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Inhibition of cardiac Kir2.1–2.3 channels by beta3 adrenoreceptor antagonist SR 59230A

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ABSTRACT

Kir2.x channels form the molecular basis of cardiac I_{K1} current and play a major role in cardiac electrophysiology. However, there is a substantial lack of selective Kir2 antagonists. We found the β_3-adrenoceptor antagonist SR59230A to be an inhibitor of Kir2.x channels. Therefore, we characterized the effects of SR59230A on Kir2.x and other relevant cardiac potassium channels.

Cloned channels were expressed in the *Xenopus* oocyte expression system and measured with the double-microelectrode voltage clamp technique.

SR59230A inhibited homomeric Kir2.1 channels with an IC_{50} of 33 μM. Homomeric Kir2.2 and Kir2.3 channels and Kir2.x heteromers were also inhibited by SR59230A with similar potency. In contrast, no relevant inhibitory effects of SR59230A were found in cardiac Kv1.5, Kv4.3 and KvLQT1/minK channels. In hERG channels, SR59230A only induced a weak inhibition at a high concentration.

These findings establish SR59230A as a novel inhibitor of Kir2.1–2.3 channels with a favorable profile with respect to additional effects on other cardiac repolarizing potassium channels.

1. Introduction

The cardiac inwardly rectifying potassium current I_{K1} is essential to maintain the resting membrane potential of cardiomyocytes [1]. I_{K1} current reduction caused by mutations in the Kir2.1 channel subunit underlies Long QT Syndrome Type 7 with a characteristic pattern of QT interval prolongation and predisposition to ventricular ectopy and ventricular tachycardia [2]. On the contrary, gain-of-function mutations in Kir2.1 leading to I_{K1} outward current increase cause Short QT Syndrome Type 3 that is associated with atrial and ventricular fibrillation [3].

There is an increasing body of evidence that heteromeric assembly of Kir2.1, Kir2.2 and Kir2.3 potassium channels is the molecular basis of cardiac I_{K1} current [4–6].

Piao and co-workers demonstrated that in the mouse heart upregulation of I_{K1} is proarrhythmic, and that I_{K1} blockade in cardiac myocytes may be a rational antiarrhythmic strategy [7,8]. Rees and Curtis showed that I_{K1} blockade with RP58866 can suppress ventricular fibrillation during reperfusion [9]. However, it was later shown that RP58866 also blocks other potassium currents [10,11].

Although selective Kir2 channel antagonists may be both a very useful research tool and a potential basis for antiarrhythmic drug development, the majority of Kir2/I_{K1} antagonists also affect other cardiac ion channels [12–22]. SR59230A is commonly used for research in the field of adrenergic signal transduction, often for differentiation between different receptor subtypes [23–29]. To date, there is no experimental data investigating direct effects of SR59230A on cardiac ion channels. Therefore, we studied the effects of SR59230A on Kir2.x and other physiologically relevant cardiac potassium channels in the *Xenopus* oocyte expression system.

Here we show that SR59230A inhibits homomeric Kir2.1 channels with an IC_{50} of 33 μM. Homomeric Kir2.2 and Kir2.3 channels and Kir2.x heteromers are also inhibited by SR59230A with similar potency. In contrast, no relevant inhibitory effects of SR59230A are found in cardiac Kv1.5, Kv4.3 and KvLQT1/minK channels. In hERG channels, SR59230A only induces a weak inhibition at a high concentration. These findings establish SR59230A as a novel inhibitor of Kir2.1–2.3 channels with a favorable profile with respect to additional effects on other cardiac repolarizing potassium channels.

2. Methods

2.1. Solutions and drug administration

Voltage clamp measurements of *Xenopus* oocytes were performed in a K^+ solution containing (in mmol/l) 5 KCl, 100 NaCl,

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1.5 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES (pH 7.4 with NaOH). Electrodes were filled with 3 mol/l KCl solution. All measurements were carried out at room temperature (20 °C). *Xenopus* oocytes were incubated in the drug solution. Recordings were made prior to incubation and after 40 min. SR59230A (Sigma, Germany) was dissolved in DMSO to a stock solution of 100 mmol/l and stored at −20 °C. On the day of experiments, aliquots of the stock solution were diluted to the desired concentrations with the bath solution.

2.2. Electrophysiology and data analysis

The two-microelectrode voltage-clamp configuration was used to record currents from *Xenopus laevis* oocytes. Data were low-pass filtered at 1 to 2 kHz (−3 dB, four-pole Bessel filter) before digitalization at 5 to 10 kHz. Recordings were performed using a commercially available amplifier (Warner OC-725A, Warner Instruments, Hamden, U.S.A.) and pCLAMP software (Axon Instruments, Foster City, U.S.A.) for data acquisition and analysis. No leak subtraction was performed during the experiments. Statistical data are presented as mean ± standard error. Statistical significance was evaluated using ANOVA. Differences were considered to be significant if the p-value was <0.05. The concentration response curves were fitted with the Hill equation: $I/I_0 = 1/(1 + X/IC_{50})^{n_H}$, with I/I_0 being the relative current, I_0 the unblocked current amplitude, X the drug concentration, IC_{50} the concentration for half maximal block and n_H the Hill coefficient.

2.3. Heterologous expression

Complementary RNA was prepared from Kir2.x cDNA with the mMESSAGE mMACHINE in vitro transcription kit (Ambion) by use of T7 Polymerase (Kir2.1 and Kir2.2) and T3 Polymerase (Kir2.3). Injection of RNA into stage V and VI defolliculated oocytes was performed using a Nanoject automatic injector (Drummond, Broomall, USA). Measurements were made 1 to 5 days after injection. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996).

3. Results

3.1. SR59230A inhibits Kir2.1 channels

As the Kir2.1 channel subunit is the most relevant Kir2 subunit in the myocardium, we first characterized the effects of SR59230A on homomeric Kir2.1 channels. Channels were heterologously expressed in *Xenopus laevis* oocytes and currents were measured using the voltage-clamp technique. Representative current traces under control conditions (Fig. 1A) and after application of 100 μmol/l SR59230A (Fig. 1B) over a period of 40 min are shown in Fig. 1. A standardised voltage protocol was used to measure Kir2 currents: From a holding potential of −80 mV, test pulses to from −120 mV to +40 mV were applied in 10 mV increments (400 ms each). Inward current amplitudes at −120 mV were determined to quantify effects. Under control conditions, Kir2.1 currents remained stable with 100 ± 6.9% of initial current amplitudes after 40 min in the bath solution (n = 5).

Concentration–response relations were obtained as described above. *Xenopus* oocytes were inserted into a bath solution containing SR59230A at concentrations ranging from 0.1 μmol/l to 200 μmol/l. Incubation time was 40 min for all concentrations except for 200 μmol/l that caused cytotoxic effects on the oocytes during long exposure. It was only applied for an incubation time of 15 min. Current amplitudes at −120 mV were determined to quantify relative block. The dose–response curve for Kir2.1 channels yielded an IC_{50} of 33.2 μmol/l (n = 6 − 10; Fig. 1D).

Onset and wash-out of the inhibitory effect of SR59230A on Kir2.1 currents were investigated with a voltage protocol which was repeated at start-to-start intervals of 10 s. A test pulse to −120 mV (400 ms) was applied to elicit large inward currents. The holding potential was −80 mV. Mean values of the inward current amplitudes during wash-in of SR59230A at a concentration of 40 μmol/l (i.e., close to the estimated IC_{50}) are plotted versus time in Fig. 1E (n = 8). The onset of block was very slow and did not reach steady-state conditions after 40 min. However, we did not lengthen incubation times because, according to experience, the cells do not tolerate longer experiments. Upon wash-out with the bath solution, the effect was almost not reversible. After 40 min, a recovery of peak current amplitudes of merely 15% was observed (n = 7, Fig. 1F).

3.2. Inhibition of Kir2.2 and Kir2.3 channels by SR59230A

In order to investigate the specificity of SR59230A with respect to the different cardiac Kir2 subunits, we also examined its effects on homomeric Kir2.2 and Kir2.3 channels. Concentration–response relations were obtained analogously to those of Kir2.1 as described above. Under control conditions, Kir2.2 and Kir2.3 currents showed a small run-up of initial currents to 106 ± 5% (n = 7) and 105.4 ± 6.2% (n = 9), respectively. Current–voltage curves of representative measurements before and after exposure to 150 μmol/l SR59230A are shown in Fig. 2A for Kir2.2 channels and in Fig. 2C for Kir2.3 channels. Again, dose–response relationships were obtained as explained for Kir2.1. The dose–response curves for Kir2.2 and Kir2.3 channels yielded IC_{50} values of 46.4 μmol/l and 14.6 μmol/l, respectively (n = 6 – 10; Fig. 2B and D).

3.3. Inhibition of heteromeric Kir2 channels by SR59230A

It has been demonstrated that heteromeric assembly of Kir2.1, Kir2.2 and Kir2.3 probably is the main molecular correlate of ventricular I_{K1} current. Furthermore, it has been shown that co-expression of Kir2.1 and Kir2.2 in *Xenopus* oocytes gives rise to distinct currents with biophysical properties that resemble those of human native I_{K1} current better than those of homomeric Kir2.1 or Kir2.2 currents [5].

Hence, Kir2.x heteromeric channels were generated by co-injection of RNA in *Xenopus* oocytes according to Schram et al., [5]. Under control conditions, Kir2.1/2.2, Kir2.1/2.3 and Kir2.2/2.3 currents increased to 114.8 ± 6.8% (n = 6), 105.8 ± 6.8% (n = 7) and 108.5 ± 9.6% (n = 5), respectively. Current–voltage curves of representative measurements before and after exposure to 100 μmol/l SR59230A are shown in Fig. 3A, C and E. Dose–response relationships were studied analogous to the experiments described above. Dose–response curves yielded IC_{50} values of 30 μmol/l for Kir2.1/2.2 heteromers, 32.2 μmol/l for Kir2.1/2.3 heteromers and 49.5 μmol/l for Kir2.2/2.3 heteromers, respectively (n = 6 – 11; Fig. 3B, D and F).

3.4. Effects of SR59230A on other cardiac potassium channels

Many ion channel antagonists exert effects on several different channels. This effect profile has major implications for the use of these compounds both in research and in clinical medicine. Thus, we also screened for effects of SR59230A on other physiologically important cardiac potassium channels in the *Xenopus* oocyte expression system (Fig. 4A–E). In order to clearly identify antagonistic effects, we chose a high concentration of SR59230A (200 μmol/l) for these experiments.

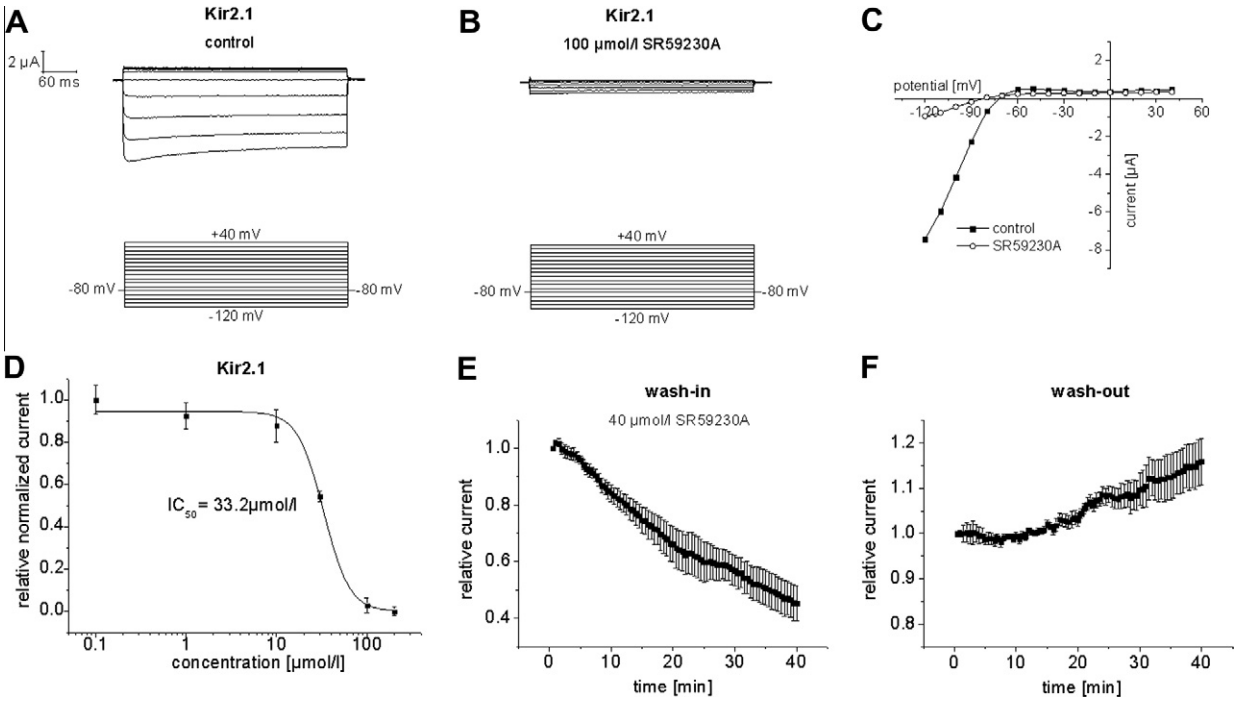

Fig. 1. Typical Kir2.1 currents under control conditions and after exposure to SR59230A (100 µmol/l) are displayed (A and B). The corresponding current–voltage curves are shown in C. Dose–response relationship of the inhibitory effect of SR59230A on Kir2.1 channels is shown in D (IC50 33.2 µmol/l). Inward current amplitudes during wash-in of 40 µmol/l SR59230A are plotted as a function of time (E, $n = 8$). Experiments during wash-out were analyzed analogously and are shown in F ($n = 7$). Onset of blockade was slow and did not reach steady-state conditions within 40 min. Wash-out of the effect was even slower with only about 15% recovery during 40 min.

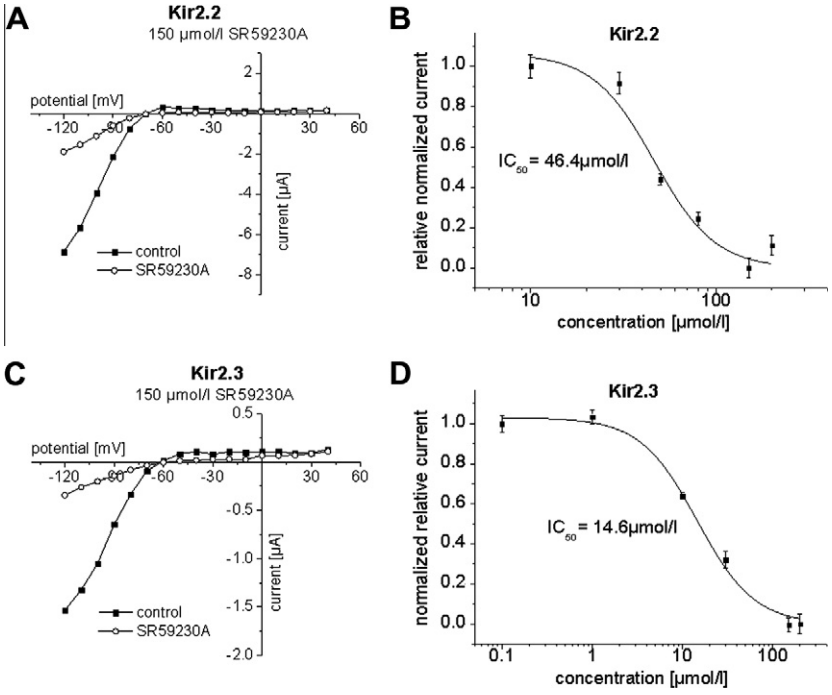

Fig. 2. Current–voltage curves of representative measurements of Kir2.2 and Kir2.3 channels before and after incubation with 150 µmol/l SR59230A (A and C). Dose–response curves of the inhibitory effect of SR59230A on Kir2.2 and Kir2.3 channels are shown in B and D (IC50 values: 46.4 and 14.6 µmol/l).

First, we investigated the effect of SR59230A on Kv1.5 channels which conduct the ultrarapid delayed rectifier current (I_{Kur}) [30]. From a holding potential of −80 mV, cells were subject to a long (600 ms) variable depolarizing test pulse ranging from −80 to +120 mV (increment 20 mV). The holding potential was −80 mV. Under control conditions, Kv1.5 currents remained stable and current amplitudes were 102.1 ± 3.2% after 15 min in the bath solution ($n = 8$). SR59230A only slightly affected Kv1.5 currents. After 15 min of exposure to SR59230A at a concentration of 200 µmol/l, currents were reduced to 87.6 ± 6.7% of the respective initial

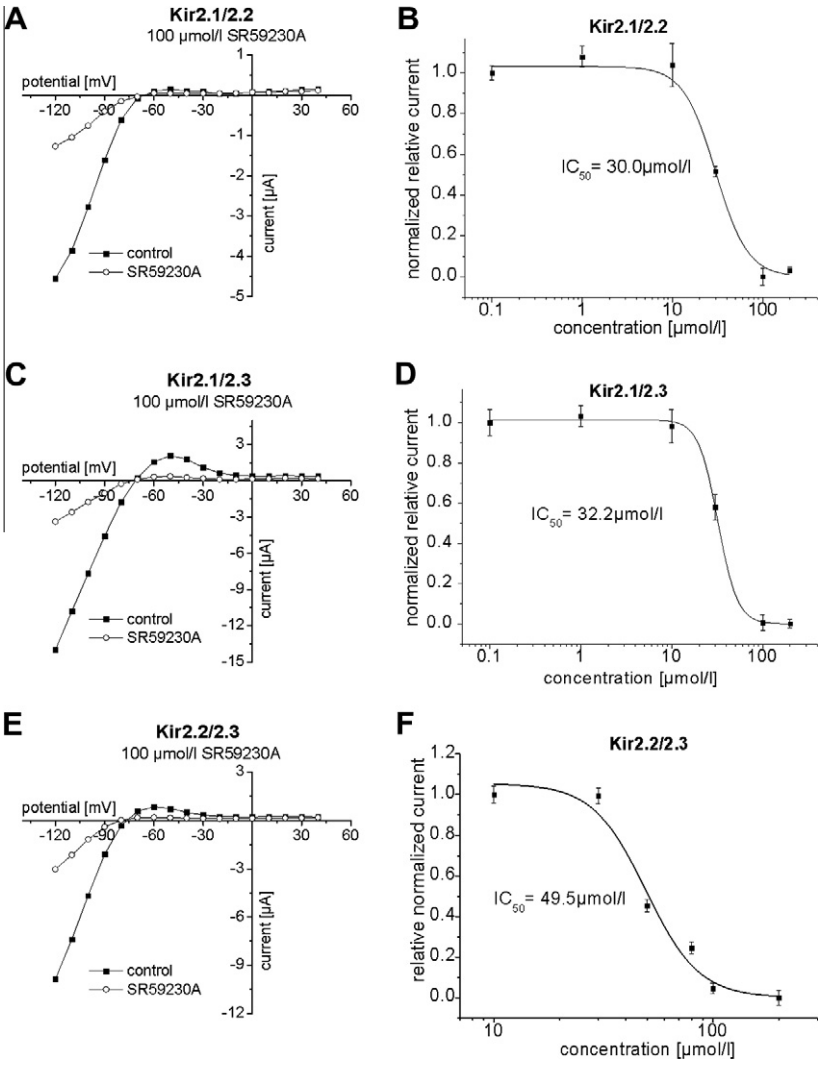

Fig. 3. Current–voltage curves of measurements of Kir2.1/2.2, Kir2.1/2.3 and Kir2.2/2.3 channels under control conditions and after exposure to SR59230A (100 µmol/l) are displayed in A, C and E. Dose–response curves of the inhibitory effect of SR59230A on Kir2.1/2.2, Kir2.1/2.3 and Kir2.2/2.3 channels are shown in B, D and F. IC50 values were 30.0, 32.2 and 49.5 µmol/l, respectively.

values ($n = 5$). A representative experiment displaying current traces at +120 mV under control conditions and after exposure to 200 µmol/l SR59230A is shown in Fig. 4A.

Kv4.3 channels are major molecular components of I_{to} currents in human hearts [31]. In order to elicit typical Kv4.3 currents, the following voltage protocol was used: Test pulses from −100 to +50 mV in 10 mV-increments were applied to induce large outward currents (holding potential was −100 mV). Traces at +50 mV under control conditions and after exposure to SR59230A in a representative experiment are shown in Fig. 4B. In control experiments, peak outward currents remained stable with 99.0 ± 2.9% of the respective initial values ($n = 13$). After SR59230A had been applied at a concentration of 200 µM for 15 min, currents were only slightly reduced to 93.6 ± 4.6% of initial values ($n = 10$, $p = 0.37002$).

The molecular basis of human I_{Ks} current is formed by KvLQT1 and minK subunits [32,33]. Co-injection of KvLQT1/minK resulted in outward potassium currents characterized by a linear current–voltage (I–V) relationship [32,33]. Currents were activated during depolarizing steps to potentials from −60 to +120 mV (2 s), and tail currents were recorded at −40 mV (2 s). The holding potential was −80 mV. In control experiments, peak outward tail current amplitudes increased to 121.9 ± 11.3% of the respective initial values ($n = 10$). Representative current traces at +120 mV under control conditions and after exposure to SR59230A are shown in Fig. 4C. KvLQT1/minK currents were not affected by SR59230A and were found to increase to 111.5 ± 10.4% of the respective initial values ($n = 6$).

hERG potassium channels are the molecular correlate of the cardiac repolarizing delayed rectifier potassium current I_{Kr} [34]. Characteristic hERG currents were elicited with the following voltage protocol: A first step to potentials ranging from −100 mV to +100 mV (10 mV-increments, 400 ms) was followed by a return pulse to −120 mV (400 ms) eliciting large inward tail currents (holding potential −80 mV). Peak inward tail currents were determined to quantify effects. Under control conditions, we observed a current run-up to 121.7 ± 3.2% of respective initial values ($n = 11$). In contrast, SR59230A inhibited hERG currents with a reduction to 70.1 ± 5.2% of the respective initial values after 15 min of exposure to 200 µmol/l SR59230A ($n = 15$; $p < 0.05$). Current traces from a representative experiment are displayed in Fig. 4D. Summary data of all experiments is shown in Fig. 4E.

4. Discussion

Although cardiac I_{K1} current and its molecular basis – Kir2.x channels – are of major relevance for cardiac electrophysiology,

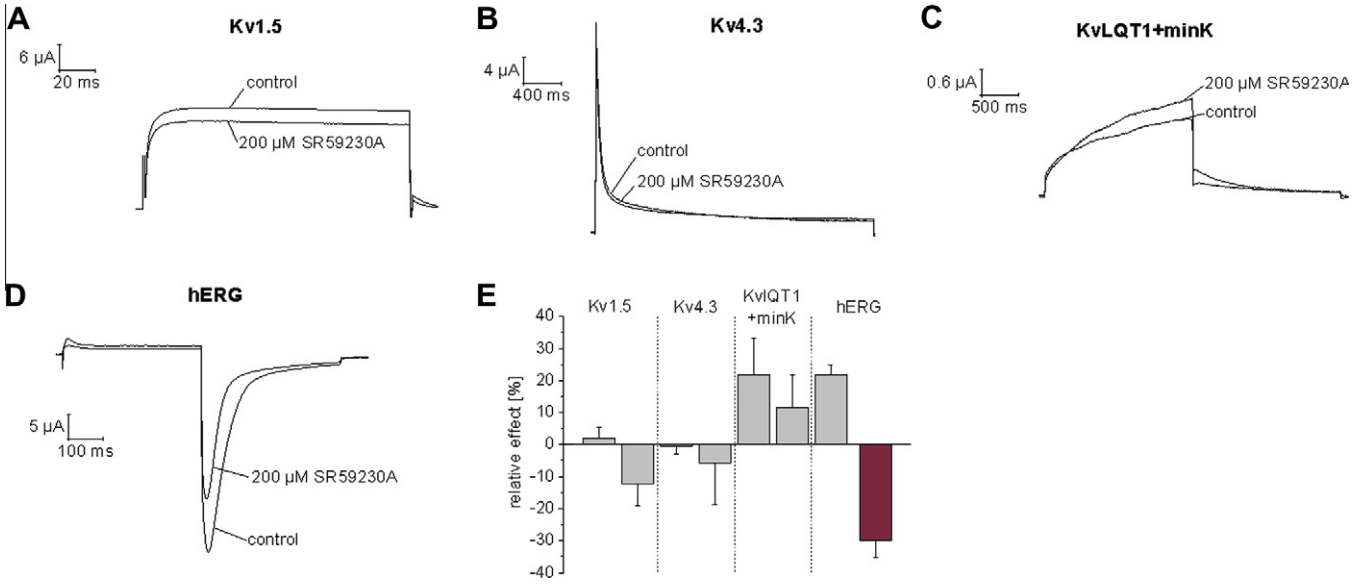

Fig. 4. Kv1.5, Kv4.3, KvLQT1/minK and hERG were screened for effects of SR59230A. A single high concentration (200 µmol/l) with an incubation time of 15 min was chosen for this purpose. Selected current traces of representative experiments before and after incubation with SR59230A are displayed for Kv1.5 channels in A, for Kv4.3 channels in B, for KvLQT1 + minK channels in C and for hERG channels in D. Summary data of those experiments is shown in E. SR59230A did not exert relevant effects on Kv1.5, Kv4.3 and KvLQT1/minK, but it induced an inhibition of hERG currents by 29.9 ± 5.2% (n = 15).

only few and mostly unspecific pharmacological antagonists that target these channels have been characterized to date. After having noticed incidentally that the β_3 adrenoceptor antagonist SR59230A exerts inhibitory effects on Kir2 channels, we provide an experimental characterization of its pharmacological properties with respect to the inhibition of Kir2.x channels and its effects on other cardiac potassium channels.

In *Xenopus* oocytes, we observed a dose-dependent inhibition of Kir2.1 channels with an IC_{50} of 33 µmol/l which correlates to a moderate to low affinity in this expression system [35–37]. The IC_{50} values of inhibition for the other Kir2 channel subunits and for the Kir2.x heteromers were comparable. Hence, the effect of SR59230A on Kir2 channels does not exhibit subtype-specific properties. Due to the follicular membranes and the yolk of *Xenopus* oocytes, higher drug concentrations are needed than in mammalian cells or *in vivo* experiments, often by a factor of 5–20 [38]. Typically applied concentrations of SR59230A for experimental use in cell lines or animal models range from 1 to 10 µmol/l. Thus, it can be expected that SR59230A also exerts Kir2 channel blocking effects in this concentration range.

Most I_{K1} current antagonists characterized to date also block other cardiac ion channels. Based on a screening with a high concentration of SR59230A, we could exclude relevant effects on Kv1.5, Kv4.3 and KvLQT1/minK channels. We found an inhibitory effect on hERG channels that due to their peculiar pore structure are a target of a broad range of diverse drugs [34]. However, the observed effect was only small and with a high concentration. Hence, at lower concentrations that are sufficient for almost complete Kir2 current inhibition, SR59230A is unlikely to exert relevant effects on other major cardiac channels. Compared to other recently described inhibitors of Kir2 channels such as chloroquine and carvedilol [13,18], this relative selectivity may be an advantage for the use of SR59230A in cardiac electrophysiology.

Several compounds have been shown to exert inhibitory effects on Kir2.x channels or I_{K1} current. Recently, two principal underlying mechanisms have been identified: First, a group of compounds comprising for example Tamoxifen, Mefloquin, Carvedilol and quinacrine interferes with the interaction of the channels with the membrane phospholipid PIP_2 [12,18–20]. As Kir channels require the PIP_2 interaction to stabilize the open state, this interference leads to a current reduction [12,18–20]. Second, another group of small molecule inhibitors such as chloroquine and pentamidine directly block Kir2 channels through binding to the cytoplasmatic pore region [13,14]. Interestingly, in the case of quinacrine it has even been shown that its inhibitory effect on Kir2.1 channels is based on both direct channel blockade and interference with channel-PIP_2 interaction [12]. Furthermore, for several other inhibitors of Kir2 channels such as genistein the underlying pharmacological mechanisms have not been elucidated to date [17].

We did not observe relevant variation of IC_{50} values between different channel subtypes, which argues against an exclusively PIP_2-based effect that is typically associated with a markedly higher potency in Kir2.3 than in Kir2.1 [12,18–20]. Hence, further study will be needed to elucidate the detailed underlying mechanisms.

4.1. Limitations

The *Xenopus* oocyte expression system has several advantages, however, it is a relevant limitation that due to the structure of the oocytes higher drug concentrations are needed than in mammalian cells to induce similar effects [38]. Hence, IC_{50} values are higher in oocytes and deduction of associated IC_{50} value ranges in native tissues is only possible to a limited extent. Furthermore, we found some minor variation of drug potency with respect to the Kir2 channel subtype and we cannot exclude that the molecular Kir2 composition of the respective cell type may also influence drug potency.

4.2. Conclusions

SR59230A is a moderate to low affinity antagonist at human Kir2.x channels, which form the molecular basis of cardiac I_{K1} current. Unlike other Kir2 channel inhibitors, SR59230A does not exert major inhibitory effects on other cardiac potassium channels. SR59230A may be useful for more selective Kir2/I_{K1} block for experimental purposes and could serve as a model compound for the development of novel Kir2 antagonists.

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