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Blockade of cardiac outwardly rectifying K^+ channels by TEA and class III antiarrhythmics – evidence against a single drug-sensitive channel site

I. Benz, M. Kohlhardt

Physiological Institute, University of Freiburg, Hermann-Herder-Strasse 7, D-79104 Freiburg, Germany

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Abstract. Elementary K^+ currents through cardiac outwardly rectifying K^+ channels were recorded in inside-out patches excised from cultured neonatal rat cardiocytes at 19 °C and at 9 °C. By studying the inhibitory effects of tetraethylammonium (TEA), quinidine and verapamil, the properties of this novel type of K^+ channel were further characterized. Internal TEA (50 mmol/l) evoked a reversible decline of i_{unit} to 62.7 ± 2.7% of control (at −7 mV), without significant changes of open state kinetics, indicating a blockade of the open K^+ pore with kinetics too fast to be resolvable at 1 kHz. This TEA blockade was e-fold voltage-dependent, with a decrease of the apparent $K_{D(TEA)}$ from 102 mmol/l at −37 mV to 65 mmol/l at +33 mV and, furthermore, became accentuated on lowering the internal K^+ concentration. Thus, TEA competes with the permeant K^+ for a site located in some distance from the cytoplasmic margin, within the K^+ pore. Quinidine (100 μmol/l), like verapamil (40 μmol/l) reversibly depressed i_{unit} to about 80% of the control value (at −7 mV), but drug-induced fast flicker blockade proved voltage-insensitive between −27 mV and +23 mV. These drugs gain access to a portion of the pore distinct from the TEA binding site whose occupancy by drugs likewise blocks K^+ permeation. Both drugs showed a greater potency to depress P_o which, with quinidine, decreased reversibly to 38.6 ± 11.1% (at −7 mV) and, with verapamil to 24.9 ± 9.1% (at −7 mV), mainly by an increase of the prolonged closed state (C_2). This alteration of the gating process also includes a sometimes dramatic shortening of the open state. Most probably, cardiac $K^+_{(outw.-rect.)}$ channels possess a second drug-sensitive site whose occupancy by quinidine or verapamil may directly or allosterically stabilize their non-conducting configuration.

Key words: Single cardiac K^+ channels – Gating – Quinidine – Verapamil – Channel-associated binding site – Heart muscle

Correspondence to: M. Kohlhardt

Introduction

The fascination of the K^+ channel family relies on the extreme heterogeneity of its members. Although K^+ selective, these channels differ tremendously from each other in permeation properties and in their susceptibility to modulating agents including intracellular Na^+ and Ca^{++} ions, neurotransmitters, G-proteins, protein kinase A and C, or metabolites. This diversity is of important biological significance since it enables K^+ channels to be employed in controlling very distinct cellular functions. The principal subunit of voltage-gated K^+ channels consists of 6 helical segments which form a membrane-spanning domain (for review see Catterall 1988) with the outstanding S4-segment. The highly conserved S4-segment bears a positively charged residue at every third position and is, thus, proposed to act as voltage sensor. Inward-rectifying K^+ channels differ in their proposed membrane topology in that they can be structurally modelled by two (M1 and M2) membrane-spanning segments flanking a pore-forming inner core (Kubo et al. 1993). Recent expression studies with RCK1 K^+ channels provided evidence that functional K^+ channels are tetramers (Liman et al. 1992).

In heart muscle, K^+ channels regulate important cellular functions by setting the resting potential and by mediating repolarization. The latter process is intimately involved in controlling the shape of the cardiac action potential and, thus, influences refractoriness of the heart cell. At least 6 distinct types can be distinguished (for review see Pennefather and Cohen 1990), a number which may well rise further. Taking permeation properties as the discrimination criterion, an inward rectifying 3.6 pS channel (Sakmann and Trube 1984), a non-rectifying 15 pS channel (Clapham and Logothetis 1988; Yue and Marban 1988) and, just identified as a novel type, an outwardly rectifying 66 pS channel (Benz et al. 1991) can be classified. They share the common property of being K^+ selective. The 15 pS channel and the 66 pS channel are candidates that may contribute to a major extent in shaping the ventricular action potential unless ATP-sensitive K^+ channels become activated owing to ATP de-

pletion or, in atrial cardiocytes, acetylcholine-regulated K^+ channels open.

A wide-spread property of K^+ channels is their susceptibility to quaternary ammonium ions. A variety of them becomes blocked by the presence of TEA at the external as well as at the internal surface. Particularly the internal TEA blockade served as a tool in probing structural properties of the pore (for review see Stanfield 1983; Hille 1984). This strategy has been adopted in the present inside-out experiments to further characterize the novel member of the cardiac K^+ channel family, $K^+_{(outw.-rect.)}$ channels. The sensitivity of $K^+_{(outw.-rect.)}$ channels to certain drugs such as quinidine and verapamil was another topic of interest, not only with respect to their specific response to these particular agents. Although quinidine is established as an inhibitor of several K^+ conductances in heart muscle, including the delayed rectifier K^+ current (Colatsky 1982; Hiraoka et al. 1986; Roden et al. 1988; Furukawa et al. 1989), the transient outward current (Imaizumi and Giles 1987) and the inward rectifier K^+ current (Hiraoka et al. 1986; Salata and Wasserstrom 1988), little was done at the single channel level. Thus, the nature of this blockade is only poorly understood, if at all. It will be shown that quinidine, like verapamil, exerts two distinct inhibitory actions with functional consequences not consistent with the hypothesis that there is only a single channel-associated binding site, at least in cardiac $K^+_{(outw.-rect.)}$ channels.

Methods

Elementary K^+ currents through single outwardly rectifying K^+ channels were recorded in the inside-out configuration with an L-M/EPC 5 amplifier by employing the standard patch clamp technique (Hamill et al. 1981). The patches were excised from short-time (18–24 h) cultured neonatal cardiocytes of rats. Disaggregation of the hearts, cell culture and handling of the cultured cardiocytes were essentially the same as described in detail elsewhere (Kohlhardt et al. 1986). Rod-shaped cardiocytes were selected for the patch clamp experiments since the likelihood to detect $K^+_{(outw.-rect.)}$ channels is larger in this cell type than in spherical cardiocytes. After patch formation, an initial equilibration in the cell-attached mode of about 10 min was allowed before the patch was excised in isotonic K^+ solution supplemented with 0.5–1 mmol/l ATP. ATP served to increase the open probability of $K^+_{(outw.-rect.)}$ channels (Benz et al. 1991) and was used for practical reasons since P_0 is usually very low in the absence of this adenine nucleotide.

The patch clamp recordings were filtered at 1 kHz using a 8-pole Bessel filter, stored on tape and digitized with a sampling rate of 5 kHz to be analyzed. The dead time was 0.2 ms under these recording conditions. The analysis was based on the 50% threshold method (Colquhoun and Sigworth 1983) to evaluate open probability (analyzed for periods of 30 s), unitary current size (i_{unit}, obtained from a Gaussian histogram analysis), open and closed kinetics. The latter were obtained from open and closed time histograms, constructed from non-overlapping single events and fitted by the least square method to yield τ_{open} and τ_{closed}, respectively. Closed time analysis was restricted to experiments with only one $K^+_{(outw.-rect.)}$ channel.

Apparent dissociation constants for TEA, quinidine and verapamil were calculated from

$$K_D = [\text{blocking agent}] \times \frac{i_{\text{unit (blocking agent)}}}{i_{\text{unit (control)}} - i_{\text{unit (blocking agent)}}}$$

Whenever possible, the data are expressed as mean ± SEM.

Solutions (composition in mmol/l)

A. Isotonic K^+ solution (depolarizing bath solution to avoid spontaneous activity of the cardiocytes, facing the cytosolic membrane surface after patch excision): KCl 140 (or 70 plus 70 sucrose); $MgCl_2$ 2; glucose 20; Hepes 10; EGTA 2; pH 7.4; temperature 19 ± 0.5 °C or 9 ± 0.5 °C.
B. Pipette solution (facing the external side of the membrane): KCl 5; NaCl 135; $MgCl_2$ 2; Hepes 10; pH 7.4.

Compounds

ATP, TEA (as tetraethylammonium bromide), quinidine and verapamil were purchased from Sigma Chemie, München. All compounds were freshly dissolved in isotonic K^+ solution just before use.

Results

Consistent with earlier observations (Benz et al. 1991), cardiac $K^+_{(outw.-rect.)}$ channels are characterized by a low density, at least in cultured neonatal cardiocytes, since they could be detected in the present experiments in only 10% or less of the patches, regardless of the recording mode, cell-attached or inside-out. Although they preserve their initial activity in cell-free conditions over a long period of time, 60 min or more, without any signs of run-down, only the cytoplasmic treatment with 0.5–1 mmol/l ATP caused $K^+_{(outw.-rect.)}$ channels to attain a high activity mode with a P_0 level of 30% (at −7 mV) or more. As shown in Fig. 1, openings mostly occur in bursts and, more interestingly, the channel can occasionally leave the dominating full conductance state to reach a substate where i_{unit} has only 50% of its normal size. Openings showing this particular permeation mode were usually attained from the closed channel configuration which is consistent with the reaction scheme C–O. They can persist over several tens of milliseconds but substate openings may be the less stable open configuration. This becomes evident from distinct open state kinetics (Fig. 1): τ_{open} in the substate was found to be reduced to about one third when compared with τ_{open} in the fullstate. Other experiments, however, yielded corresponding values for τ_{open} which excludes the possibility that substate openings are regularly left with an increased exit rate. The nature

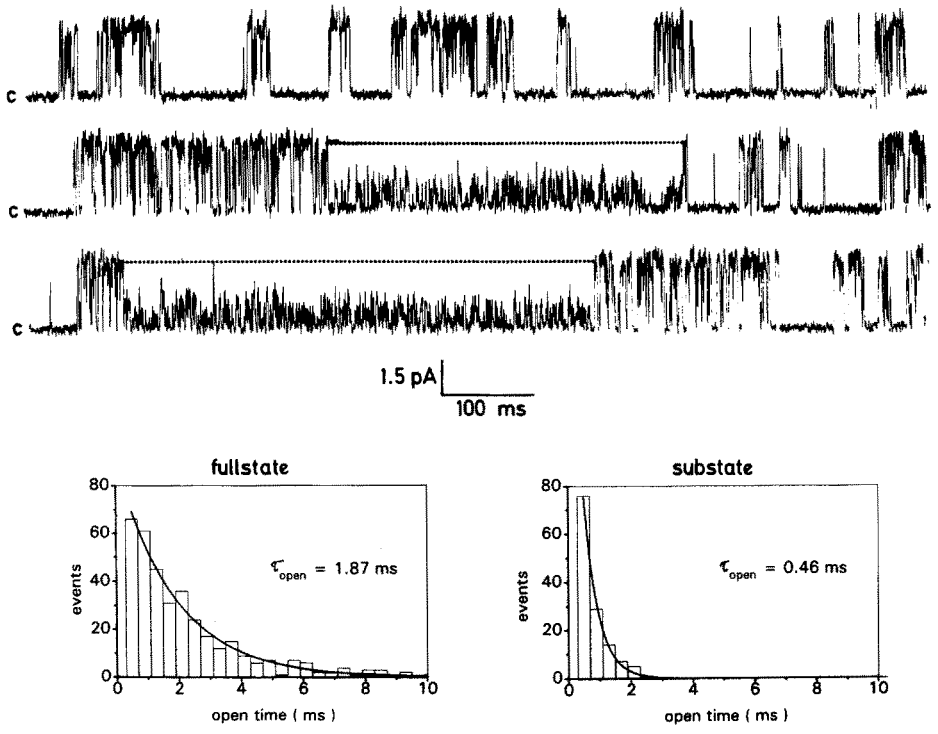

Fig. 1. Two conductance levels in cardiac $K^+_{(outw.-rect.)}$ channels. *Upper part:* Continuous recordings of elementary K^+ currents, upward deflections mean outward current. *C* means closed state. The dashed lines indicate the fully sized event level. *Lower part:* Open time histograms from fully sized (*left*) and from subconductance events (*right*). By neglecting the first bin of 0.2 ms, the best fit was $N = 70 \exp(-t/0.00197)$ and $N = 71 \exp(-t/0.00046)$, respectively. Exp. 401; membrane potential −7 mV; temperature 19 °C

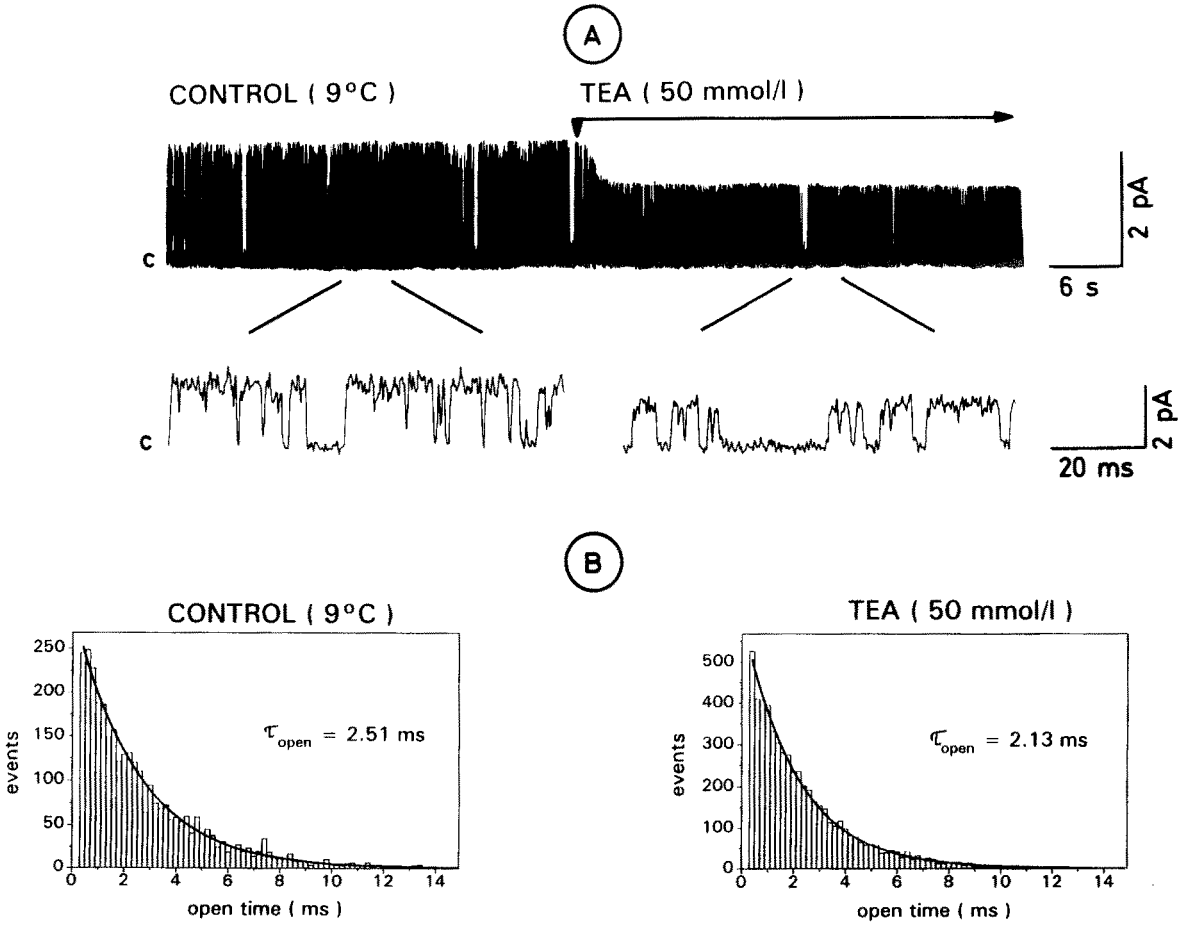

Fig. 2. The effect of internal TEA on cardiac $K^+_{(outw.-rect.)}$ channels. *Part A:* Continuous recording of elementary K^+ currents before and after internal TEA application (50 mmol/l). Note the persistence of long-lasting openings in the presence of TEA (see expanded traces). *C* means the close state. *Part B:* Open time histograms before (*left*) and after (*right*) internal TEA application. By neglecting the first bin of 0.2 ms, the best fit was $N = 286 \exp(-t/0.00251)$ and $N = 611 \exp(-t/0.00213)$, respectively. Exp. 303; membrane potential −7 mV; temperature 9 °C

of the subconductance state and its potentially distinct open state kinetics remain unknown.

A first series of experiments dealt with the influence and the mode of action of TEA. Figure 2 demonstrates the typical response of cardiac $K^+_{(outw.-rect.)}$ channels when exposed internally to quaternary ammonium ions (50 mmol/l), namely a substantial reduction in unitary current size, an effect proven to be rapidly reversible on washout (not shown). That the i_{unit} reduction specifically reflects a TEA action and is not related to the accompanying rise in osmolarity was demonstrated in 3 control experiments. Exposing the cytoplasmic channel surface to 100 mmol/l sucrose and, thus, to the same hyperosmolaric environment had no influence on i_{unit}. Obviously, TEA interferes with $K^+_{(outw.-rect.)}$ channels to repetitively block the open pore with a rate too fast to be resolvable even at low temperature, 9 °C. No evidence could be obtained that TEA might eventually exert a second blocking action, i.e. with slower reaction kinetics, since long-lasting openings remained in the presence of TEA and were not chopped (see Fig. 2 A) in multiple transitions between a conducting and a non-conducting state. A resolvable, slow flicker blockade was reported to occur as another manifestation of the TEA interaction in cloned K^+ brain channels (Kirsch et al. 1991) and in ATP-sensitive K^+ channels from skeletal muscle (Davies et al. 1989). In the present experiments, τ_{open} proved insensitive or changed only slightly in a 10% range (see Fig. 2 B).

The theoretical arguments justifying an analysis of unresolved channel flickering from amplitude distributions has been presented by Yellen (1984). Basically, there exists a proportionality between the reduction of i_{unit} and the strength of flicker blockade but, in a quantitative aspect, this relationship depends fundamentally on the recording conditions, i.e. on the actual filter frequency. The present analysis (Fig. 3 A) of the internal TEA blockade ignored this problem and will, therefore, only yield semiquantitative block data suitable to describe modulating influences on the TEA action but not necessarily comparable with block data, including K_D values of the literature.

The internal TEA blockade can be modulated by variations of the cytosolic K^+ concentration (Fig. 3 B) and is also a function of voltage (Fig. 3 C). At a quasi physiological internal K^+ concentration (140 mmol/l), 50 mmol/l TEA, at −7 mV, caused i_{unit} to decline to 62.7 ± 2.7% ($n=5$) of the control value, indicating a blockade of 37.3 ± 2.7%. A separate set of experiments tested the effectiveness of the same TEA concentration at reduced internal K^+ (70 mmol/l): channel blockade was found to be stronger and amounted to 47.9 ± 2.6%. Consequently, the apparent $K_{D\,(TEA)}$ decreased from 80 ± 5 mmol/l to 55 ± 6 mmol/l. This is consistent with the attenuation of the blocking tetramethylammonium action in Ca^{++}-activated K^+ channels after elevation of internal K^+ (Villarroel et al. 1988) and represents the expected result when TEA competes with K^+ for a common site. Shifting the membrane potential in the positive direction accentuated the TEA action. The apparent $K_{D\,(TEA)}$ declined from 102 ± 2 mmol/l at −37 mV to 65 ± 6 mmol/l at +33 mV to exhibit an exponential voltage dependence (see Fig. 3 C). In the presence of 70 mmol/l internal K^+, this voltage

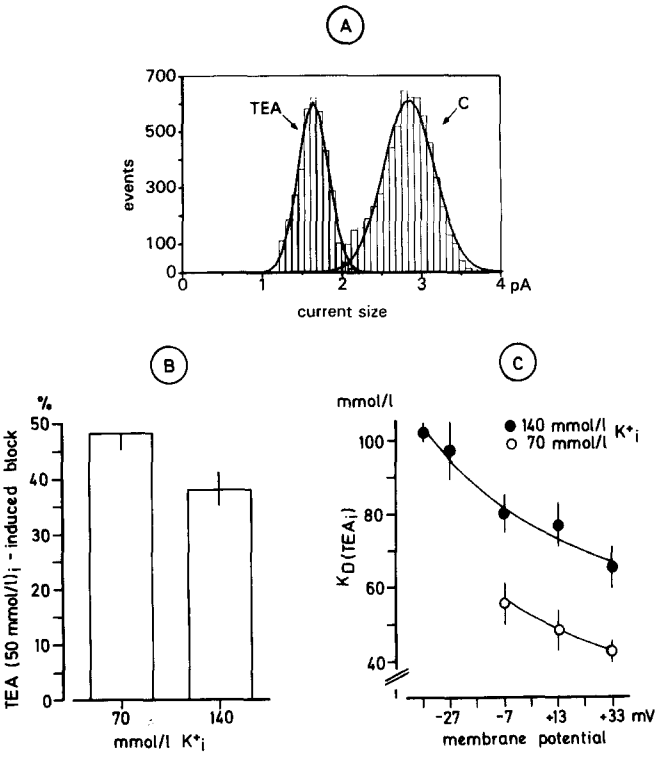

Fig. 3. *Part A:* Superimposed Gaussian amplitude distributions obtained under control conditions (*C*) and after internal TEA application (50 mmol/l; TEA). Exp. 401; membrane potential −7 mV; temperature 9 °C. *Part B:* The dependence of internal TEA blockade on the cytosolic K^+ concentration. Each column represents an individual set of experiments and symbolizes the mean of 5 experiments (at 140 mmol/l internal K^+) and of 3 experiments (at 70 mmol/l internal K^+), respectively; vertical bars indicate SEM. Membrane potential −7 mV; temperature 19 °C. *Part C:* Voltage dependence of the apparent $K_{D\,(TEA)}$ (calculated from the equation given in the methods) at 140 mmol/l internal K^+ (*filled circles*, $n=5$) and at 70 mmol/l internal K^+ (*open circles*, $n=3$). Vertical bars are SEM. The lines relating K_D to E_M were drawn by eye. Temperature 19 °C

dependence was studied only between −7 mV and +33 mV to avoid analytical problems which may arise from the rather small i_{unit} size at more negative potentials. It remains, therefore, to be seen if the slope of the $K_{D\,(TEA)}-E_m$ relationship is actually insensitive to internal K^+ variations.

Some other organic molecules, structurally unrelated to TEA, were found to cause the same type of channel blockade. Quinidine, a naturally occuring cinchona alkaloid, proved effective in blocking open $K^+_{(outw.-rect.)}$ channels. After cytosolic administration of 100 μmol/l, for example, i_{unit} declined in the experiment illustrated in Fig. 4 A from 3.9 pA to 2.9 pA (see also Fig. 6 A) within a few tens of milliseconds. In a total of 7 inside-out patches, unitary current size decreased, at +13 mV, from 3.93 ± 0.23 pA to 3.34 ± 0.22 pA, i.e. to 85% of the control. Experiments with well-resolved substate openings (Fig. 5) showed that quinidine remained effective when the channel switches in this particular permeation mode. Obviously, the substate responded as strongly to the drug as the dominating fullstate: in the experiment illustrated in

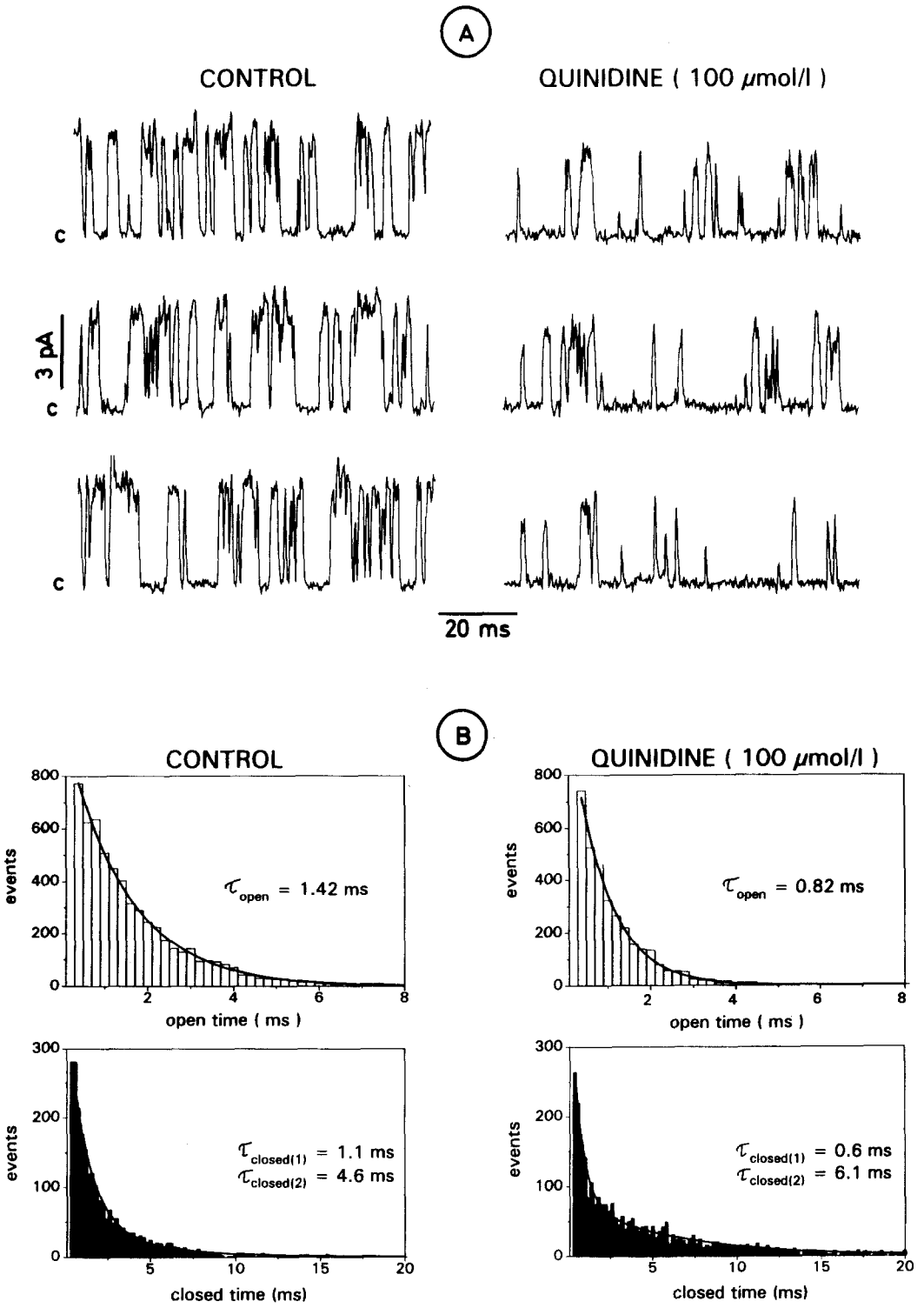

Fig. 4. Multiple effects of quinidine on cardiac $K^+_{(outw.-rect.)}$ channels. *Part A:* Selected continuous records of elementary K^+ currents before and after cytosolic quinidine application (100 μmol/l). *C* means closed state. *Part B: Open* (*light columns*) and closed (*dark columns*) time histograms before (*left*) and after quinidine treatment (*right*). By neglecting the first bin of 0.2 ms, the open time histograms could be best fitted by $N=1\,064 \exp(-t/0.00142)$ and $N=727 \exp(-t/0.00082)$, respectively. The closed time histograms obey $N=341 \exp(-t/0.00114)+39 \exp(-t/0.00463)$ and $N=370 \exp(-t/0.0006)+80 \exp(-t/0.00612)$, respectively. Exp. 422; membrane potential +13 mV; temperature 19 °C

Fig. 5, the ratio $i_{unit\ (substate)}/i_{unit\ (fullstate)}$ was approximately 0.52 before and 0.53 after quinidine treatment.

However, as can also be seen in Fig. 4, the quinidine action on $K^+_{(outw.-rect.)}$ channels is rather complex and is not restricted to i_{unit}. Consistent with recent observations of Balser et al. (1991) with cardiac inward-rectifying K^+ channels, P_0 also declined, in this case from 40% to 19%, mainly due to a prolongation of the long-lasting closed state, C_2 (see Fig. 4B). $\tau_{closed\ (2)}$ rose in 4 experiments at −7 mV from 3.1 ± 1.7 ms to 8.2 ± 4.5 ms, i.e. to 271 ± 5%

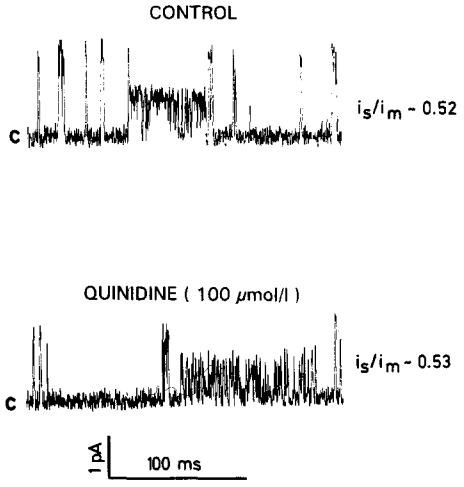

Fig. 5. The sensitivity of the subconductance state to quinidine. Records of elementary K^+ currents through $K^+_{(outw.-rect.)}$ channels before (*upper registration*) and after quinidine treatment (*lower registration*). From the arithmetic mean of 10 events with a minimum open time of 1 ms, the unitary current size was determined for the main state (i_m) and for the substate (i_s). *C* means closed state. Exp. 421; membrane potential −7 mV; temperature 19 °C

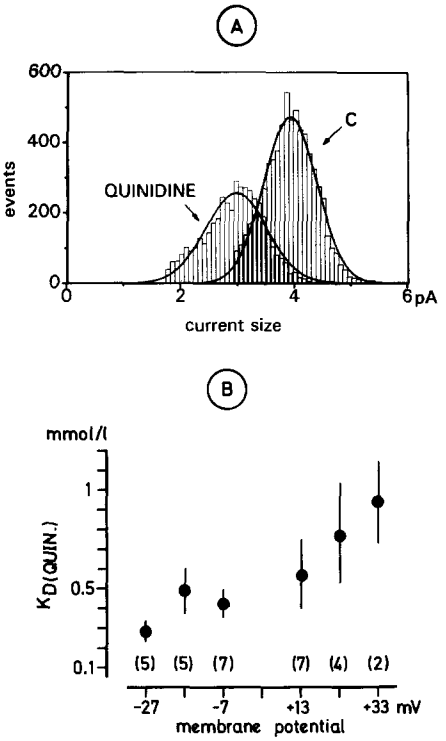

Fig. 6. *Part A:* Superimposed Gaussian amplitude distributions obtained under control conditions (*C*) and after cytosolic quinidine application (100 µmol/l). Exp. 422; membrane potential +13 mV; temperature 19 °C. *Part B:* The voltage dependence of the apparent $K_{D\,(quinidine)}$ (calculated from the equation given in the methods). Each circle symbolizes the mean of experiments whose numbers are given in brackets, vertical bars indicate SEM. The data at −27 mV and +23 mV are not statistically different from each other. Temperature 19 °C

of the predrug value whilst $\tau_{closed\,(1)}$ varied only insignificantly to 114±11% of the control. Open state kinetics reacted less sensitively (see Fig. 4B) and declined to 74±7% of the control. Open probability as well as unitary current size rapidly returned to the initial control values on washout, an important observation with respect to the partial reversibility of the quinidine action in cardiac inward-rectifying K^+ channels (Balser et al. 1991) and with respect to the reported irreversible quinidine-induced depression of steady-state outward currents in ventricular myocytes (Salata and Wasserstrom 1988). Readmission of quinidine was found to promptly reestablish i_{unit} depression and P_0 reduction.

The phenylalkylamine verapamil, structurally unrelated to quinidine and shown in voltage-clamped cardiac Purkinje fibers to reduce slow outward current activation (Kass and Tsien 1975), exerted a very similar influence on cardiac $K^+_{(outw.-rect.)}$ channels (Fig. 7). Exposing the cytoplasmic membrane surface to 40 µmol/l verapamil caused both i_{unit} and P_0 to decline, the former from 2.4±0.3 pA to 1.9±0.2 pA (at −7 mV) and the latter from 50±2% to 12±5% (at −7 mV). The open state was also affected and became shortened (see Fig. 7B) from 1.3±0.2 ms to 0.5±0.2 ms (at −7 mV; $n=3$), i.e. to 38±11% of the control value. The necessity to use multichannel patches for the verapamil experiments invalidated any closed state analysis because, in this particular case, long-lasting gaps between openings cannot be unambigiously interpreted as closing events of an individual channel.

In an attempt to elucidate the mode of action of both drugs on i_{unit} in grater detail, and thereby also stressing similarities or dissimilarities with the blocking action of internal TEA, the voltage dependence of the drug effect on i_{unit} was studied. With quinidine, the depression of i_{unit} and, thus, the apparent $K_{D\,(quinidine)}$ varied only insignificantly in a broad potential range, between −27 mV and +23 mV (see Fig. 6B). This could be confirmed for the verapamil blockade: in 2 experiments with 40 µmol/l, i_{unit} was close to 80% of the predrug value over the entire voltage range from −27 mV to +23 mV, in striking contrast to the voltage-dependent internal TEA blockade described above.

A quantitative comparison of the drug effects (Fig. 8 A, B) suggests a differential channel sensitivity since quinidine as well as verapamil depressed P_0 more strongly than i_{unit}. This figure also shows that 40 µmol/l verapamil is equipotent with 100 µmol/l quinidine, i.e. verapamil is more effective than quinidine. This is, however, a point of minor importance in the present context. An objection against this quantitative comparison may arise from methodological considerations since i_{unit} depends essentially on the recording conditions, in contrast to P_0, the latter single channel parameter being less critical in this respect. Consequently, any quantification of the fast flicker blockade from an i_{unit} reduction will be complicated by the filter frequency employed. However, assuming higher filter frequencies, e.g. 10 kHz instead of the 1 kHz used in the present study, such improved recording conditions would yield, in a tendency to resolve fast flicker events, a unitary current size which would better approach its true value. Consequently, the error to be envisaged is not an

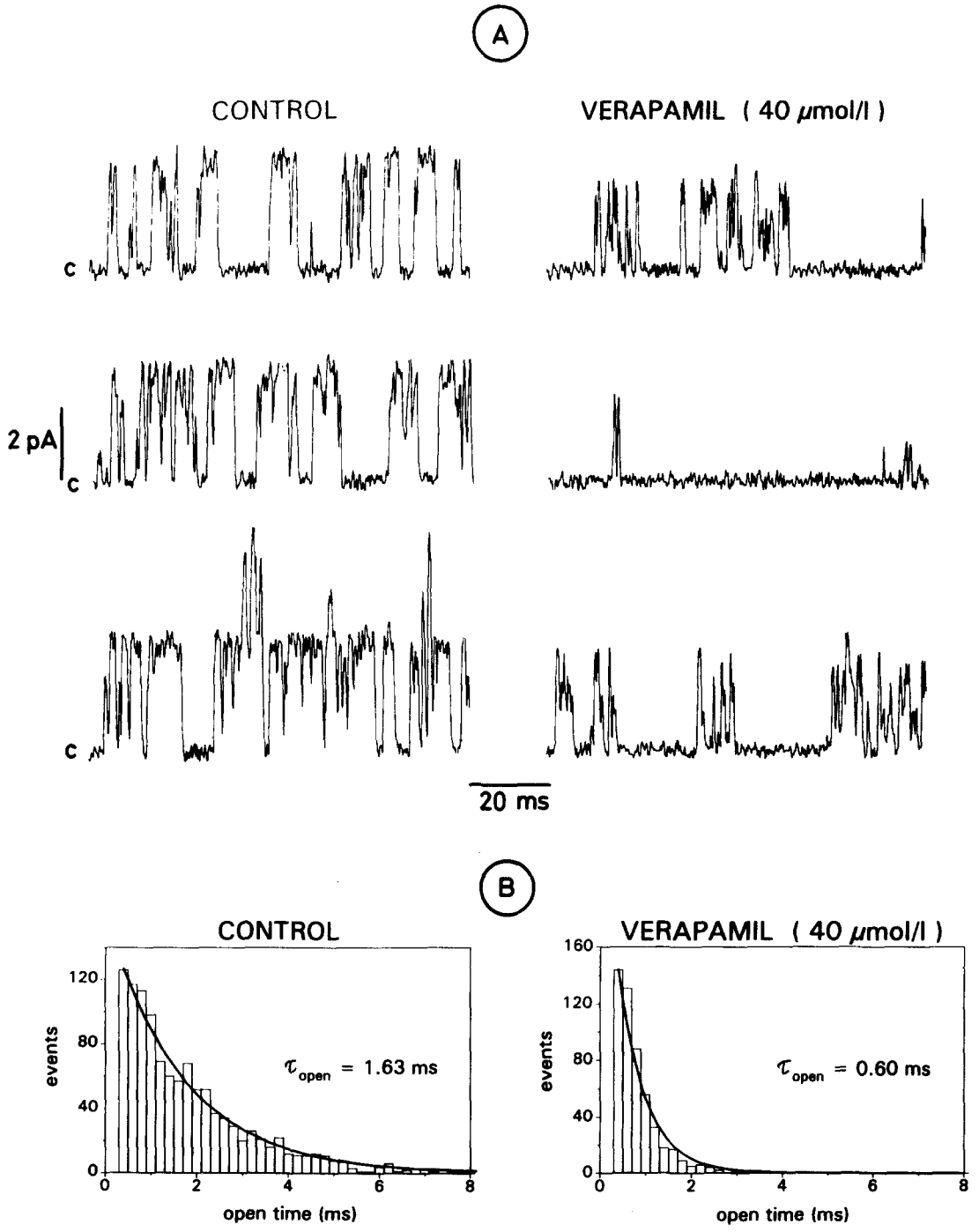

Fig. 7. Multiple effects of verapamil on cardiac $K^+_{(outw.-rect.)}$ channels. *Part A:* Continuous records of elementary K^+ currents under control conditions (*left*) and after verapamil treatment (40 μmol/l; *right*). *C* means closed state. *Part B:* Open time histograms before (*left*) and after (*right*) verapamil treatment. By neglecting the first bin of 0.2 ms, they could be best fitted by $N = 130 \exp(-t/0.00163)$ and $N = 164 \exp(-t/0.0006)$, respectively. Exp. 440; membrane potential −7 mV; temperature 19 °C

underestimate but rather an overestimate of the drug action on i_{unit}. This stresses the conclusion that either drug, in interacting with $K^+_{(outw.-rect.)}$ channels, preferentially affects open probability.

Discussion

The present inside-out patch clamp experiments have characterized the internal TEA blockade of cardiac $K^+_{(outw.-rect.)}$ channels and have shown that this novel K^+ channel shares some important features with other members of the K^+ channel family. Thus, the observed $K_{D\,(TEA)}$ matches the range from 27 mmol/l in K^+ channels from mouse neuroblastoma cells (Im and Quandt 1992) to 60 mmol/l in Ca^{++}-activated K^+ channels from rat muscle (Blatz and Magleby 1984) at comparable environmental ionic conditions and a membrane potential close to 0 mV. Rat brain RCK2 K^+ channels expressed in Xeno-

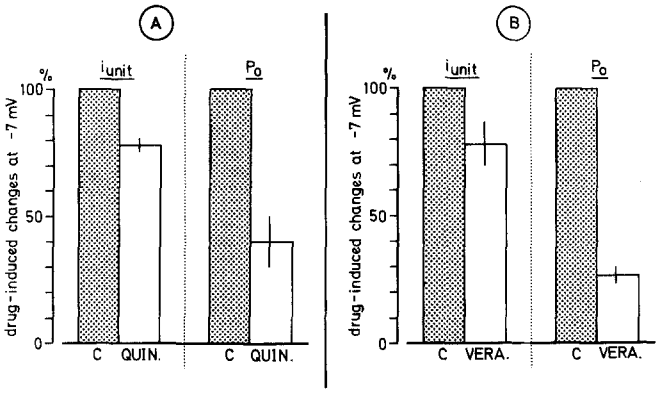

Fig. 8. Differential effects of quinidine (100 μmol/l; *Part A*) and of verapamil (40 μmol/l; *Part B*) on i_{unit} and P_o of $K^+_{(outw.-rect.)}$ channels. *Dotted columns* symbolize the control level (=100%) and the open columns indicate the mean changes in 5 experiments in Part A and of 3 experiments in Part B; *vertical bars* represent SEM. Membrane potential −7 mV; temperature 19 °C

pus oocytes reportedly have a higher TEA affinity (Kirsch et al. 1991). Moreover, the voltage sensitivity and the modulation of the TEA interaction with the open K^+ pore by the internal K^+ concentration is not specific for cardiac $K^+_{(outw.-rect.)}$ channels but is a general feature recently defined by Blatz and Magleby (1984); Kirsch et al. (1991) and Villarroel et al. (1988) at the single channel level.

TEA and related quaternary ammonium ions which specifically block K^+ channels have provided insights into the structure of the inner K^+ channel entrance. Although there is a theoretical debate about the size of the inner mouth, just large enough to accomodate TEA (having a diameter of 0.8 nm) as initially proposed by Armstrong (1975) or larger, with an assumed diameter of 1.2 nm (Swenson 1981) recently also proposed for Ca^{++}-activated K^+ channels (Villarroel et al. 1988), the TEA binding site clearly lies in some distance from the cytoplasmic channel surface. The voltage dependence of the internal TEA blockade indicates a binding site location within the electrical field of the membrane at a point 25–30% distant from the cytoplasmic margin (Blatz and Magleby 1984; Villarroel et al. 1988; Kirsch et al. 1991). In binding, TEA competes for K^+, thereby occluding the K^+ permeation pathway with rates too fast to be resolvable.

Recent site-directed mutagenesis experiments (Yellen et al. 1991) with K^+ channels encoded by the Drosophila shaker gene have identified the SS1-SS2 region as containing the internal TEA binding site. The SS1-SS2 stretch is proposed to cross the membrane twice, thereby providing a link between the segments S5 and S6. A point mutation in position 441 of the SS1-SS2 stretch from threonine to serine led to a drastically reduced internal TEA affinity at about one tenth of the wild type K^+ channel. Consequently, Yellen et al. (1991) suggested that this conserved region is intimately involved in forming the K^+ pore. Since, interestingly, the reported internal TEA affinity ($K_{D\ (TEA)}$ 0.7 mmol/l) of the wild type channel is 50- to 100-fold larger when compared with many other mammalian K^+ channels, including cardiac $K^+_{(outw.-rect.)}$ channels, it seems tempting to assume that position 441 cannot be homologous through the whole K^+ channel family. Likewise unusual in wild type Shaker H4 K^+ channels is their apparently reversed TEA affinity, namely externally lower than internally (Yellen et al. 1991), contrasting with the TEA affinity in other K^+ channels. Moreover, in delayed rectifying K^+ channels from Drosophila neurons, internal TEA was reported (Yamamoto and Suzuki 1989) to evoke two distinct responses, besides fast flicker blockade, a reduction in open probability as if the channel were stabilized in its inactivated state.

It was interesting to see that cardiac $K^+_{(outw.-rect.)}$ channels are sensitive to small organic molecules such as quinidine and verapamil and respond with a fast flicker blockade. However, their blocking effect differs fundamentally from internal TEA blockade by its failing voltage dependence. Both drugs interact with the open pore at a site obviously not located within the electrical field of the membrane and being distinct from the internal TEA binding site. That Balser et al. (1991) could not detect any changes of i_{unit} in inward rectifying cardiac K^+ channels after quinidine treatment may be related to the lower drug concentration they used, 50 μmol/l, or, alternatively, could indicate that individual K^+ channels may behave differently. The location of the drug binding site, at the internal or external channel mouth, remains to be elucidated. This uncertainty relies on the physico-chemical properties of quinidine and verapamil employed as blockers in the present experiments: about 10% of the drug molecules exist at pH 7.4 in the neutral form and will diffuse, after cytoplasmic administration, through the lipid matrix of the membrane to reach the external channel mouth. The latter may well accomodate a drug binding site whose occupancy blocks the K^+ permeation pathway. Another open question is the number of drug binding sites, a common single site for both quinidine and verapamil or two distinct sites for the two drugs. Both hypotheses would be consistent with the experimental result that 40 μmol/l verapamil caused almost the same reduction of i_{unit} as 100 μmol/l quinidine.

Fast flicker blockade of cardiac $K^+_{(outw.-rect.)}$ channels has particular interest because this drug action seems to be lacking in other cardiac ionic channels. Voltage-gated Na^+ channels, for example, preserve not only their permeation properties when exposed to quinidine (Benz and Kohlhardt 1991) but are also protected against any other antiarrhythmic drugs so far studied (Kohlhardt and Fichtner 1988). Na^+ channels share this drug-resistant open state with L-type Ca^{++} channels since the latter do not respond to Ca^{++} blockers, including phenylalkylamines and dihydropyridines, with a reduction of i_{unit}. That both conductance states of $K^+_{(outw.-rect.)}$ channels have apparently the same quinidine sensitivity can be explained with both hypotheses, a double-barelled or a single-barelled channel. In the latter, which fits the putative channel models most plausibly, a subconductance level is thought to arise from a jump-like increase of K^+ affinity of groups lining the pore to be involved in K^+ permeation.

Drug-treated cardiac $K^+_{(outw.-rect.)}$ channels can be modelled, in the simplest form, by a Markovian reaction

scheme as follows

$$\begin{array}{ccccc} C_2 & \underset{k_3}{\overset{k_1}{\rightleftharpoons}} & C_1 & \underset{k_4}{\overset{k_2}{\rightleftharpoons}} & O \\ \Updownarrow & & & & \Updownarrow \\ C_2D & & & & OD \end{array}$$

where C_2 and C_1 mean the long-lasting and the short-lasting closed state, O is the open state whilst C_2D and OD are drug-associated states. Accordingly, the P_0 reduction seen with quinidine and verapamil may emerge predominantly from trapping the channel in the more stable C_2D-state but flicker blockade arises from drug binding to give the OD-state. Clearly, this formalism adopts a scheme initially proposed by Hille (1977) and Hondeghem and Katzung (1977) to describe the interaction of local anesthetics with voltage-gated Na^+ channels. The drug sensitivity of τ_{open} suggests a rather complex change of the gating process and indicates that quinidine and particularly verapamil favour the transition from the open to the closed configuration, by increasing the exit rate, with verapamil, from 787 s^{-1} to 2 000 s^{-1} (at -7 mV).

When compared with the response of i_{unit}, the major effect of quinidine and verapamil was the depression of P_0. This differential channel sensitivity is most easily explained by assuming two distinct channel-associated binding sites, one of them involved in fast flicker blockade whilst drug occupancy of the other binding site stabilizes the closed channel configuration, either directly or by inducing an allosteric reaction. It is tempting to speculate that the latter binding site is not located in the channel mouth but lies in some distance from this pore-forming portion. Again, for the reasons mentioned above and related to the lipophilicity of quinidine and verapamil at pH 7.4, the cytoplasmic channel surface cannot be definitely identified as the area which bears this drug-sensitive region. It also remains to be shown whether quinidine and verapamil or other related drugs interact with the same site to influence channel gating.

Although quinidine was reported to have the capability to reduce the cellular cAMP content (Mirro 1981) so that the P_0 decline might eventually result from changes in channel phosphorylation, this possibility is meaningless in the present context because the drug response of isolated $K^+_{(outw.\text{-}rect.)}$ channels has been studied. Regarding quinidine and verapamil as class III antiarrhythmic agents, their depressing effect on P_0 would be most important in defining the class III efficacy if other K^+ channels also contributing to the repolarization process could react as sensitively as $K^+_{(outw.\text{-}rect.)}$ channels. However, as another manifestation of their heterogeneity, cardiac K^+ channels may be characterized by an individual drug sensitivity which discriminates them from each other.

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