, 30 μM and 100 μM quinine. *O*, open state; *C*, closed state; "–" indicates current at the closed state of the channel. Recordings were low-pass filtered at 500 Hz.

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