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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.