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A kinetic study on the stereospecific inhibition of KCNQ1 and I_{Ks} by the chromanol 293B

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1 Recently we and others have demonstrated a stereoselective inhibition of slowly activating human I_{Ks} (KCNQ1/MinK) and homomeric KCNQ1 potassium channels by the enantiomers of the chromanol 293B. Here, we further characterized the mechanism of the 293B block and studied the influence of the 293B enantiomers on the gating kinetics of both channels after their heterologous expression in *Xenopus* oocytes.

2 Kinetic analysis of currents partially blocked with 10 μM of each 293B enantiomer revealed that only 3R,4S-293B but not 3S,4R-293B exhibited a time-dependent block of I_{Ks} and KCNQ1 currents, indicating preferential open channel block activity.

3 Inhibition of both KCNQ1 and I_{Ks} channels by 3R,4S-293B but not by 3S,4R-293B increased during a 2 Hz train of stimuli.

4 At high extracellular potassium concentrations the inhibition of KCNQ1 by 3R,4S-293B and 3S,4R-293B was unaffected. Drug inhibition of KCNQ1 and I_{Ks} by both enantiomers also did not display a significant voltage-dependence, indicating that 293B does not strongly interact with permeant ions in the pore.

5 The inhibitory properties of 3R,4S-293B on I_{Ks}-channels but not those of 3S,4R-293B fulfill the theoretical requirements for a novel class III antiarrhythmic drug, i.e. positive use-dependency. This enantiomer therefore represents a valuable pharmacological tool to evaluate the therapeutic efficiency of I_{Ks}blockade.

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Abbreviations: Chromanol 293B is a racemate whose enantiomers are 3R,4S-293B, (+)-[3R,4S]-6-cyano-4-(N-ethylsulfonyl-N-methylamino)-3-hydroxy-2,2-dimethyl-chromane and 3S,4R-293B, (-)-[3S,4R]-6-cyano-4-(N-ethylsulfonyl-N-methylamino)-3-hydroxy-2,2-dimethyl-chromane; I_{Ks}, slow delayed rectifier current; KCNQ1, the α-subunit of I_{Ks}; MinK (also called KCNE1 or IsK), a β-subunit of I_{Ks}.

Introduction

The MinK protein (Takumi *et al*., 1988) interacts with the K^+ channel subunit KCNQ1 to form the cardiac I_{Ks}-conductance which is defective in patients with Long QT1 and Long QT5 syndrome, an inherited cardiac arrhythmia (Wang *et al*., 1996; Barhanin *et al*., 1996; Sanguinetti *et al*., 1996). Interestingly, in *Xenopus laevis* oocytes and mammalian cells, KCNQ1 alone expresses rapidly activating Kv-potassium-channel type currents of small amplitude. Co-expression with the MinK protein slows activation rate, shifts the voltage-dependence of activation to more depolarized potentials and dramatically increases macroscopic current amplitude which is partially due to an increased single-channel conductance (Barhanin *et al*., 1996; Sanguinetti *et al*., 1996; Pusch, 1998). Additionally, the MinK protein suppresses or masks KCNQ1 channel inactivation (Pusch *et al*., 1998; Tristani-Firouzi & Sanguinetti, 1998; Seebohm *et al*., 2001), confers regulation of I_{Ks} by PKC, and alters the pharmacology of KCNQ1 (Busch *et al*., 1997; Suessbrich & Busch, 1999).

In earlier studies it has been suggested that block of I_{Ks} would constitute a promising class III antiarrhythmic approach (Sanguinetti *et al*., 1991; Yang *et al*., 2000) because of an increased prominence of the I_{Ks}-current during β-adrenergic sympathetic stimulation (Sanguinetti *et al*., 1991). Accordingly, Schreieck *et al*. (1997), Fadayel *et al*. (1998) and Bosch *et al*. (1998) showed that the specific I_{Ks} blocker chromanol 293B antagonizes the action potential shortening seen after isoproterenol treatment. Most known class III drugs block the rapid component, I_{Kr}, of the delayed rectifier potassium current, I_K, and cause prolongation of cardiac action potentials (Sanguinetti & Jurkiewicz, 1992; Roden, 1993; Singh, 1996). Unfortunately, I_{Kr} blockade typically causes excessive prolongation of action potentials at slow heart rates, whereas at higher rates blockade is much less effective (Hondeghem & Snyders, 1990). This so-called reverse use-dependent action can lead to life-threatening arrhythmias (Roden, 1993; Singh, 1996). Hence selective I_{Ks} blockade might be more promising with less pro-arrhythmic potential.

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Two I_{Ks}-specific blocker classes have been described, namely distinct benzodiazepines (Selnick *et al*., 1997) and chromanols. The benzodiazepine L-364,373 has a stereo-specific opener/blocker effect on I_{Ks} by a complex mechanism (Salata *et al*., 1998). The second class of I_{Ks} blockers, the chromanols, are derived from a modified K_{ATP} channel opener structure. The lead compound is the chromanol 293B (293B).

We recently reported an enantio-specific block by 293B on I_{Ks} and KCNQ1 (Lerche *et al*., 2000b). Fujisawa *et al*. (2000) analysed the block of I_{Ks} by the chromanol 293B racemate in guinea-pig ventricular myocytes and reported a time-dependence of block. Here we demonstrate that the time-dependence of block of KCNQ1 and I_{Ks} is mediated by 3R,4S-293B but not by 3S,4R-293B. To further characterize the blocking mechanism of 293B enantiomers we investigated the use-dependence on both channels and analysed the kinetic properties while blocking the currents partially with 10 μM of 293B enantiomers to show state-dependency. Use-dependence describes the development of block during a train of stimulations. This parameter has a high predictive value for the effect of a blocker *in vivo*. Finally, the voltage-dependence of block of both currents and the potassium-dependence of KCNQ1 block were investigated at different 293B concentrations.

Methods

Handling of *Xenopus* oocytes, synthesis of cRNA (mMESSAGE mMACHINE mRNA synthesis Kit, Ambion), two-electrode voltage-clamp experiments and the analysis of current traces have already been described in detail (Lerche *et al*., 2000a). Oocytes were co-injected with 50 nl of cRNA encoding hKCNQ1 (100 ng μl^{-1}) and hMinK (100 ng μl^{-1}). Standard two-electrode voltage-clamp recordings were performed 3–4 days after injection. Oocytes were perfused continuously with ND-96 buffer containing (mM) NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1 and HEPES 5 (pH 7.5 with NaOH). Comparative experiments were done in oocytes of the same batch and on the same day of injection. To test the effects of high extracellular K^+ on KCNQ1, the potassium concentration was increased to 96 mM by substitution with the same amount of sodium in ND96. The high potassium solution (high K^+ solution) contained (in mM): KCl 96, NaCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, pH 7.4. Pipettes were filled with 3 M KCl and had resistances of 0.5–1.5 MΩ.

Chromanol 293B (trans-6-cyano-4-(N-ethylsulfonyl-N-methylamino)-3-hydroxy-2,2-dimethyl-chromane) enantiomers were synthetized in-house. The chromanol enantiomers were added from respective 100 mM stock solutions in DMSO to the recording solutions ND96 or high K^+ solution (final DMSO concentration in the bath solution did not exceed 0.1%).

Standard two-electrode voltage-clamp recordings were performed with a Turbo Tec 10CX (NPI) amplifier and an ITC-16 interface combined with Pulse software (HEKA) for data recording. Data analyses were done with PulseFit (HEKA), Igor Pro software (Wavemetrix Inc.) and Origin 5.0/6.0 software (Microcal). The exponential fitting procedures were based on the simplex algorithm with the formulae:

$$I(t) = A_0 + A_t * \exp(-t/\tau_t) \text{ for a single exponential or}$$
$$I(t) = A_0 + A_{t1} * \exp(-t/\tau_{t1}) + A_{t2} * \exp(-t/\tau_{t2}) \text{ for double exponential fits.} \quad (1)$$

The IC_{50} values were calculated from the measured current amplitudes at the end of test pulses with the formula:

$$I/I_{max} = 1/(1 + [c/IC_{50}]^H) \quad (2)$$

Where I is the measured current and I_{max} is the maximal measured current amplitude, c is the concentration of the blocker, H is the Hill-coefficient and IC_{50} is the concentration of half maximal block.

The voltage-dependence of channel activation was fitted with the Boltzmann equation:

$$I = I_{max}/\{1 + \exp[(V_t - V_{1/2})/k]\} \quad (3)$$

Where I_{max} is the maximal measured current amplitude, V_t is the membrane potential at which the test was performed, k is the slope factor which equals RT/zF (R,T and F have their usual thermodynamic meanings and z is the apparent equivalent electrical valence) and $V_{1/2}$ is the membrane potential of half maximal current amplitude.

Student's *t*-test was used to test for statistical significance, which was assumed if $P<0.05$ and indicated by *. Numerical values are reported as $\pm$s.e.mean; the number of experiments (n) was always at least four.

Results

Kinetic analysis of chromanol block of KCNQ1 and I_{Ks} channels

A specific and enantio-specific blockade of I_{Ks} and KCNQ1 by chromanol 293B has previously been characterized by us and others (Suessbrich *et al*., 1996, Lerche *et al*., 2000b). To investigate further the details of the blocking properties of chromanol enantiomers, homomeric hKCNQ1 and heteromeric hKCNQ1/hMinK (I_{Ks}) potassium channels were expressed in *Xenopus* oocytes. The IC_{50} values of the chromanols on I_{Ks} and KCNQ1 were (mean$\pm$s.e.mean) for 3R,4S-293B: 2.6$\pm$0.2 μM and 16.4$\pm$0.7 μM for 3S,4R-293B: 15.6$\pm$0.8 μM and 30.1$\pm$1.3 μM (Lerche *et al*., 2000b). Figure 1 shows the activation of KCNQ1 (Figure 1A,B) or I_{Ks} (Figure 1D,E) currents in the presence and absence of 10 μM of the respective 293B enantiomer. For both currents 3R,4S-293B showed time-dependent block kinetics (Figure 1B,C,E,F), whereas 3S,4R-293B did not. Activation and deactivation were fitted with double exponential functions. The results are summarized in Table 1. Activation of KCNQ1 and deactivation kinetics of KCNQ1 and I_{Ks} were only marginally affected by the chromanols. However, 3R,4S-293B slightly decreased the time constant of fast activation in KCNQ1 and KCNQ1/MinK, whereas the other enantiomer increased fast time constant of I_{Ks} channels (Table 1, Figure 1).

In order to determine the effects of chromanol enantiomers on KCNQ1 inactivation two types of pulse protocols were employed. A double-pulse protocol was used to measure the onset of inactivation (Figure 2A,B). During a 3-s prepulse to

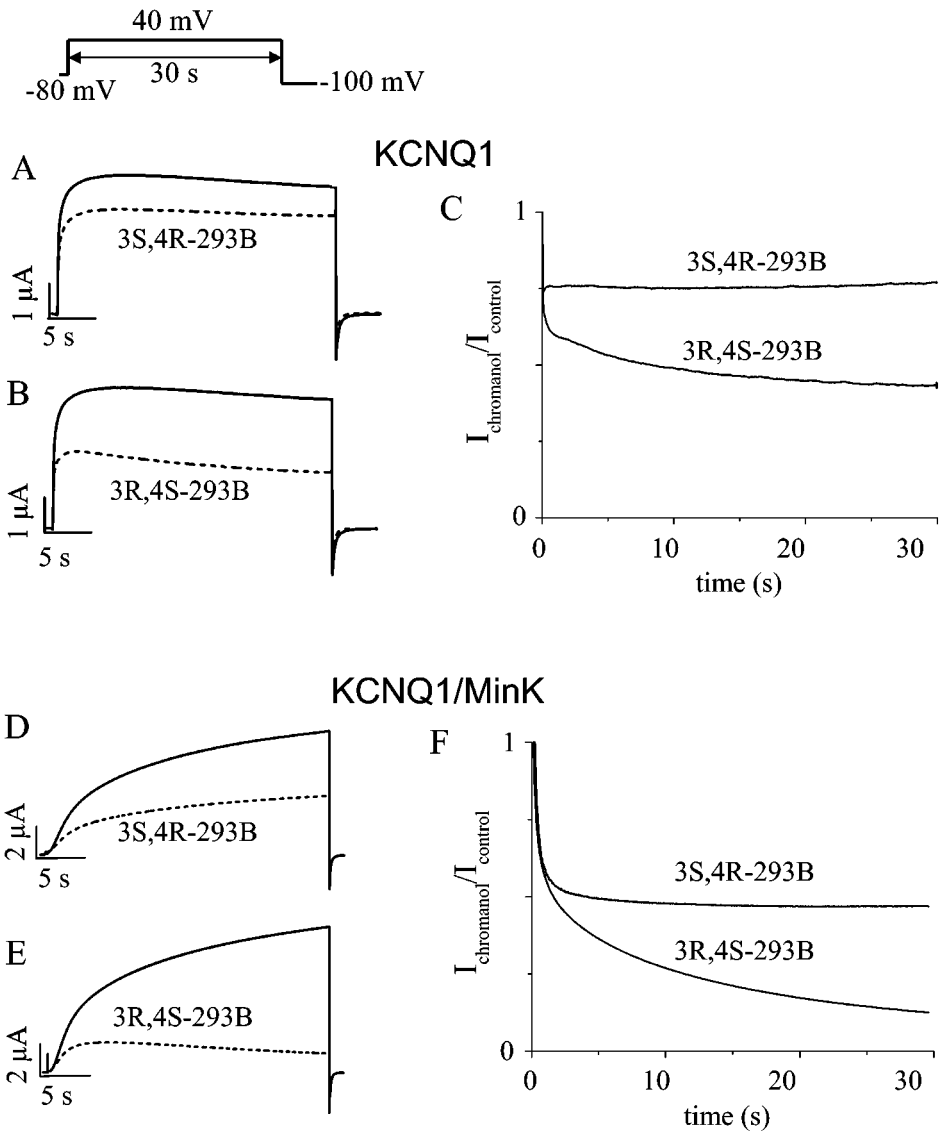

Figure 1 Effects of chromanol 293B enantiomers differ on both homomeric KCNQ1 and heteromeric KCNQ1/MinK (I_{Ks}) channels. Representative current traces from oocytes expressing KCNQ1 alone (A,B) or together with MinK (D,E). KCNQ1 currents and KCNQ1/MinK (I_{Ks}) currents were recorded with the pulse protocol indicated. Dotted lines show currents measured after perfusion of oocytes for 5 min with 10 μM 3S,4R-293B (A,D) or 10 μM 3R,4S-293B (B,E). The fraction of blocked currents per control currents ($I_{chromanol}/I_{control}$) was plotted against the time, and the data of KCNQ1 and KCNQ1/MinK are shown in C and F, respectively.

40 mV channels activate and partially inactivate. After a 20 ms interpulse at a potential of -60 mV a significant fraction of channels had recovered from inactivation and channels could then subsequently be inactivated again by a second pulse to 40 mV (Figure 2A,B; Tristani-Firouzi & Sanguinetti, 1998). The kinetics of onset of inactivation was not significantly influenced by the enantiomers (Figure 2A,B; Table 1). Recovery of inactivation was measured with the pulse protocol shown in Figure 2 (lower inset). Tail currents measured after pulses to 40 mV were studied at -60 mV to analyse recovery from inactivation (Pusch *et al*., 1998; Tristani-Firouzi & Sanguinetti, 1998). Kinetics of recovery from inactivation for KCNQ1 channels were also not significantly influenced by 293B enantiomers (Figure 2C,D; Table 1).

In KCNQ1/MinK channels a fast reactivation has been reported (Tzounopoulos *et al*., 1998). Fast reactivation occurs from an intermediate closed state and its time constants are smaller than those for 'normal' activation. For analysis of fast reactivation properties of KCNQ1/MinK currents, a double pulse protocol as shown in Figure 3 was used. During the first pulse channels were activated and were allowed to partially close again during the interpulse to -100 mV. The fast reactivation was then analysed in the rising phase of currents during the second pulse. With intervals shorter than 1 s separating the two pulses the fast reactivation became most obvious, indicating that channels were likely activating from an intermediate closed state. Kinetics of fast reactivating currents were not significantly affected by 293B enantiomers measured at interpulse intervals shorter than

1 s (Figure 3D). In contrast, currents measured with longer interpulse intervals approaching the situation of 'normal' activation were affected (Figure 3).

Table 1 Kinetic analysis

	KCNQ1		I_{Ks}	
activation	τ_{fast} (ms)	τ_{slow} (s)	τ_{fast} (s)	τ_{slow} (s)
control	103±2	1.65±0.30	1.3±0.05	37.5±5.6
	(n=15)	(n=15)	(n=18)	(n=9)
3S,4R-293B	97±1*	1.45±0.33	2.0±0.01*	31.6±2.7
	(n=13)	(n=13)	(n=14)	(n=7)
3R,4S-293B	82±3*		1.1±0.07*	
	(n=13)		(n=14)	
deactivation	τ_{fast} (ms)	τ_{slow} (s)	τ_{fast} (ms)	τ_{slow} (s)
control	222±2	1.7±0.4	170±1	3.2±0.5
	(n=19)	(n=19)	(n=18)	(n=8)
3S,4R-293B	210±1	1.6±0.7	150±4	3.7±1.6
	(n=14)	(n=14)	(n=14)	(n=9)
3R,4S-293B	193±1*	1.4±0.7	120±3*	5.5±2.6
	(n=13)	(n=13)	(n=14)	(n=9)
inactivation	onset (ms)	recovery (ms)		
control	15±3	29±3		
	(n=5)	(n=5)		
3S,4R-293B	13±0.8	29±5		
	(n=5)	(n=5)		
3R,4S-293	15±3	27±2		
	(n=5)	(n=5)		

(Significant alterations ($P<0.05$) are indicated by *. Errors in s.e.m. Numbers of experiments in parentheses.

Use-dependence of KCNQ1 and I_{Ks} channel block by chromanol

Class III antiarrhythmics bearing use-dependent characteristics should be particularly efficient during long lasting periods of elevated frequency of action potentials. To test if chromanols have such use-dependent properties the currents were elicited at 2 Hz and were partially blocked. As shown in Figure 4, no use-dependence was found for 3S,4R-293B. Weak use-dependence was found in 3R,4S-293B inhibition of homomeric KCNQ1 (Figure 4A,B). 3R,4S-293B but not 3S,4R-293B exhibited strong use dependence in KCNQ1/MinK channels (Figure 4C,D).

Evaluation of the chromanol 293B binding site

Even if chromanol 293B is uncharged, block of potassium channels close to the selectivity filter might be voltage-dependent due to interaction with permeating ions. Varying the voltage of the activating pulse between −80 mV and 60 mV had no clear effect on the half maximal activation values ($V_{1/2}$) of both KCNQ1 and I_{Ks} (Figure 5).

If chromanol block occurs in the pore near the selectivity filter like an ion block from the inside, the inhibition by 293B enantiomers might decrease in high extracellular potassium. We increased the potassium concentration in the bath solution up to 96 mM and tested this hypothesis on KCNQ1

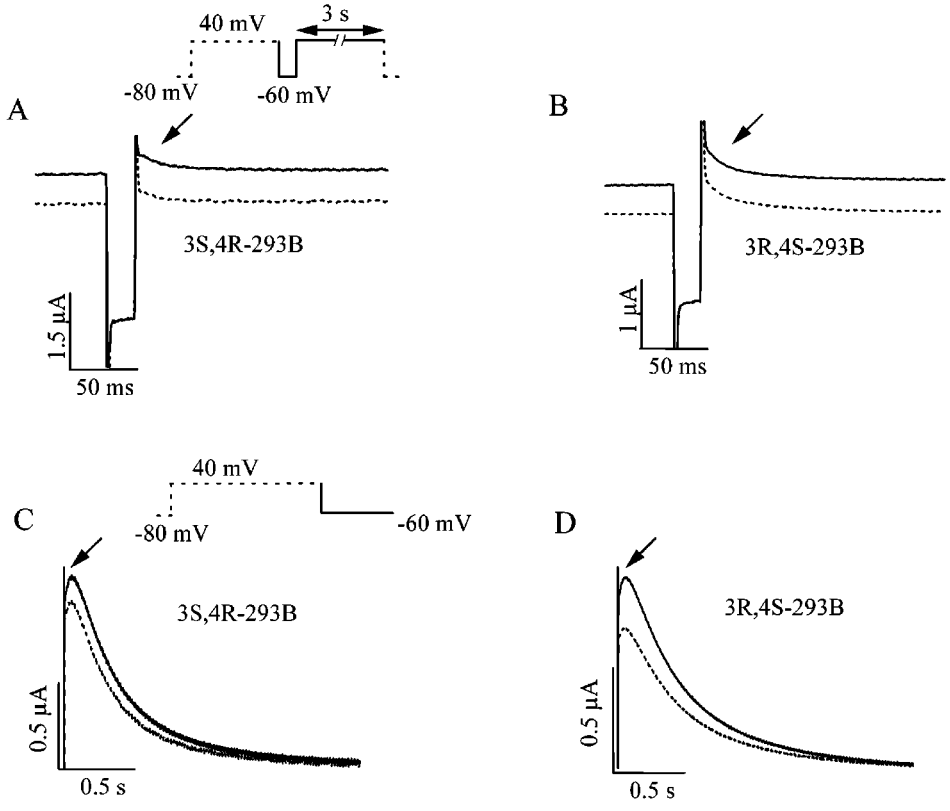

Figure 2 Chromanol 293B enantiomers do not influence inactivation of homomeric KCNQ1. Inactivation properties were compared in the absence (continuous lines) and the presence (dotted lines) of 10 μM 293B enantiomer. Current traces produced by the pulse protocol during the period indicated by the solid line are shown. A and B show the fast onset of inactivation after a 20-ms pulse to −60 mV during which channels partially recover from inactivation. In C and D tail currents after a 3-s pulse to 40 mV are shown. The rising phase of the tail currents (arrows) represents the recovery from inactivation. Note the similar kinetics with and without 293B enantiomers for the onset and the recovery of inactivation.

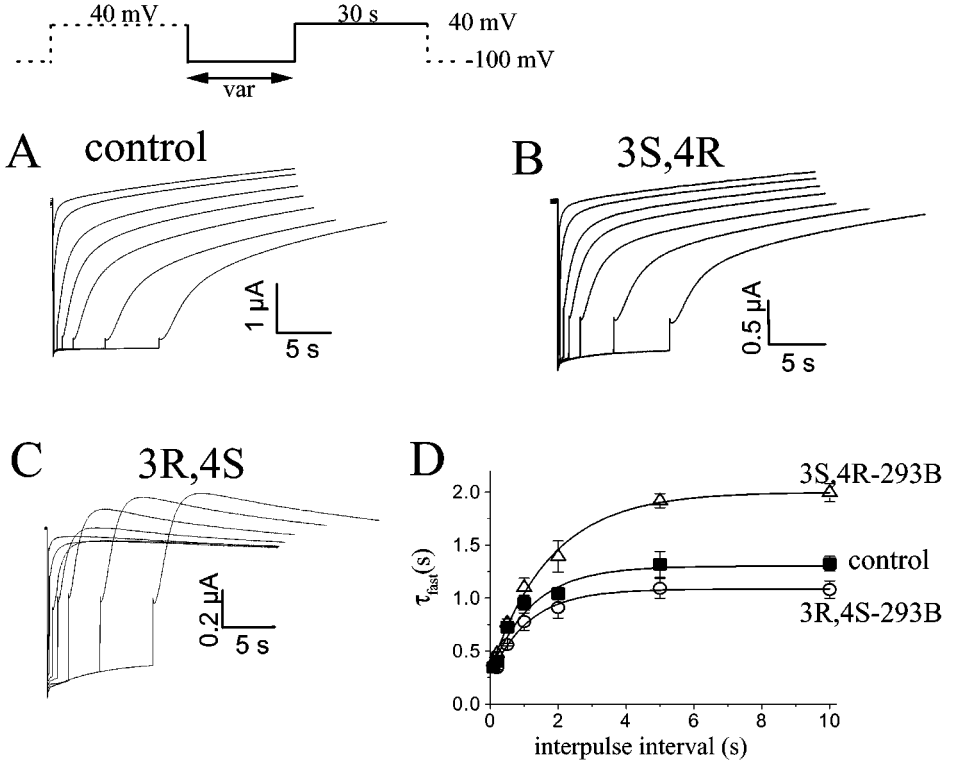

Figure 3 Activation but not fast reactivation kinetics of KCNQ1/MinK (I_{Ks}) were affected by chromanol 293B enantiomers. Representative current traces from the interpulse and the following activating pulse (solid line in the pulse protocol) recorded from oocytes of the same preparation co-injected with cRNA encoding KCNQ1 and MinK in the absence of 293B (A) and after perfusion of 10 μM of 3S,4R-293B (B) and 3R,4S-293B (C) for 5 min. For analysis of the reactivation at 40 mV channels were allowed to deactivate at −100 mV for different durations (0.1–10 s) after a 30-s pulse to 40 mV. Activation was fitted by the sum of two exponential components. After longer interpulses where the activation time course was sigmoidal, the initial few msec of the traces were excluded from the fit. In D mean values ($n=4-6$) of the fast time constants are shown. Note that the fast time constants obtained for interpulse durations shorter than one second that describe fast reactivation are very similar with and without either enantiomer. The differences seen for longer interpulses reflect the effect of chromanol enantiomers on the normal activation kinetics.

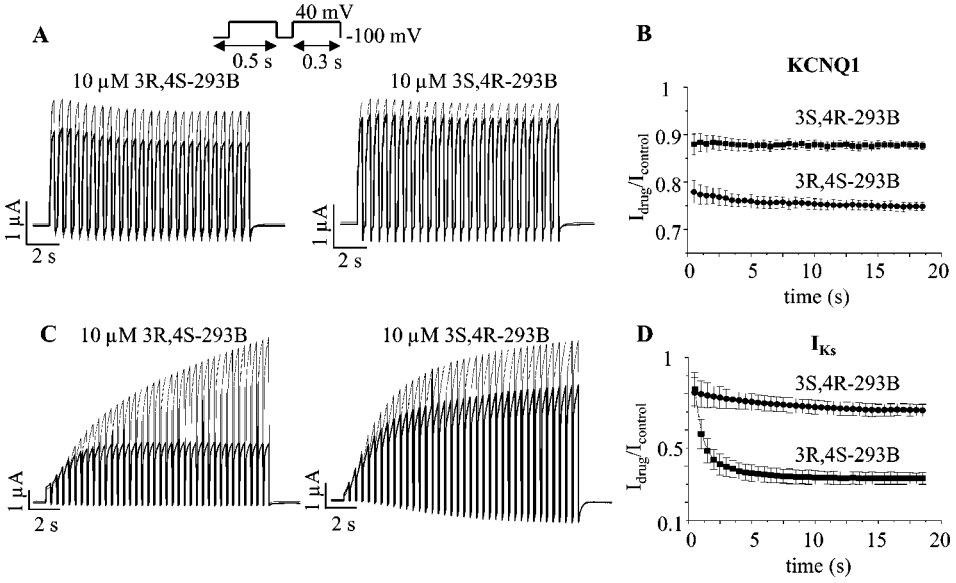

Figure 4 Use-dependence of chromanol inhibition of homomeric KCNQ1 and heteromeric KCNQ1/MinK (I_{Ks}) channels. Oocytes were perfused with 10 μM of 3S,4R-293B and 3R,4S-293B. Currents were recorded by repetitive stepping the membrane potential from −100 mV to 40 mV for 0.3 s at 2 Hz. Control currents (thin lines) and currents partially blocked (thick lines) are shown superimposed (A, KCNQ1; C, KCNQ1/MinK). The current amplitudes were measured at the end of the 0.3-s test pulses and the fraction of blocked current per control current ($I_{chromanol}/I_{control}$) was plotted against the time. Mean values ($n=4-6$) for KCNQ1 and KCNQ1/MinK are shown in B and D, respectively.

channels. Under identical conditions the inhibition by chromanol 293B enantiomers were slightly but not significantly changed (3R,4S-293B, $P=0.6$) or not (3S,4R-293B, $P=0.95$) changed in high extracellular potassium solution (Figure 6). The Hill-coefficients were between 0.9 and 1.2 and were not significantly different for alternative enantiomers.

Discussion and conclusions

In this study we examined the block of chromanol 293B enantiomers of homomeric KCNQ1 and of heteromeric I_{Ks} currents with the help of ionic current measurements. Blocking efficiency of chromanol 293B on KCNQ1 is modulated by MinK in *Xenopus* oocytes (Busch *et al*., 1997; Suessbrich *et al*., 1996; Yang *et al*., 2000; Lerche *et al*., 2000a, b). Fujisawa *et al*. (2000) showed that block of I_{Ks} by the chromanol 293B racemate in guineapig ventricular myocytes was unaffected by pulse voltage. In contrast, Loussouarn *et al*. (1997) reported a voltage-dependence for chromanol 293B blockade of I_{Ks} heterologously expressed in COS-7 cells. We also could not detect a significant voltage-dependence of inhibition by chromanol 293B enantiomers of either KCNQ1 or KCNQ1/MinK when expressed in *Xenopus* oocytes.

The chromanol block of both KCNQ1 and I_{Ks} revealed a Hill-coefficient of around 1, indicating a lack of cooperativity of chromanol 293B enantiomers and rather supporting a 1 : 1 binding stoichiometry. This was also proposed for the racemate by Fujisawa *et al*. (2000).

The changes in fast activation and deactivation kinetics of KCNQ1 channels observed in the presence of 10 μM chromanol enantiomers were small. Onset of inactivation and recovery from inactivation in KCNQ1 were not

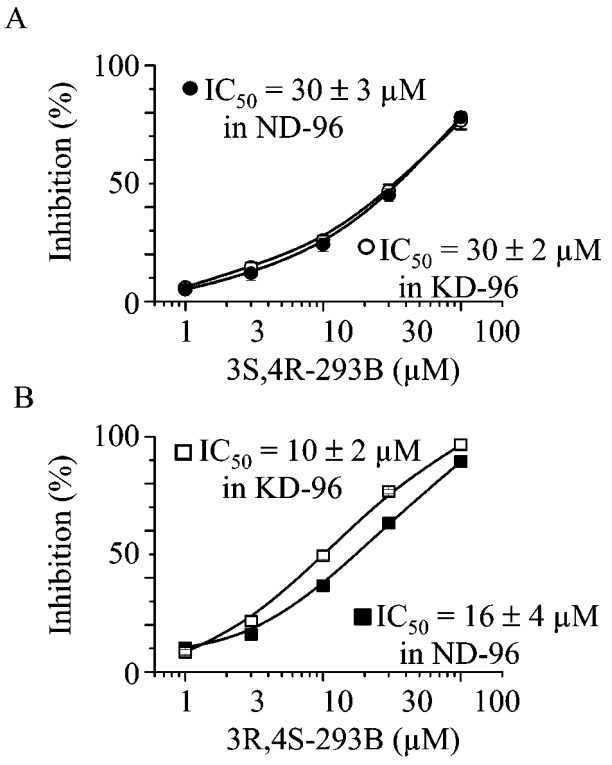

Figure 6 Lack of effect of extracellular K^+ on 293B block. Oocytes expressing KCNQ1 were perfused with 3S,4R-293B and 3R,4S-293B at different concentrations. KCNQ1 currents were recorded stepping the membrane potential from −80 mV to 40 mV for 3 s with a interpulse interval of 3 s. The dose inhibition relation is shown in ND-96 and high K^+ solution for 3S,4R-293B (A) and for 3R,4S-293B (B), respectively. IC_{50} values are indicated and the Hill-coefficients were between 1 – 1.2.

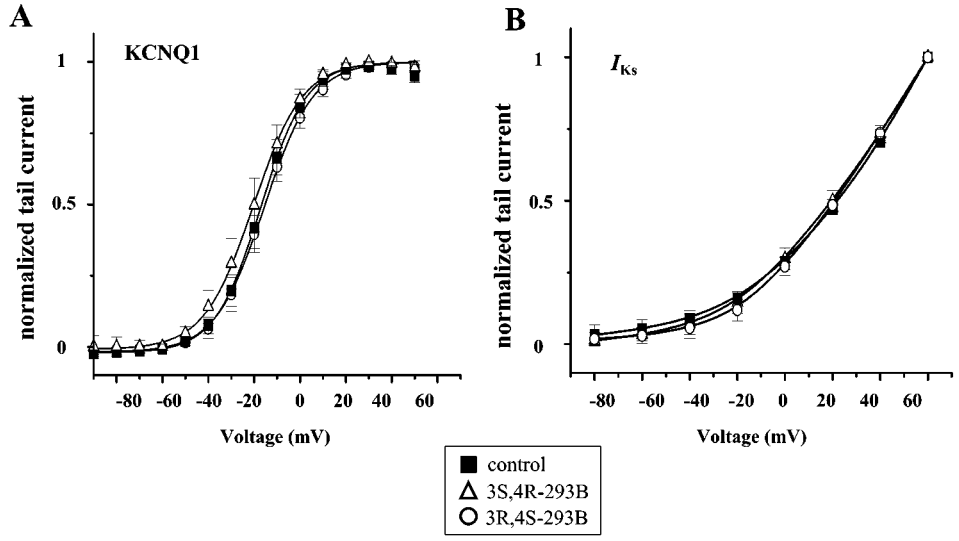

Figure 5 Voltage-dependence of block by chromanol 293B enantiomers of homomeric KCNQ1 and heteromeric KCNQ1/MinK (I_{Ks}). KCNQ1 and KCNQ1/MinK (I_{Ks}) currents expressed in oocytes were recorded at 0.1 Hz with 3-s test pulses to 40 mV ($V_m = -80$ mV) and tail currents were measured at −100 mV. KCNQ1 and KCNQ1/MinK (I_{Ks}) tail currents were measured at perfusion with ND-96, with 3S,4R-293B (KCNQ1: 20 μM, I_{Ks}: 10 μM) and 3R,4S-293B (KCNQ1: 20 μM, KCNQ1/MinK: 10 μM). Current values normalized to the maximal currents were plotted against voltage. The voltage of half maximal activation ($V_{1/2}$) of KCNQ1 was calculated by fit of the data to the Boltzmann equation (A, $n=5$). The $V_{1/2}$ values of KCNQ1 were 17 ± 2 mV for control currents, 20 ± 3 mV for currents partially blocked 3S,4R-293B and 16 ± 3 mV for 3R,4S-293B blocked currents, respectively. Results for I_{Ks} are shown in B.

influenced by either enantiomer. Inactivation could not be detected in heteromeric KCNQ1/MinK channels and could thus not be investigated. This indicates that inactivated states are not stabilized by chromanol. The chromanols failed to affect fast reactivation that is only present in I_{Ks}. The biphasic current traces in the presence of 3R,4S-293B (Figure 1B,E) argues for a decreased affinity for closed states. The intermediate state enabling fast reactivation was also insensitive to the chromanol enantiomers.

Taken together we believe that 3R,4S-293B binds preferentially to open channels and therefore exerts a use-dependent block. Compared to KCNQ1 it seems likely that MinK in the I_{Ks} channel complex stabilizes the open channel in a blockable state and thereby increases affinity to the open channel for 3R,4S-293B.

Blockers are thought to block the pore and by this inhibit the permeation of ions whereas gating modifiers by altering their gating reduce the current through ion channels. A pore plugging mechanism of 293B inhibition is strongly suggested by two basic findings. First, 293B does not significantly modify the voltage-dependence of gating, and secondly, almost complete inhibition was achieved also at very large positive voltages (40 mV) at concentrations of 100 μM (Lerche *et al*., 2000b). If 293B enantiomers inhibit currents by stabilizing a closed state rather than block directly ion conduction, the voltage-dependence of gating should be changed and block should be less pronounced at positive voltages that favour the open state.

An ion block of potassium channels, like that by Mg^{2+} can account for inward rectification and occurs within the pore near the selectivity filter. This results in a dependence of block on the extracellular potassium concentration, since in the case of Mg^{2+} the ions block the pore from the cytosolic side and can be pushed out of the ion pathway when extracellular potassium is increased (Armstrong, 1969; Bara *et al*., 1993). Since chromanol 293B is effective in the cell-attached mode (Loussouarn *et al*., 1997) lipophilic chromanol 293B may act from the cytosolic side of the membrane. If chromanols block the inner pore very close to the selectivity filter, their inhibitory effects might similarly be decreased by elevation of the extracellular potassium concentration. In contrast to this, high extracellular potassium solution left the inhibitory potency of 3S,4R-293B and 3R,4S-293B unaffected (Figure 6). These findings, together with the lack of a significant voltage-dependence of inhibition, would argue against a binding site close to the selectivity filter. However, since 293B is uncharged, block of the open pore cannot be excluded. Actually, the postulated chromanol binding pocket is likely to be located in the inner vestibule and parts of the P-loop (H5) and S6 transmembrane domain may contribute to the binding site whereas MinK seems not directly be involved in the binding pocket of chromanols (Lerche *et al*., 2000b). Summarizing, it is most likely that chromanol blocks the channels by binding within the inner vestibule at a distance away from the selectivity filter.

Blockers of I_{Ks} have been discussed as promising class III antiarrhythmic drugs (Sanguinetti *et al*., 1991; Yang *et al*., 2000). For this reason the increased prominence of I_{Ks} during β-adrenergic stimulation as well as the blocking characteristics of the drug are of great importance. The open channel affinity, the positive use-dependence of 3R,4S-293B render it superior to 3S,4R-293B. Thus 3R,4S-293B should be the preferred enantiomer, for development and testing of an antiarrhythmic drug targeting repolarizing I_{Ks} currents in the heart.

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