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Characterization of the Driving Force as a Modulator of Gating in Cardiac ATP-sensitive K^+ Channels — Evidence for Specific Elementary Properties

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Abstract. Single cardiac ATP-sensitive K^+ channels and, comparatively, two other members of the inwardly rectifying K^+ channel family, cardiac $K^+_{(ir)}$ and $K^+_{(ACh)}$ channels, were studied in the inside-out recording mode in order to analyze influence and significance of the electrochemical K^+ gradient for open-state kinetics of these K^+ channels.

The conductive state of $K^+_{(ATP)}$ channels was defined as a function of the electrochemical K^+ gradient in that increased driving force correlates with shortened open-channel lifetime. Flux coupling of gating can be largely excluded as the underlying mechanism for two reasons: (i) τ_{open} proved identical in 23 pS, 56 pS and 80 pS channels; (ii) $K^+_{(ATP)}$ channel protonation by an external pH shift from 9.5 to 5.5 reduced conductance without a concomitant detectable change of τ_{open}. Since gating continued to operate at E_K, i.e., in the absence of K^+ permeation through the pore, K^+ driving force cannot be causally involved in gating. Rather the driving force acts to modulate the gating process similar to Rb^+ whose interference with an externally located binding site stabilizes the open state. In $K^+_{(ir)}$ and $K^+_{(ACh)}$ channels, the open state is essentially independent on driving force meaning that their gating apparatus does not sense the electrochemical K^+ gradient. Thus, $K^+_{(ATP)}$ channels differ in an important functional aspect which may be tentatively explained by a structural peculiarity of their gating apparatus.

Key words: Cardiac inward rectifier K^+ channels — Kinetics — Permeation — Electrochemical K^+ gradient — pH — Rubidium

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Introduction

As in many other excitable tissues, inward rectifier K^+ channels play an important role in myocardium. By regulating excitability, they control vital cardiac functions. Cardiac cells express mainly three types with considerable density: the strong rectifier ($K^+_{(ir)}$) which keeps the resting potential near E_K, muscarinic K^+ channels ($K^+_{(ACh)}$) generating acetylcholine-activated K^+ currents and acting to modulate resting K^+ conductance as well as the shape of the action potential in the atrium, and the weakly rectifying ATP-sensitive K^+ channels. $K^+_{(ir)}$ channels can be functionally distinguished from $K^+_{(ACh)}$ and $K^+_{(ATP)}$ channels by a ligand-independent activity mode.

These highly K^+-selective inward rectifiers belong to a superfamily with a unique transmembrane topology. Compared with voltage-gated K^+ channels, they are characterized by a simplified architecture consisting of only two membrane-spanning hydrophobic domains (M1 and M2) which are connected by the highly homologous, pore-lining H5-segment (Kubo et al., 1993*a*,*b;* Dascal et al., 1993; Ho et al., 1993). Four M1-H5-M2 domains assemble to form a functional channel with a central pore (Yang, Jan & Jan, 1994). Evidence for a more complex structure of $K^+_{(ATP)}$ channels emerges from the coexpression of Kir6.2 channels with the sulfonylurea receptor, a member of the ATP-binding cassette superfamily, which is ATP-sensitive and which can be blocked by sulfonylureas (Inagaki et al., 1995). Each Kir6.2 subunit requires one SUR1 subunit to form a functional channel (Clement et al., 1997) in an octameric or tetradimeric structure (Shyng & Nichols, 1997).

After their discovery in metabolically exhausted myocardium by Noma (1983), ATP-sensitive K^+ channels attracted growing interest. During the last 15 years, their elementary properties including the modalities of

permeation and channel kinetics had been analyzed in great detail so that this weakly inward rectifier K^+ channel is biophysically now well defined (for review *see* Noma & Takano, 1991; Terzic, Jahangir & Kurachi, 1995). Single file diffusion through the pore which simultaneously accommodates multiple K^+ ions can be modelled by a three barrier energy profile (Davies et al., 1996). Surprisingly, an important aspect is not yet understood, namely a sensitivity of the open state to K^+ driving force (Zilberter et al., 1988). This might suggest a correlation between permeation and open channel lifetime as it characterizes the behavior of some other ionic channels (Kolb & Bamberger, 1977; VanHelden, Hamill & Gage, 1977; Ascher, Marty & Neild, 1978; Benneton & Christopherson, 1990). Whether the driving force plays actually a causal role for $K^+_{(ATP)}$ channel kinetics as proposed by Zilberter et al. (1988) remains to be clarified. Another intriguing question relates to the general importance of the K^+ driving force for open state kinetics in inward rectifier K^+ channels.

The present inside-out patch-clamp experiments focus on this problem and established the electrochemical K^+ gradient as a modulating factor for gating in cardiac $K^+_{(ATP)}$ channels. A comparison with cardiac $K^+_{(ir)}$ and $K^+_{(ACh)}$ channels studied likewise in cell-free conditions excluded the possibility that gating in other inward rectifier K^+ channels follow this principle.

Materials and Methods

Elementary K^+ currents through $K^+_{(ATP)}$, $K^+_{(ir)}$ and $K^+_{(ACh)}$ channels from cultured ventricular and atrial cardiocytes of neonatal rats were recorded in the inside-out patch configuration by employing the standard patch-clamp technique (Hamill et al., 1981) and an L-M/EPC 5 amplifier. Disaggregation of the cardiac tissue, cell culture and the handling of the short-time (18–24 h) cultured cardiocytes were identical with procedures described in detail earlier (Kohlhardt, Fichtner & Fröbe, 1989). Rod-shaped cardiocytes were selected for the patch-clamp experiments because they represent the cell type in the more advanced developmental stage in this primary cell culture.

Experiments with isolated $K^+_{(ATP)}$ channels were performed in the continued cytosolic presence of a nucleotide mixture (10 μmol/l ATP, 100 μmol/l each of ADP and GDP) to stabilize channel activity in cell-free conditions. Stable activation of isolated $K^+_{(ACh)}$ channels was achieved by the external presence of 10–100 nmol/l acetylcholine and the simultaneous cytosolic presence of 50–100 μmol/l GTP. Likewise in the inside-out experiments with $K^+_{(ir)}$ channels, ATP (1 mmol/l) was added to the internal solution to abolish $K^+_{(ATP)}$ channel activity.

The records were filtered at 1 kHz with a 8-pole Bessel filter, stored on tape and digitized with a sampling rate of 5 kHz to be analyzed. Single-channel analysis concentrated on i_{unit}, open and closed times. In some one-channel patches, the apparent gating frequency was estimated from the number of resolved openings. The latter parameter was obtained from bursts by counting resolved openings or, in $K^+_{(ir)}$ channels, by counting resolved events during periods when P_o attained a level of about 0.9. The 50% threshold method was employed to determine open and closed times; τ_{open} and τ_{closed} resulted from the best weighted fit of probability density functions (by neglecting the first bin of 0.4 msec). i_{unit} was obtained from Gaussian event distributions. No attempts were done to correct the data for missed events.

Whenever possible, the data are expressed as mean ± SEM. Statistically significant difference between means was judged by Student's *t*-test; $P < 0.05$ was considered to indicate statistical significance.

Solutions (composition in mmol/l)

(A) Bathing solution (and facing the cytoplasmic membrane surface in the inside-out recording mode); (i) isotonic K^+ solution: KCl 140; $MgCl_2$ 2 (or 0); glucose 20; HEPES 10; EGTA 2; pH 7.4; (ii) hypertonic K^+ solution: K^+ aspartate 230; KCl 20; $MgCl_2$ 2 (or 0); glucose 20; HEPES 10; EGTA 2; pH 7.4; (B) Pipette solutions (facing the external membrane surface); (i) KCl 5; NaCl 135; $MgCl_2$ 2; HEPES 10; pH 7.4; (ii) KCl 140; $MgCl_2$ 2; HEPES 10 (or Tris 5); pH 7.4 (or 9.5, 6.5, 5.5); (iii) KCl 250; $MgCl_2$ 2; HEPES 10; pH 7.4; (iv) KCl 140; $MgCl_2$ 2; RbCl (4.7–47); HEPES 10; pH 7.4; (v) KCl 140; $MgCl_2$ 2; CsCl (0.47–4.7); HEPES 10; pH 7.4; Temperature (controlled by a Peltier element): 19°C (or 29°C)

Compounds

ATP, ADP, GDP, acetylcholine (purchased from Sigma Chemie, München) were freshly dissolved just before use.

Results and Discussion

Isolation of $K^+_{(ATP)}$ channels from their natural environment is usually followed by rundown of channel activity. This well-known phenomenon is due to dephosphorylation and the loss or a degradation of metabolites needed at the cytosolic channel surface for maintaining an active channel state even at a critically reduced ATP concentration. Exposing the channels to stimulatory nucleotides (ADP, GDP) could not always prevent channel deactivation. An initial equilibration period of 5 min was, therefore, expected to ensure that channel activity had attained a quasi-steady state, also a requirement for stable channel kinetics.

Open state kinetics of $K^+_{(ATP)}$ channels reacted sensitively to changes in membrane potential but they cannot be defined as a function of voltage (Fig. 1*A* and *B*) as reported in the literature (for review *see* Terzic et al., 1995). The evidence comes from experiments at different transmembrane K^+ gradients (5 mmol/l external, 140 mmol/l internal K^+; or 140 mmol/l symmetrical K^+): at a given membrane potential, τ_{open} can differ tremendously. Consequently, the electrochemical K^+ gradient, $(E_m - E_K)$, is precisely the factor which determines open state kinetics (Fig. 1*C*). Since $i = \gamma (E_m - E_K)$, this dependence of τ_{open} on K^+ driving force might reflect flux coupling as reported from gramicidin channels (Kolb & Bamberger, 1977) and some natural ionic channels (VanHelden et al., 1977; Ascher et al., 1978; Benneton & Christopherson, 1990) where τ_{open} correlates inversely with conductance. This possibility could be excluded in experiments at different external K^+ concentrations (5,

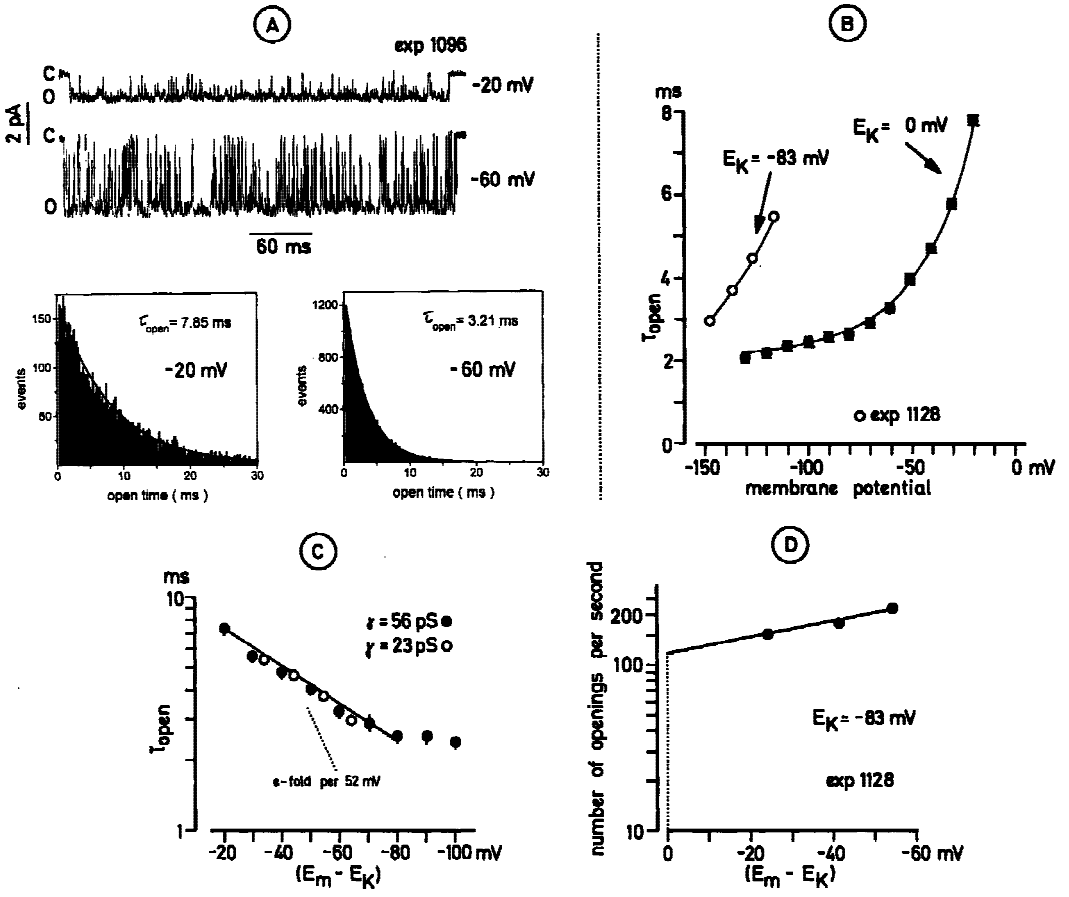

Fig. 1. (*A*) Records of elementary K^+ currents through a single $K^+_{(ATP)}$ channel at membrane potentials more negative than E_K (0 mV); symmetrical (140 mmol/l) K^+ concentration. *C* means closed and *O* open state. Open time histograms (lower part) could be best fitted by a single exponential at any membrane potential indicating a single conducting state in inwardly permeating channels. Exp1096. (*B*) The voltage dependence of τ_{open} in $K^+_{(ATP)}$ channels is determined by E_K. The filled circles represent mean values of at least 4 individual experiments at symmetrical (140 mmol/l) K^+ concentration ($E_K = 0$ mV) and a mean channel conductance of 56 pS; horizontal bars indicate the SEM range. The open circles symbolize a single experiment (exp1128) at asymmetrical (5 mmol/l external, 140 mmol/l internal) K^+ concentration ($E_K = -83$ mV), channel conductance was 23 pS. (*C*) Semilogarithmic plot of τ_{open} from 56 pS $K^+_{(ATP)}$ channels (filled circles, vertical bars are SEM) and from a 23 pS $K^+_{(ATP)}$ channel (open circles) against K^+ driving force, $(E_m - E_K)$. Shown are the same data points than in *B* of this figure. τ_{open} varies e-fold per a 52-mV change in driving force over a broad potential range but note the saturation tendency at very strong driving forces. (*D*) Semilogarithmic plot of the apparent number of openings per second of a $K^+_{(ATP)}$ channel vs. $(E_m - E_K)$. Asymmetrical (5 mmol/l external, 140 mmol/l internal) K^+ concentration; $E_K = -83$ mV.

140, 250 mmol/l) which analyzed the influence of conductance on τ_{open} at a given K^+ driving force. Conductance varied with external K^+ according to $\gamma = 13.9$ pS $[K^+]_o^{0.30}$, a somewhat steeper relationship than observed by Kakei, Noma & Shibasaki (1985). At an $(E_m - E_K)$ of −70 mV, for example, τ_{open} was 2.6 ± 0.15 msec ($n = 3$) at 23 pS, 2.9 ± 0.11 msec ($n = 4$) at 56 pS, and 2.5 ± 0.18 msec ($n = 3$) at 80 pS. Consequently, the $\tau_{open} - (E_m - E_K)$ relationship of 23 pS and 56 pS $K^+_{(ATP)}$ channels obeys the same slope factor (*see* Fig. 1*C*). This is noteworthy with respect to the response of stretch-activated channels and endothelial inward rectifier K^+ channels where external K^+ can stabilize the open state (Yang & Sachs, 1990; Pennefather & DeCoursey, 1994). The independence of τ_{open} on external K^+ in $K^+_{(ATP)}$ channels argues against a modulatory K^+ binding site in a superficial channel region as postulated to exist in endothelial inward rectifier K^+ channels.

$K^+_{(ATP)}$ channel gating is an intrinsic process that can basically operate in the absence of an electrochemical K^+ gradient. Extrapolating the $\tau_{open} - (E_m - E_K)$ relationship to zero driving force (Fig. 1*C*) yields a value for τ_{open} of close to 10 msec, the intrinsic dwell time in the open state. Accordingly, the channel continues to gate in the absence of K^+ through the pore (Fig. 1*D*). Apparent values of the gating frequency between 100 and 140 openings/sec were found but, for methodological reasons, should be considered as an only rough estimate of the true kinetics. Clearly, the electrochemical K^+ gradient is not causally involved in gating of $K^+_{(ATP)}$

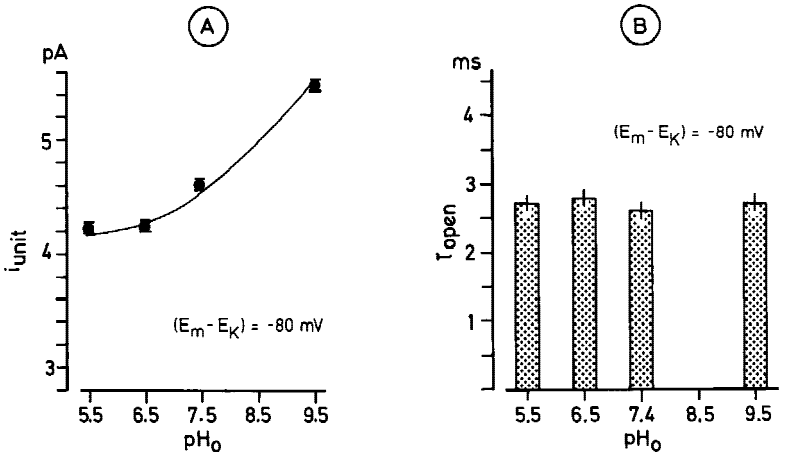

Fig. 2. The influence of external pH on $K^+_{(ATP)}$ channels: the response of i_{unit} (*A*) and τ_{open} (*B*) to a pH shift from 9.5 to 5.5. Each symbol represents the mean value of at least 3 individual experiments (vertical bars are SEM) separately performed at a given pH and the same K^+ driving force (−80 mV).

channels as proposed by Zilberter et al. (1988) but has a modulating influence.

Additional evidence for a dissociation of gating and permeation was obtained in pH experiments. External channel protonation by a stepwise decrease of external pH from 9.5 to 5.5 reduced conductance to 77% (Fig. 2*A*) but failed to induce a detectable change of τ_{open} (Fig. 2*B*). This seems consistent with the notion that distinct channel domains are involved in gating of and permeation through $K^+_{(ATP)}$ channels. The gating domain either might be insensitive to the pH range tested or is not accessible for external protons.

$K^+_{(ATP)}$ channels are permeable for Rb^+ ions (Ashcroft, Kakei & Kelly, 1989; Spruce, Standen & Stanfield, 1987) although blockade complicates the Rb^+ passage through the pore. The reaction of $K^+_{(ATP)}$ channel kinetics in this particular situation was of primary interest in the present experiments with external Rb^+ (Fig. 3). A comparison with control experiments likewise performed at symmetrical 140 mmol/l K^+ and inward permeation elucidated two prominent effects, depression of i_{unit} and prolongation of the open state. As expected from bi-ionic conditions (140 mmol/l external K^+ plus 47 mmol/l external Rb^+; 140 mmol/l internal K^+), the iv-relationship inwardly rectifies (Fig. 3*B*), and a reversal potential of +42 mV was calculated. As tested at −70 mV in a cumulative concentration response analysis (Fig. 3*C*), external Rb^+ prolonged the open state with a K_m of about 16 mmol/l and saturation close to 47 mmol/l. External Rb^+ caused a shift of the $\tau_{open} - (E_m - E_{rev})$ relationship along the abscissa without a detectable change of the slope factor (Fig. 3*B*). This means that Rb^+ interferes with the open state in a voltage-independent fashion. Moreover, the unchanged slope factor of this relationship is remarkable with respect to the non-ohmic iv-relationship. In the presence of the saturating Rb^+ concentration, about a 4-fold increased intrinsic open time was obtained by extrapolating the $\tau_{open} - (E_m - E_{rev})$ relationship to zero driving force. Internal Rb^+, however, failed to prolong the open state as observed in 5 experiments at asymmetrical (5 mmol/l external, 140 mmol/l internal) K^+ concentration and outward permeation although i_{unit} declined to 38 ± 4% of the control. This excludes that blockade of the pore by Rb^+ has a stabilizing influence on the open state.

Obviously, $K^+_{(ATP)}$ channels possess an externally located, superficial Rb^+ binding site whose occupancy can stabilize the open state, either directly or by an allosteric reaction. The Rb^+ interference was examined in greater detail in pH experiments to exclude that external acidification from 9.5 to 5.5 modulates the Rb^+ effect on τ_{open}. Another series of experiments was concerned with a possible Rb^+–K^+ competition. At symmetrical 250 mmol/l K^+ and 47 mmol/l external Rb^+, τ_{open} was 5.5 ± 0.4 msec ($n = 4$) compared with a value of 6.5 ± 0.3 msec ($n = 2$) at symmetrical 140 mmol/l K^+ and 47 mmol/l external Rb^+ at the same driving force, −101 mV.

Cs^+ experiments should test the cation specificity of the Rb^+-sensitive external channel region. As in inward rectifier K^+ channels (Quayle, Standen & Stanfield, 1988; Matsuda, 1996) external Cs^+ caused fast, unresolved flicker blockade with a K_i of 3.5 mmol/l. Although the values for τ_{open} (3.2 ± 0.1 msec; $n = 3$; −70 mV) did not significantly differ ($P > 0.05$) from control (2.9 ± 0.1 msec; $n = 5$; −70mV) in presence of 2.35 mmol/l Cs^+, this result cannot be unambiguously interpreted when fast flicker blockade occurs. A classification of Cs^+ as a cation lacking the potency to prolong the open state is difficult for still another reason. As mentioned above, external Rb^+ prolongs the open state with a K_m of about 16 mmol/l. This concentration range, however, could not be tested with Cs^+.

$K^+_{(ATP)}$ channels are distinguishable by their driving force-dependent open state kinetics from other cardiac inward rectifier K^+ channels. A comparative analysis of $K^+_{(ir)}$ and $K^+_{(ACh)}$ channels likewise in cell-free condi-

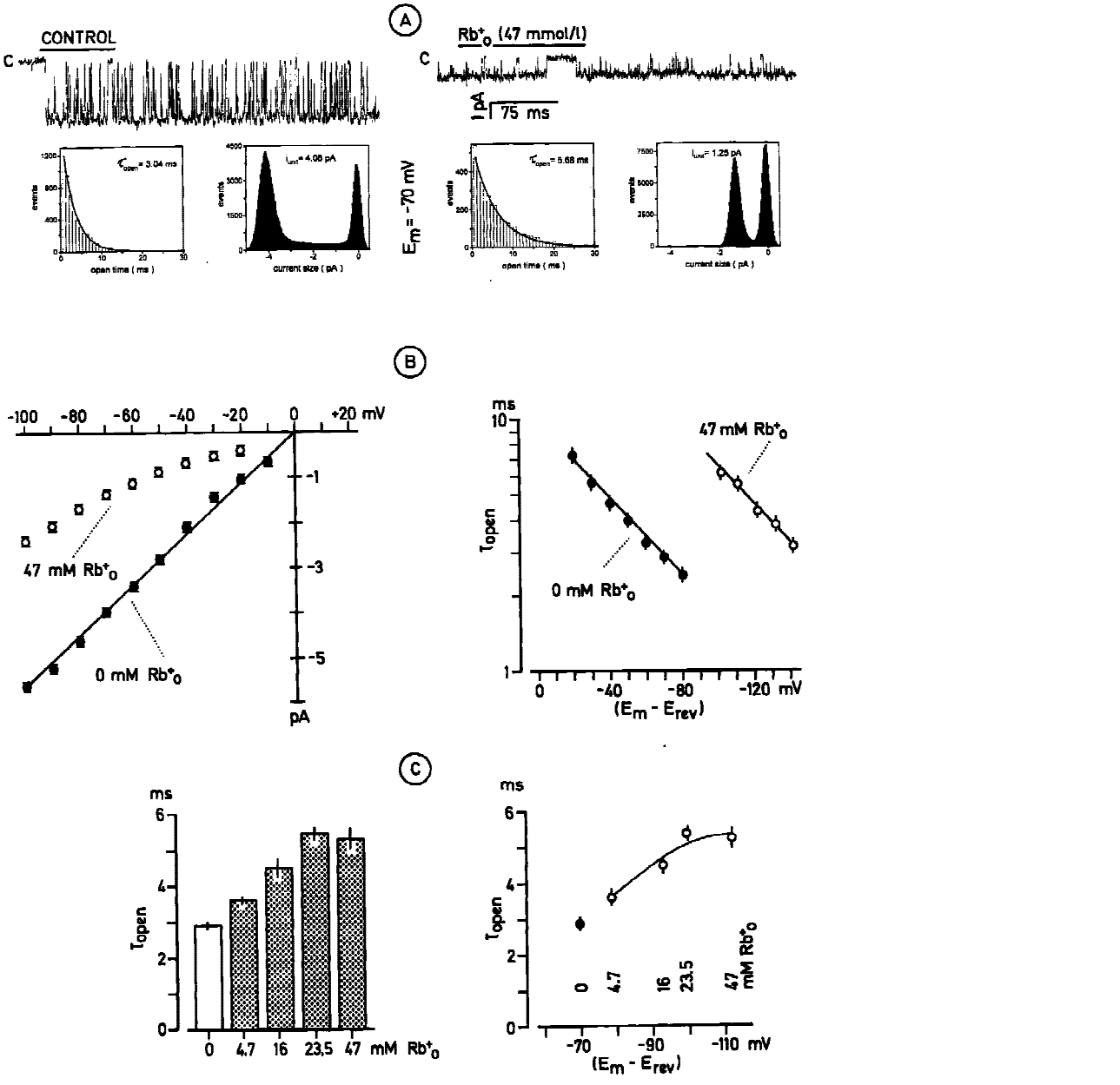

Fig. 3. Sensitivity of $K^+_{(ATP)}$ channels to external Rb^+. (*A*) Records of elementary K^+ currents (c means closed configuration), open time histograms and amplitude distributions in the absence (left, exp1112) and in the presence of external Rb^+ (right, exp1212). Symmetrical (140 mmol/l) K^+ concentration; membrane potential −70mV. (*B*) *iv*-relationships under control conditions (filled circles, $n = 4$ as a minimum) and with 47 mmol/l external Rb^+ (open circles, $n = 4$ as a minimum); horizontal bars are SEM. Symmetrical (140 mmol/l) K^+ concentration. (*right*) Semilogarithmic plot of τ_{open} vs. $(E_m - E_{rev})$ in the absence (filled circles, $n = 4$ as a minimum) and in the presence of external Rb^+ (47 mmol/l) (open circles, $n = 4$ as a minimum), vertical bars are SEM. Ionic conditions as in the left part. (*C*) *left:* Concentration-dependent increase of τ_{open} by external Rb^+. *right:* Driving-force-dependent increase of τ_{open}. Shown are the same data as in the left part to establish saturation of the Rb^+ effect. Each symbol is the mean of at least 3 individual experiments, vertical bars are SEM.

tions and K^+ inward permeation revealed essentially voltage-independent open state kinetics (Fig. 4). Open time in $K^+_{(ir)}$ channels was 36.2 ± 3.9 msec ($n = 5$) at −50 mV and 36.6 ± 4.0 msec ($n = 3$) at −100 mV. Interestingly, these values correspond to the intrinsic open time in $K^+_{(ATP)}$ channels when externally exposed to a saturating Rb^+ concentration. An exceptionally low gating rate with an apparent value of close to 10 openings/sec is an outstanding property of $K^+_{(ir)}$ channels: $K^+_{(ACh)}$ channels exhibit the same voltage independence of open

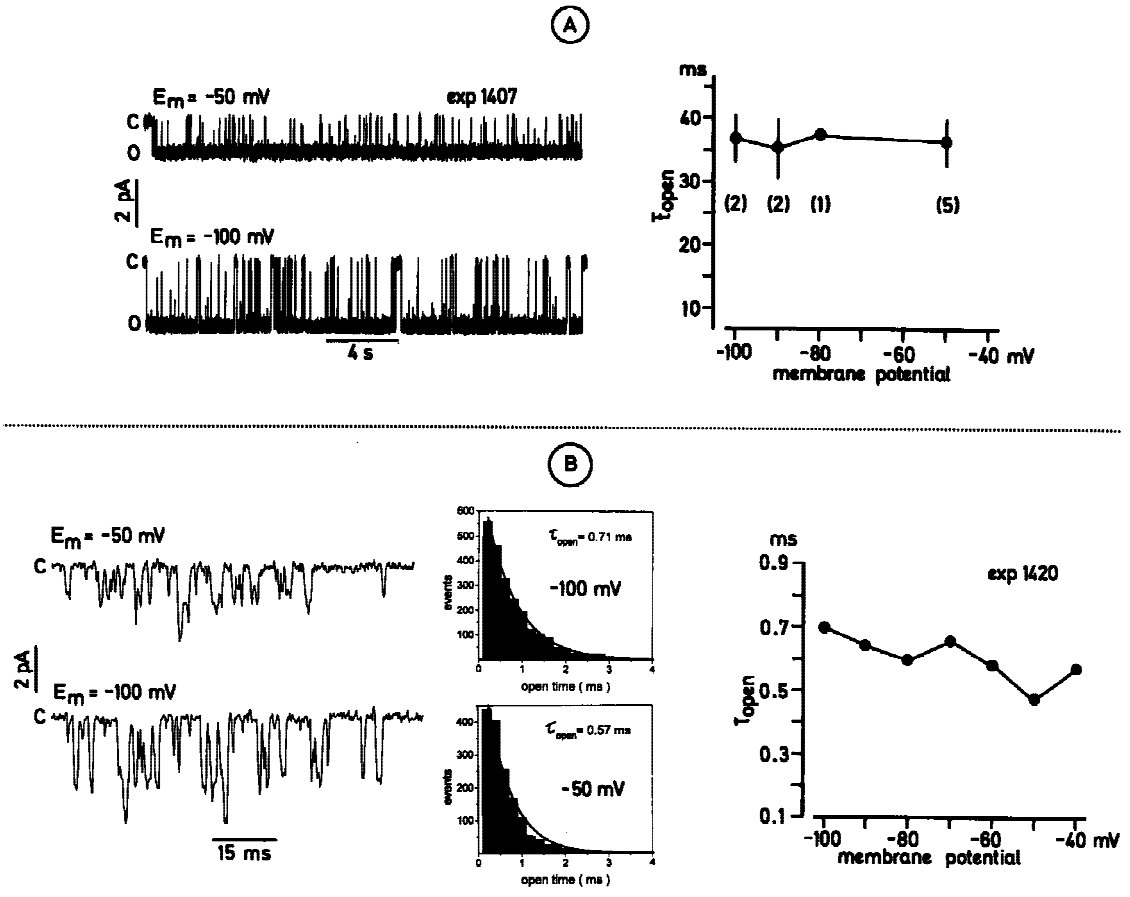

Fig. 4. (*A*) Records of elementary K^+ currents through a single $K^+_{(ir)}$ channel (*left*) and the voltage independence of open time in $K^+_{(ir)}$ channels (*right*). In the graph, the number under each data point refers to the number of experiments. Symmetrical (250 mmol/l) K^+ concentration. (*B*) Records of elementary K^+ currents through activated $K^+_{(ACh)}$ channels (*left*). Note the very different time scale compared with the record in *A*. Only one exponential is needed to fit the open time histograms (*middle*). (*Right*) The dependence of τ_{open} on membrane potential; voltage independence was stressed by the mean values for τ_{open} at −110 mV (1.06 ± 0.14 msec; $n = 5$) and at −50 mV (1.31 ± 0.24 msec; $n = 4$; $P > 0.05$). Symmetrical (140 mmol/l) K^+ concentration.

state kinetics but dwell very short-lasting in the conductive configuration, for about 1 msec. The hydrophobic core (M1-H5-M2) but not $G_{\beta\gamma}$-stimulation of muscarinic GIRK1 channels was recently recognized to be important for these brief openings (Slesinger et al., 1995).

Individual intrinsic open times in $K^+_{(ACh)}$, $K^+_{(ATP)}$ and $K^+_{(ir)}$ channels with an observed rank order for $1/\tau_{open}$ of roughly 30 : 3 : 1 can be barely expected in channels sharing exactly the same structural motif of the gating apparatus. Moreover, as found in experiments performed to analyze the temperature dependence of the apparent gating frequency between 19°C and 29°C, an activation energy for gating of 0.74 kcal/mol was obtained in $K^+_{(ATP)}$ channels, but of 5.2 kcal/mol in $K^+_{(ir)}$ channels. The high activation energy points to relatively large structural and energetic differences between the conducting and the nonconducting configuration in $K^+_{(ir)}$ channels. This is also noteworthy with respect to the large homology of the pore-lining H5-segment and disagrees with the hypothesis that a physical collapse of this part of the pore would be the event which structurally underlies a transition from the open to the closed channel configuration. Still more complicated seems the situation in $K^+_{(ATP)}$ channels because their gating apparatus must be supposed to sense the force created by the electrochemical K^+ gradient.

Structural peculiarities in $K^+_{(ATP)}$ channels are also predicted by their very individual K^+ sensitivity of conductance when compared with $K^+_{(ir)}$ and $K^+_{(ACh)}$ channels (Fig. 5). The present conductance measurements at inward K^+ permeation in $K^+_{(ir)}$ and $K^+_{(ACh)}$ channels yielded values which nicely fit the conductance − $[K^+]_o$ relationship obtained by Sakmann and Trube (1984) in cardiac inward rectifier K^+ channels. Interestingly, the slope factor of this function is in $K^+_{(ATP)}$ channels only half as large, 0.30 instead of 0.62. To consider a functional consequence for physiologically relevant *in situ* conditions, even major fluctuation of the K^+ concentra-

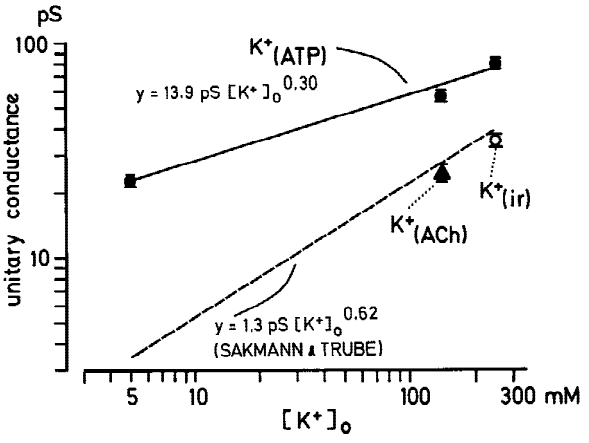

Fig. 5. The dependence of conductance on external K^+ in $K^+_{(ATP)}$ channels (filled circles), in $K^+_{(ir)}$ channels (open circles), and in $K^+_{(ACh)}$ channels (filled triangles). Vertical bars are SEM. The solid line was drawn from $\gamma = 13.9 \text{ pS} \times [K^+]_o^{0.30}$ (calculated from the experimental data). The broken line follows $\gamma = 1.3 \text{ pS} \times [K^+]_o^{0.62}$ published by Sakmann and Trube (1984) and obtained in cardiac inward rectifier K^+ channels.

tion in the extracellular space will only minimally affect $K^+_{(ATP)}$ channel conductance, if at all. Even more striking is the difference in the proportionality factor of both $\gamma - [K^+]_o$ relationships, 13.9 pS instead of 1.3 pS which indicates distinct permeation characteristics. $K^+_{(ATP)}$ channels, therefore, possess a still considerable conductance in the hypothetical case that K^+ homeostasis cannot be maintained in the extracellular space and external K^+ concentration would fall to drastically reduced levels. Externally located K^+ binding sites that are separate from the pore were proposed to activate or to control conductance in inward rectifier K^+ channels (Horowics, Gage & Eisenberg, 1968; Ciani et al., 1978). In $K^+_{(ATP)}$ channels, the K^+ interaction with these sites seems to follow specific rules. This tentative explanation does not disagree with the observation that an exchange of the pore-lining H5-segment between the less-conductive IRK and the more-conductive ROMK1 channels has little or no influence on K^+ conductance (Taglialatela et al., 1994). Unfortunately, these authors did not present kinetic data. It remains, therefore, an intriguing question whether an exchange of the H5-segment alters gating properties or not. Nevertheless, $K^+_{(ir)}$ and $K^+_{(ACh)}$ channels share with $K^+_{(ATP)}$ channels a unifying principle, namely failing flux coupling of open channel lifetime.

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