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Effects of (+)- and (±)-sotalol on repolarizing outward currents and pacemaker current in sheep cardiac Purkinje fibres*

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Summary. This study was aimed to differentiate the action of (+)- and (±)-sotalol (10–1000 μmol/l) on membrane currents which are active during the repolarization of cardiac action potentials. Effects where studied in shortened sheep cardiac Purkinje fibres with the two-microelectrode voltage-clamp technique. Action potentials were activated at a frequency of 0.25 Hz and membrane currents at 0.03 Hz or 0.05 Hz in most experiments.

Out of the currents investigated the transient outward current (i_{to}) reacted most sensitively to (+)- and (±)-sotalol. I_{to}-amplitude was decreased on the average to 77% of reference at 10 μmol/l and to 53% at 1000 μmol/l (+)- or (±)-sotalol. The maximally available i_{to}-current was decreased but the voltage-dependent control of inactivation was left nearly unchanged. The initial inwardly rectifying current (i_{K1}), which propels the last repolarization phase of the action potential and controls resting potential to a large extent was reduced on the average to 93% of reference at 10 μmol/l and to 62% at 1000 μmol/l (+)- or (±)-sotalol. Time-dependent (delayed) outward current (i_K) was on the average not affected by (+)- or (±)-sotalol up to 100 μmol/l and was decreased to 84% of reference current under the influence of 1000 μmol/l. An initial outward current, which is activated at positive membrane potentials (i_{inst}) was not clearly affected by (+)- or (±)-sotalol at concentrations up to 1000 μmol/l. Pacemaker current (i_f) was not influenced by the drugs up to 100 μmol/l. Only at 1000 μmol/l was the amount of available i_f-current decreased to 79% of reference. The potential-dependent control of activation was not affected. Time constants of time-dependent currents i_{to}, i_K and i_f did not change in concentrations up to 1000 μmol/l of the drug.

Action potential duration increased at (+)- or (±)-sotalol concentrations ≥ 10 μmol/l and maximal prolongation was achieved at concentrations of 100–300 μmol/l. Resting potential remained nearly unchanged at these concentrations, but the membranes depolarized at 1000 μmol/l. According to our data action potential prolongation in sheep Purkinje fibres under the influence of (+)- and (±)-sotalol correlates to the drug-induced block to i_{to}-current and inwardly rectifying i_{K1}-current.

Key words: Sheep Purkinje fibre – Outward currents – Pacemaker current – (+)-Sotalol – (±)-Sotalol

Introduction

(±)-Sotalol is a clinically available beta-adrenergic blocking agent, which also has class III antiarrhythmic properties. The drug prolongs cardiac action potentials in vitro in mammalian species (e.g.: Kaumann and Olson 1968; Singh and Vaughan Williams 1970; Strauss et al. 1970; Carmeliet 1985; Borchard et al. 1985; Hafner et al. 1988). Monophasic action potentials recorded via suction electrodes in human patients are prolonged as well (e.g.: Edvardsson et al. 1980; Echt et al. 1982; Hayward and Taggart 1986). In humans suppression of both supraventricular (e.g.: Rizos et al. 1984; Campbell et al. 1985) and ventricular arrhythmias (Senges et al. 1984; Lidell et al. 1985; Nademanee et al. 1985) by (±)-sotalol has been reported. Concerning the effects of the optical isomers of sotalol it has been shown that both enantiomers possess similar class III antiarrhythmic activity, whereas the β-sympatholytic effect is largely restrained to (−)-sotalol [D(−)-sotalol], commonly referred to as l-sotalol (Patil 1968; Somani and Watson 1968; Carmeliet 1985). Recent reports established the antiarrhythmic activity of (+)-sotalol [L(+)-isomer, also referred to as d-sotalol] in humans (McComb et al. 1987; Schwartz et al. 1987).

Only few investigations were done in order to explain the action of (+)- and (±)-sotalol on the cardiac action potential in terms of the drug's action on ionic currents. In rabbit Purkinje fibres a substantial reduction of the time-dependent K-outward current (i_K) and a reduction of the background K-current (i_{K1}) has been observed (Carmeliet 1985). Also in frog atrial fibres a reduction of outward currents has been reported (Kern et al. 1983) under the influence of sotalol. In general reduction of outward currents will explain the action potential prolongation caused by the drug.

Our study was aimed to differentiate the action of (+)- and (±)-sotalol on repolarizing outward currents in shortened sheep Purkinje fibres. We used the two-microelectrode voltage-clamp technique and analysed the transient outward current (i_{to}), an initial outward current activated at positive membrane potentials (i_{inst}), the delayed outward current (i_K) and the initial inwardly rectifying current (i_{K1}). Additionally (+)- and (±)-sotalol's action on the pacemaker current (i_f) was studied. A preliminary report has been published in abstract form (Borchard et al. 1988).

Methods

Sheep cardiac Purkinje fibres were used in the experiments. The hearts were removed at the slaughterhouse immediately after exsanguination of the animals and transferred to the

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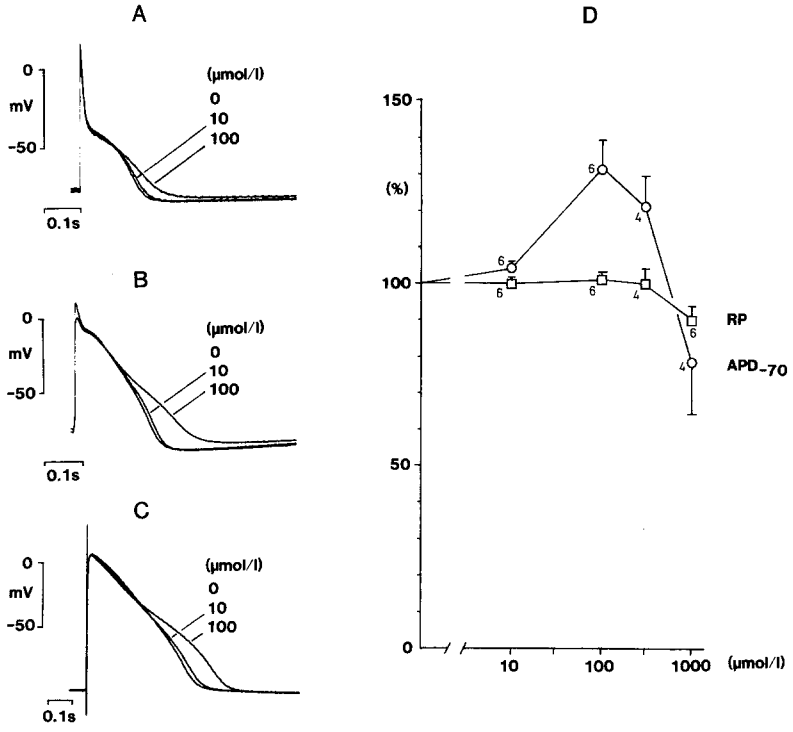

Fig. 1. (+)- and (±)-sotalol prolongs cardiac action potentials. Signals were recorded from **A**: sheep Purkinje fibre; **B**: sheep Purkinje fibre under the influence of 1 mmol/l 4-aminopyridine; **C**: human ventricular muscle. Electrical stimuli were applied extra- (**A**, **C**) or intracellularly (**B**) at a frequency of 0.25 Hz (**A**, **B**) or 1 Hz (**C**). Concentration-response relationship of (+)- and (±)-sotalol's effect on action potential duration (stimulus frequency: 0.25 Hz) measured at −70 mV (APD-70) and resting potential (*RP*) in sheep Purkinje fibres is shown in **D**. *Abscissa:* drug concentration. *Ordinate:* mean values of *APD-70* and *RP* as percentage of the respective control (100%: *RP*: −78.2 ± 1 mV, $n = 7$; *APD-70:* 191.6 ± 16 ms, $n = 7$). Number of experiments and + or − SEM are indicated at the data points. Data from experiments with or without 4-aminopyridine (0.5 or 1 mmol/l) in the bathing solution were pooled

laboratory in a Thermos bottle containing a modified Krebs-Henseleit solution (mmol/l: NaCl 118, KCl 4.7, $CaCl_2$ 1.8, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2, glucose 10.1 and Na-pyruvate 2.0).

Fine Purkinje strands were excised from both ventricles and mounted in an experimental chamber which was similar to that described by Aronson et al. (1973). A grid was used to crush the fibres in short segments (< 1 mm) suitable for voltage-clamp experiments. The chamber contained 1 ml of solution. The solution was vigorously bubbled with a mixture of 95% O_2 and 5% CO_2 before it flowed through the bath at a rate of 1.6 ml/min. The pH of the solution was 7.4. The temperature was in the range of 35 − 37° C for different experiments but constant throughout the course of each experiment (sensor PT 1000, Degussa, Hanau, FRG).

The two-microelectrode technique (Deck et al. 1964) was used to trigger and record action potentials and perform voltage-clamp experiments. A current-passing glass micropipette filled with 2 mol/l K-citrate was placed in the middle of a short fibre and a voltage recording electrode filled with 3 mol/l KCl was placed midway between the current-passing electrode and one crushed termination of the short fibre. Electrode resistances were between 10 and 30 MΩ. Sometimes the electrolyte in the electrodes contained 1 μmol/l EGTA (ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; Merck, Darmstadt, FRG) to prevent increase in the electrode resistance.

Membrane potential was controlled by a conventional voltage-clamp device (design and construction: Ing. grad. J. Springer, Institute of Pharmacology, University of Düsseldorf, FRG). Command pulses were supplied by a minicomputer (Mincal 621-X2, Dietz, Mühlheim, FRG). Total applied current was measured by a virtual ground amplifier. Membrane potential and membrane current were recorded by a digital storage oscilloscope (Explorer III, Nicolet, Offenbach, FRG) and stored on floppy discs for data analysis. Experiments with continuous impalement of the microelectrodes were evaluated. Data reported in this paper result from 16 different preparations. Action potentials were activated at a frequency of 0.25 Hz and membrane currents at 0.03 Hz or 0.05 Hz, unless otherwise stated.

A detailed description of clamp protocols and evaluation of different currents is given in the results section. Time-dependent currents were analysed with respect to their amplitudes and time constants using sums of a variable number (1 − 3) of exponential functions. The number of exponentials was kept to a minimum and was only increased if there was a statistically significant decrease in the sum of squared deviations between experimental and theoretical data. In order to construct the concentration-response curve of a current one test potential, as indicated in results, was chosen in each experiment to evaluate the current amplitude under different sotalol concentrations. Current amplitude in the absence of the drug was taken as 100% reference. Current amplitudes (in %) out of different experiments were averaged for each sotalol concentration. Arithmetic means and standard errors of the mean (SEM) were calculated in spite of the fact that the underlying statistical distribution of data is not known.

In one experiment we recorded action potentials from a human ventricular preparation, which was excised during valve replacement from a mitral stenosis patient without premedication. Anaesthesia was produced by nitrous oxide in conjunction with ethrane. Pancuronium (8 mg) was taken as neuromuscular blocking agent.

Drugs. Sotalol (4-(2-isopropylamine-1-hydroxyethyl)methanesulfonanilide) hydrochloride in the (+)-isomer or racemic form (Bristol Arzneimittel, Neu-Isenburg, FRG) was dissolved in deionized water and added cumulatively to the bathing solution to obtain concentrations of 10 to maximally 3000 μmol/l. Usually the effects of (+)- and (±)-sotalol were examined following 30 − 40 min of superfusion at each drug concentration. This wash-in period was long enough to achieve steady-state effects on action potential duration.

In some experiments barium chloride (2 mmol/l) was added to the bathing solution to block i_{K1}-current (Di-Francesco et al. 1984) or 4-aminopyridine (0.5 or 1 mmol/l; Fluka, Neu-Ulm, FRG) to block i_{to}-current (Kenyon and Gibbons 1979; Coraboeuf and Carmeliet 1982).

Results

Effects of (+)- and (±)-sotalol on cardiac action potentials

(+)- and (±)-sotalol prolonged cardiac action potentials recorded from sheep Purkinje fibres and from one human ventricular preparation [(±)-sotalol]. All records (Fig. 1) show a minor prolongation at 10 μmol/l (+)- or (±)-sotalol which could be enhanced by increasing the concentration to 100 μmol/l. A pronounced effect of (+)- and (±)-sotalol on the time course of the action potentials can be recognized especially during repolarization at membrane potentials negative to about −40 mV.

Concentration-response curves of (+)- and (±)-sotalol's effects on membrane potential in sheep Purkinje fibres are shown in Fig. 1D where data from experiments performed with and without 4-aminopyridine in the bathing solution were pooled. The action potential duration recorded at −70 mV was maximally enhanced at concentrations between 100 and 300 μmol/l (+)- or (±)-sotalol. Up to this concentration resting potential remained nearly unchanged. Higher concentrations of (+)- or (±)-sotalol resulted in a depolarization of the membrane during the resting-state and a secondary reduction of action potential duration, which according to Carmeliet (1985) may result out of a block of a slowly inactivating Na-current.

Effects of (+)- and (±)-sotalol on the isochronous current-voltage relation

At the end of 5 s lasting test pulses to different membrane potentials in the range of −110 to + 50 mV the net membrane current was measured. Test pulses were applied from a holding potential around −50 mV at a frequency of 0.03 or 0.05 Hz. A typical example of current-voltage curves measured without and under the influence of 1000 μmol/l (±)-sotalol in the bathing solution is given in Fig. 2. Application of (+)-sotalol gave similar results. At negative test potentials no effect on net current was observed in the range from 0 to −50 mV. Between −50 and −90 mV the net current was shifted in the inward direction by sotalol and between −90 mV and more negative test potentials the net current is shifted in the outward direction. This characteristic crossing-over of the current-voltage curves close to the expected potassium equilibrium potential indicates that i_{K1}-current had been reduced. At positive membrane potentials the net outward current was decreased, although this effect was not pronounced in many preparations. This might indicate at least some reduction of i_K-current, which should be fully activated at the end of the 5 s lasting test pulses.

Effects of (+)- and (±)-sotalol on i_{to}-current

The transient outward i_{to}-current was activated mostly by depolarizing the membrane from a holding potential around −60 mV to a test potential around 0 mV. The i_{to}-current developed quickly and then decayed more slowly as can be seen in original recordings in Fig. 3. A minimum

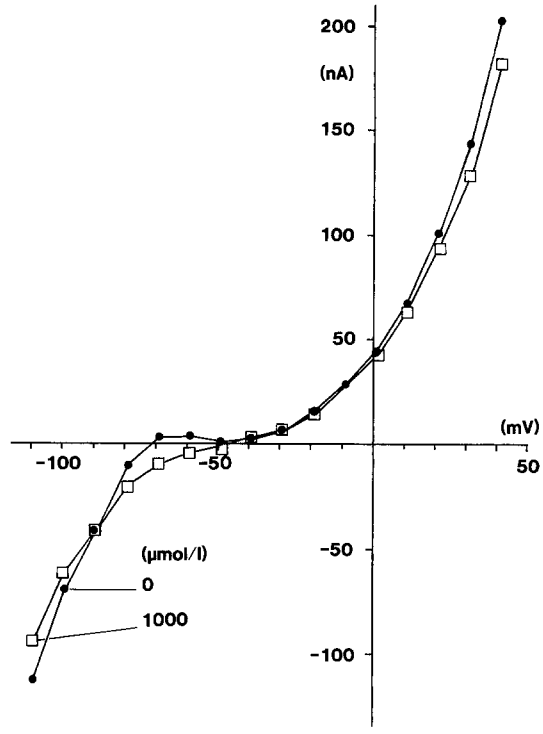

Fig. 2. Effects of (±)-sotalol on isochronous current-voltage relation of sheep Purkinje fibre. At positive test potentials net outward current was slightly decreased. At negative test potentials current was shifted in the inward direction between −50 and −90 mV and in the outward direction at test potentials negative to −90 mV. Current was measured at the end of 5 s lasting test pulses of stepwise increased amplitude applied from a holding potential of −40 mV at a frequency of 0.03 Hz. *Ordinate:* transmembrane current in nA. *Abscissa:* test potential in mV. Preparation No. 16

number (1 or 2) of exponential functions was fitted to the decreasing outward current during test potential. The difference between the value at 0.08 s and at the end of the test pulse (0.5 or 1 s) was taken as a measure of the i_{to}-amplitude.

I_{to}-current in sheep Purkinje fibres consists of two components (Coraboeuf and Carmeliet 1982). We evaluated the amplitude of the dominant and 4-aminopyridine-sensitive component which is referred to as i_{to}-current in this report.

(+)- and (±)-sotalol decreased the i_{to}-amplitude in a concentration-dependent manner (Fig. 3B). On the average i_{to}-amplitude under 10 μmol/l (+)- or (±)-sotalol was inhibited to 77 ± 5% ($n = 4$) of reference and to 53 ± 7% ($n = 5$) at 1000 μmol/l (Fig. 8).

In most recordings i_{to}-inactivation at the test potential (around 0 mV) showed a biphasic time course with τ_1 = 36 ± 8 ms and τ_2 = 240 ± 65 ms ($n = 5$). After addition of (+)- or (±)-sotalol no major changes in time constants were observed which were for example τ_1 = 26 ± 6 ms and τ_2 = 169 ± 38 ms ($n = 4$) at 100 μmol/l sotalol.

Figure 3 shows the results of an experiment which was aimed to study the effect of (+)-sotalol on the voltage-dependent inactivation of i_{to}-current. The membrane potential was held at different conditioning potentials in the range of −80 to −20 mV for 32 s and then clamped to a constant test potential of −4 mV for 1 s. A 10 ms prepulse to −50 mV was given just before the test pulse in order to inactivate the i_{Na}-current. In agreement with the published

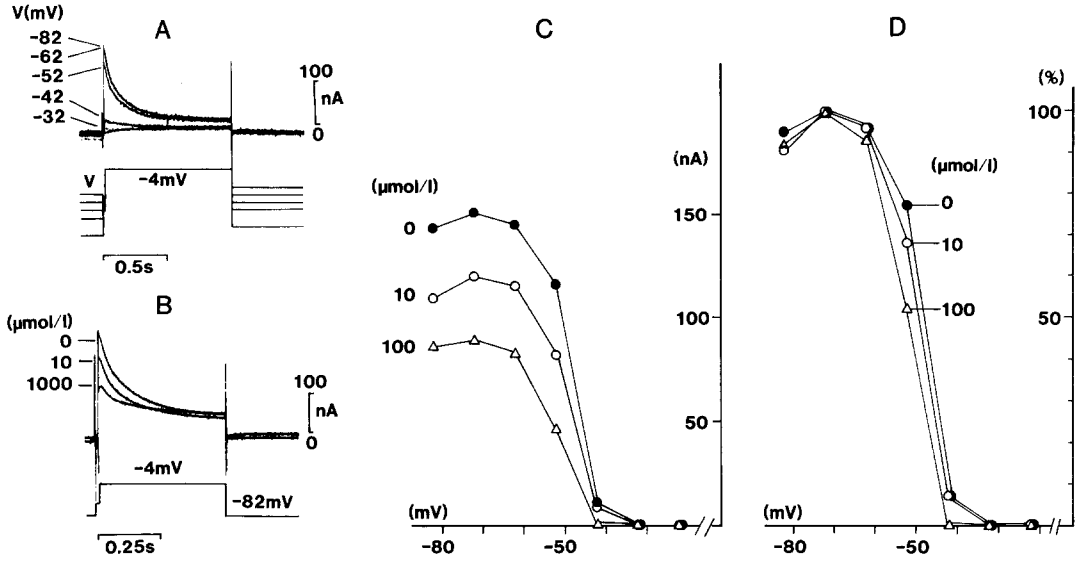

Fig. 3. Effects of (+)-sotalol on voltage-dependent inactivation of i_{to}-current. **A**: Bottom trace shows voltage protocol. After variable conditioning pulses (duration: 32 s; potentials: V, in mV) and a prepulse (duration: 10 ms; potential: −50 mV) a test pulse (duration: 1 s; potential: −4 mV) was applied to elicit i_{to}-current (*top traces*). Shift of the conditioning potential (V, in mV) to more positive values resulted in a block of i_{to}-current. **B**: (+)-sotalol decreased i_{to}-current amplitude (*top trace*) at test potential −4 mV. **C**: I_{to}-current amplitude (*ordinate*, in nA) at test potential −4 mV is plotted versus conditioning potential (*abscissa*, in mV) in the absence (0 µmol/l) and presence (10, 100 µmol/l) of (+)-sotalol. The clamp protocol was as described in **A**. (+)-Sotalol reduced maximally available i_{to}-current. **D**: (+)-Sotalol caused only minor shifts of steady-state inactivation curve of i_{to}-current along the voltage axis. *Ordinate:* normalized values of i_{to}-current amplitude. Maximal current amplitude recorded at test potential in the absence (0 µmol/l) or presence of (+)-sotalol (10, 100 µmol/l) is taken as 100% reference, respectively. *Abscissa:* conditioning potential in mV. Preparation No. 12

data on steady-state inactivation of i_{to}-current (Fozzard and Hiraoka 1973) there was no inactivation for conditioning potentials negative to −70 mV and inactivation was complete for potentials positive to −30 mV. Maximally available i_{to}-current decreased with increasing (+)-sotalol concentration (Fig. 3C) but there was no pronounced shift of the steady-state inactivation curve along the voltage axis (Fig. 3D). Similar results were achieved under the influence of (±)-sotalol.

Effects of (+)- and (±)-sotalol on persisting outward currents at positive membrane potentials

In the experiments to be described below we had to block the i_{to}-current, which otherwise would have masked the onset of delayed outward i_K-current at positive membrane potentials. We achieved block of the i_{to}-current either pharmacologically by adding 0.5 or 1 mmol/l 4-aminopyridine to the solution (e.g.: Fig. 4B) or by applying a holding potential around −30 mV which induced a voltage-dependent block of i_{to}-current (e.g.: Fig. 4A). Under both conditions outward currents were then activated by 2.5−10 s lasting test pulses to positive membrane potentials at a frequency of 0.03 Hz in most experiments.

During the test potential we recorded a slowly developing outward current (Fig. 4) which is supposed to be the potassium outward current i_K (Noble and Tsien 1969). At the onset of the test pulse an initially activating component of outward current always appeared, whose nature has not been analyzed in detail up to now. Because of it's apparent "instantaneous" activation we term it i_{inst}-current in this paper. In order to determine the amplitude of the two outward currents exponential functions were fitted to the time-

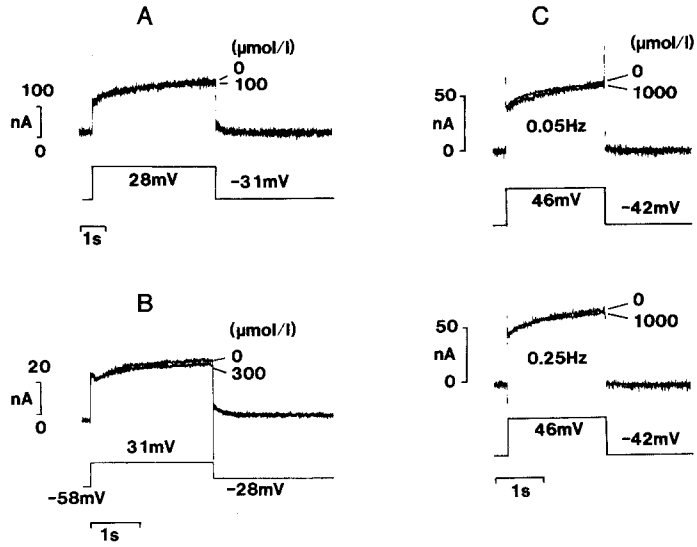

Fig. 4. (+)- and (±)-sotalol produced no clear inhibition of outward currents at positive membrane potentials. *Bottom traces* indicate voltage protocol and *top traces* show membrane currents. **A**: A very fast activating outward current (i_{inst}) and a time-dependent outward current (i_K) were triggered by depolarizing test pulses (duration: 5 s, frequency: 0.03 Hz). Holding potential was −31 mV, where i_{to}-current is inactivated. Preparation No. 6. **B**: Outward currents at positive test potentials were activated after pharmacological inactivation of i_{to}-current with 1 mmol/l 4-aminopyridine. Test pulses lasting 2.5 s where applied at a frequency of 0.05 Hz. Preceding each test pulse the membrane was clamped to −58 mV for 10 s. Preparation No. 11. **C**: I_{inst} and i_K-current were activated at a frequency of 0.05 Hz (*top half*) or 0.25 Hz (*bottom half*). I_{to}-current was blocked by 1 mmol/l 4-aminopyridine. In this preparation even 1000 µmol/l (+)-sotalol produced no distinct effects on i_{inst}- or i_K-current. Preparation No. 15

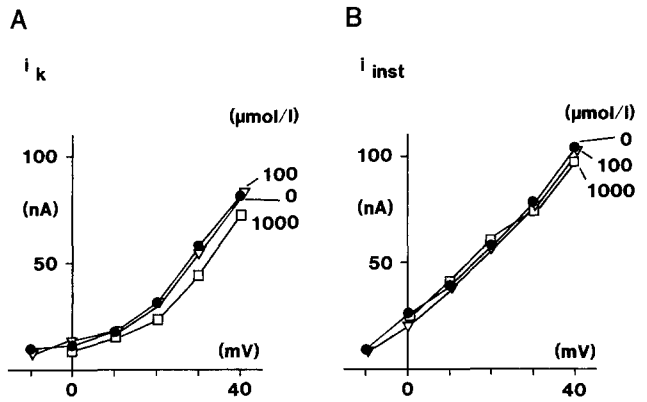

Fig. 5. Effect of (±)-sotalol on time-dependent outward current i_K (**A**) and initial outward current i_{inst} (**B**) was evaluated at different membrane potentials. From a holding potential of −40 mV 5 s lasting test pulses, whose amplitude was stepwise increased, were applied at a frequency of 0.03 Hz. I_{to}-current was blocked by 4-aminopyridine (1 mmol/l). I_K-current at positive test potentials was slightly reduced under the influence of 1000 μmol/l sotalol. *Ordinate:* current amplitude in nA. *Abscissa:* test potential in mV. Preparation No. 16

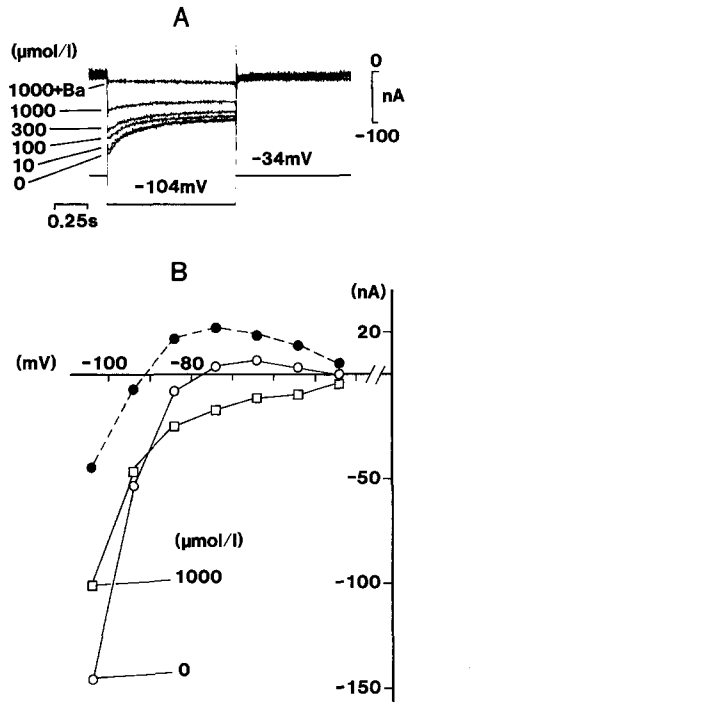

Fig. 6. Decrease of i_{K1}-current amplitude by (+)-sotalol. **A**: Hyperpolarizing test pulses (*bottom trace*) triggered i_{K1}-current (*top traces*) which was evaluated as the amplitude of the initial current jump at the beginning of the test pulse. At 1000 μmol/l (+)-sotalol the persisting i_{K1}-current was blocked after addition of 2 mmol/l barium chloride (1000 + Ba). Preparation No. 10. **B**: Initial i_{K1}-current-voltage relation recorded without and with (+)-sotalol (1000 μmol/l) in the bathing solution. From a holding potential of −34 mV hyperpolarising test potentials of 300 ms duration were applied at a frequency of 0.1 Hz. The difference curve (*filled symbols*) gives the initial (+)-sotalol-sensitive current-voltage relationship. This current is inwardly rectifying with an apparent reversal potential at about −90 mV and a region of negative slope conductance at potentials positive to −75 mV. *Ordinate:* current amplitude in nA. *Abscissa:* test potential in mV. Preparation No. 12

dependent increase in outward current during the test potential. The difference between the value at the end and at 0.1 s after the onset of the test pulse was taken as an estimate for the amplitude of the time-dependent i_K-current. As a measure for i_{inst}-amplitude we calculated the difference between the value given by the extrapolated i_K-current trace at 0 s (time of test pulse onset) and the current at the conditioning potential.

For each experiment outward currents at one test potential in the range of 30 to 50 mV were evaluated to construct concentration-response curves (Fig. 8). Typical original recordings shown in Fig. 4 reveal no strong influence of (+)- or (±)-sotalol on outward currents.

On the average of 11 experiments performed with (+)- or (±)-sotalol i_{inst}-amplitude was decreased to 93 ± 8% of reference and i_K-amplitude to 84 ± 7% at 1000 μmol/l. Minor inhibition of i_K-current at very high concentrations holds also true, when we analyzed the drug's effect on sustained outward currents over a larger potential range, as can be seen in Fig. 5.

In some experiments we activated i_K- and i_{inst}-currents repeatedly with 2 s lasting depolarizations and varied the activation frequency. Changing the activation frequency between 0.05 and 0.25 Hz had no effect on (+)-sotalol's action on the maintained outward currents (Fig. 4C).

Under reference conditions time course of activation was monoexponential or biexponential with a fast and a slow time constant (potential range: 30 to 50 mV). After pooling all data the fast component ($\tau \leq 900$ ms) showed a time constant of $\tau_{fast} = 312 \pm 37$ ms ($n = 9$) and the slow component a value of $\tau_{slow} = 2.75 \pm 0.8$ s ($n = 7$). Pooled data obtained with (+)- and (±)-sotalol show no consistent change of time constants, with values of $\tau_{fast} = 263 \pm 85$ ms ($n = 5$) and $\tau_{slow} = 2.34 \pm 0.34$ s ($n = 8$) under 1000 μmol/l.

Effects of (+)- and (±)-sotalol on i_{K1}-current

I_{K1}-current was activated by clamping the membrane potential from a holding potential around −50 mV to more negative potentials. Test pulses negative to −90 mV resulted in a strong initial inward current which was followed by a relatively slow time-dependent decrease of transmembrane current. Slow current changes are attributed to a depletion of extracellular potassium (Baumgarten and Isenberg 1977; McDonald and Trautwein 1978) and inactivation of i_{K1}-current (Sakmann and Trube 1984; Biermans et al. 1987). The amplitude of the initial current jump at the beginning of the test potential was taken as a measure for i_{K1}-amplitude.

The initial or "instantaneous" i_{K1}-current was decreased by (+)- and (±)-sotalol in a concentration-dependent manner as can be seen in a typical example of original recordings in Fig. 6A. The initial current persisting at 1000 μmol/l (+)-sotalol could be abolished by addition of 2 mmol/l barium chloride (Fig. 6A), which is known to block i_{K1}-current completely.

The concentration-response curve demonstrating the diminuation of i_{K1}-current recorded at test potentials negative to −100 mV is given in Fig. 8. In two experiments where i_{K1}-current was activated at 0.1 Hz addition of (+)-sotalol reduced reference i_{K1}-current to a similar amount as compared to nine other experiments where i_{K1}-current was activated at 0.03 or 0.05 Hz. On the average of 11 experiments performed with (+)- or (±)-sotalol i_{K1}-amplitude was

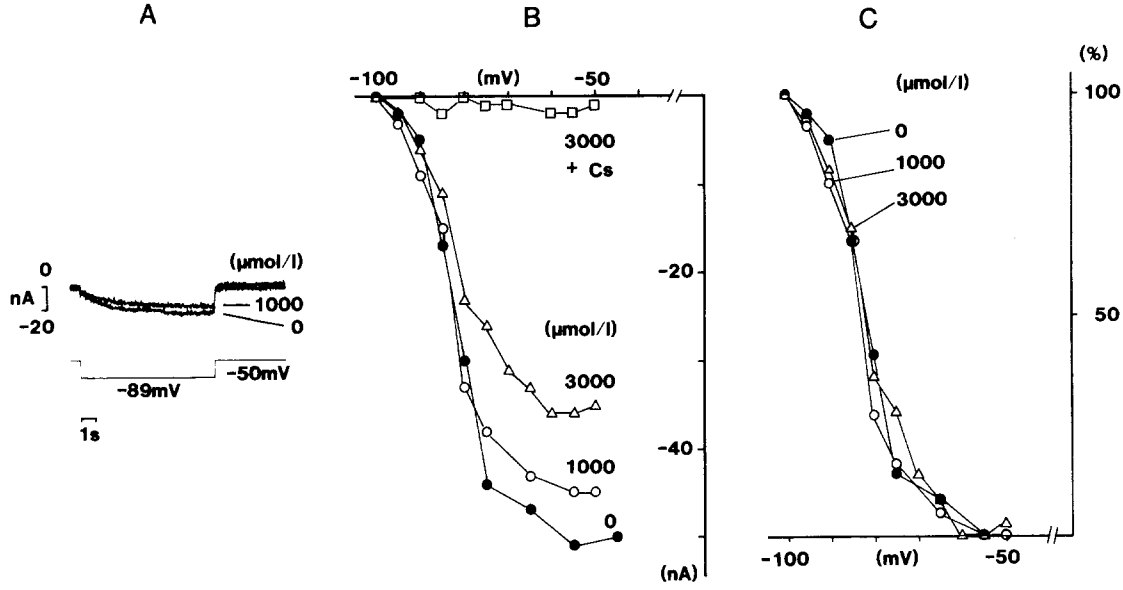

Fig. 7. Effects of (+)-sotalol on pacemaker i_f-current. **A**: I_f-current (*top trace*) was activated by hyperpolarizing test pulses (*bottom trace*) at a frequency of 0.03 Hz. Slowly activating i_f-current was slightly decreased by 1000 μmol/l (+)-sotalol. Preparation No. 2. **B**: Maximally available i_f-current was decreased by (+)-sotalol. After a conditioning pulse (duration: 10 s) to variable potentials (range: −50 to −100 mV) a constant test pulse to −100 mV was applied. Amplitude of i_f-current recorded at the test potential (*ordinate*, in nA) is plotted versus conditioning potential (*abscissa*, in mV). Throughout the experiment 2 mmol/l barium chloride was added to block i_{K1}-current. Persisting i_f-current at 3000 μmol/l (+)-sotalol could be completely abolished after addition of 2 mmol/l cesium chloride (3000 + Cs). **C**: (+)-Sotalol caused no pronounced shift of i_f-activation curve along the voltage axis. *Abscissa:* conditioning potential in mV. *Ordinate:* experimental data shown in **B** were rearranged by calculating for each data point the difference between the given i_f-amplitude and the maximal i_f-amplitude recorded for the corresponding curve. The resulting rearranged curves were normalized, by taking the amplitude of each curve as 100%, respectively. Preparation No. 9

slightly decreased at 10 μmol/l (to 93 ± 5% of reference, $n = 8$) and it was inhibited to 62 ± 5% at 1000 μmol/l ($n = 11$).

Inhibition of i_{K1}-current recorded over a larger range of test potentials is documented by an experiment with (+)-sotalol (Fig. 6B). From a holding potential of −34 mV the membrane was hyperpolarized to test potentials in the range of −104 to −44 mV. Initial currents were measured under control condition [without (+)-sotalol] and under 1000 μmol/l (+)-sotalol. The difference current at each potential was calculated. The initial (+)-sotalol-sensitive current-voltage relation showed the characteristic features of the i_{K1}-current-voltage relation. The (+)-sotalol-sensitive initial current is strongly inwardly rectifying and it is an outward current with a region of negative slope conductance at potentials positive to −90 mV. The reversal potential is close to the expected potassium equilibrium potential.

Effects of (+)- and (±)-sotalol on i_f-current

In most experiments i_f-current was activated by clamping the membrane potential from around −50 mV to potentials near −90 mV for 5 or 10 s at a test pulse frequency of 0.03 Hz. At these test potentials i_{K1}-current was very small and a slowly developing i_f-current could be measured (Fig. 7A).

The difference between the current recorded at 0.1 s and at 5 or 10 s after the onset of the test pulse was taken as a measure for i_f-current amplitude. Up to 100 μmol/l (+)- or (±)-sotalol i_f-amplitude remained nearly unchanged (99 ± 6% of reference, $n = 8$), while at 1000 μmol/l i_f-amplitude decreased to 79 ± 5% ($n = 10$) of reference (Fig. 8). I_f-current traces always showed a monoexponential time course of activation which was not changed by (+)- or (±)-sotalol. Time constant under reference conditions was 2.28 ± 0.30 s ($n = 11$). At 100 μmol/l (+)- or (±)-sotalol time constant was 2.39 ± 0.37 s ($n = 7$) and at 1000 μmol/l 2.32 ± 0.72 s ($n = 10$).

Effects on i_f-current were studied over the potential range of i_f-activation after i_{K1}-current had been blocked by 2 mmol/l barium chloride. The pulse protocol consisted of 10 s lasting conditioning pulses to potentials in the range of −50 to −100 mV followed by a test pulse to a fixed potential (−100 mV), where i_f-current should be fully activated (e.g.: Callewaert et al. 1984; DiFrancesco et al. 1986). (+)-Sotalol (1000 μmol/l) reduced the amount of i_f-current available (Fig. 7B). Persisting time-dependent current could be completely blocked by cesium chloride (2 mmol/l) which is known to be a potent i_f-blocker (Isenberg 1976; DiFrancesco 1981). Activation curve of i_f-current was not shifted along the voltage axis under the influence of (+)-sotalol (Fig. 7C) which indicates that the drug did not interfere with the voltage-dependent control of i_f-current activation.

Comparison of the concentration-dependent effects of (+)- and (±)-sotalol on ionic currents

Both isomers, (+)-sotalol and (−)-sotalol, are reported to be equally effective concerning the prolongation of cardiac action potentials (e.g.: Carmeliet 1985; Hafner et al. 1988). We, too, observed no striking differences between the racemate or the (+)-isomer in their action on the currents investigated. Therefore the data from experiments with (+)- and (±)-sotalol were pooled to construct concentration-re-

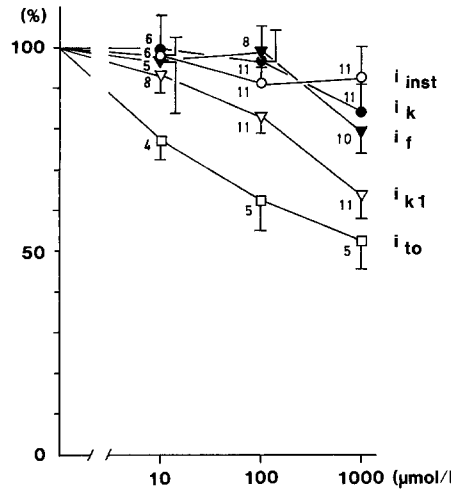

Fig. 8. Influence of (+)- and (±)-sotalol on different ionic currents in sheep cardiac Purkinje fibres. Current amplitude (*ordinate*, in %) is plotted versus sotalol concentration (*abscissa*, in µmol/l). Mean values and number of experiments are shown (for details see methods). *Bars* indicate + or − SEM. Currents evaluated are: "instantaneous" outward current at positive potentials (i_{inst}); time-dependent (delayed) outward current (i_K); pacemaker current (i_f); "instantaneous" inwardly rectifying current (i_{K1}); transient outward current (i_{to})

sponse curves. The concentration-dependent effects of (+)- and (±)-sotalol on the amplitude of different currents which are activated during the repolarizing phase of the action potential are summarized in Fig. 8. Out of the currents investigated so far i_{to}-current and i_{K1}-current were most sensitive to sotalol. The lowest (+)- or (±)-sotalol concentration used (10 µmol/l) always inhibited i_{to}-amplitude to some extent. This concentration was about the threshold concentration for i_{K1}-inhibition, because in one half of the experiments i_{K1}-current was partly inhibited and in the other half it was not. Time-dependent i_K-current and pacemaker i_f-current were only slightly inhibited at the highest concentration used (1000 µmol/l). In some preparations even this high concentration induced no pronounced inhibition of i_K-current.

Inhibition of i_{to}- and i_f-current seemed to result out of a decrease of maximally available current. The drug caused no major effects on voltage-dependent control mechanisms of these currents. Also the kinetics of the current changes of i_{to}, i_K and i_f at the respective test potentials were not altered by (+)- and (±)-sotalol.

Discussion

(+)- and (±)-sotalol in concentrations up to 300 µmol/l increased action potential duration (APD) in sheep Purkinje fibres and in one experiment [(±)-sotalol] with human ventricular muscle. The concentration-response curve of effects of (+)- or (±)-sotalol on APD measured at −70 mV (APD-70) in sheep Purkinje fibres fits very closely to the respective curve measured in guinea-pig papillary muscle (Carmeliet 1985).

APD-70 in sheep Purkinje fibres was on the average 192 ms under reference conditions which agrees with data reported by Fedida and Boyett (1985) who thoroughly studied frequency-dependence of APD in sheep Purkinje fibres. The repolarization of such rather short action potentials at low stimulation frequencies may be mainly controlled by i_{to}-, i_{inst}- and i_{K1}-currents. I_K-current might not influence repolarization to a large extent, because of it's slow activation. In order to quantify the effects of (+)- or (±)-sotalol on different membrane currents we selectively activated different currents by appropriate voltage-clamp protocols.

A considerable inhibition of i_{to}-current and i_{K1}-current was observed, while i_K-current and i_{inst}-current as well as pacemaker i_f-current were only slightly decreased on the average at high concentrations of (+)- or (±)-sotalol. Preliminary results indicate a similar pattern of current inhibition by (+)-sotalol in rat ventricular myocytes. At 30 µmol/l (+)-sotalol i_{to}-current is inhibited to about 70% and i_{K1}-current to about 50% of reference, whereas i_K-current is only reduced to about 95% of reference (unpublished results).

Decrease of i_{to}-current by (+)- and (±)-sotalol should slow down the repolarization during the early fast repolarization of the action potential (AP) in sheep Purkinje fibres. This effect is not clearly visible in the original recordings in Fig. 1 A. However, on the average of 3 experiments APD measured at −30 mV was prolonged to 114 ± 4% of control under 10 µmol/l (+)- or (±)-sotalol. In our experience abolition of i_{to}-current by the rather selective i_{to}-blocker 4-aminopyridine (0.5 or 1 mmol/l; Kenyon and Gibbons 1979) results in an increase of APD-70 to about 140% of reference (Borchard et al. 1989). Therefore partial block of i_{to}-current by (+)- and (±)-sotalol might be one mechanism to explain the AP-prolongation caused by the drug. However partial i_{to}-blockade by (+)- or (±)-sotalol may affect APD-70 only to a minor extent, because we observed no striking effects of 4-aminopyridine on APD-effects induced by (+)- or (±)-sotalol. If 4-aminopyridine was absent, APD-70 was increased to 138 ± 15% of reference ($n = 3$) by 100 µmol/l (+)- or (±)-sotalol. After inhibition of i_{to}-current under control conditions by 4-aminopyridine (0.5 or 1 mmol/l) APD-70 was increased to 125 ± 8% of the respective reference ($n = 3$) by 100 µmol/l (+)- or (±)-sotalol.

Prolongation of cardiac action potentials caused by a blockade to i_{to}-current might occur in any cardiac tissue where i_{to}-current is present, as for example in human atrial cells (Escande et al. 1987).

In sheep cardiac Purkinje fibres i_{K1}-current was reduced by (+)- and (±)-sotalol. Similar results have been reported for rabbit Purkinje fibres (Carmeliet 1985). Inhibition of i_{K1}-current by (+)- and (±)-sotalol in sheep Purkinje fibres correlates with some effects of the drug on the action potential. Firstly the AP-prolongation becomes clearly visible only at potentials negative to about −40 mV, a potential range where i_{K1}-outward current is activated. Secondly the negative slope conductance of i_{K1}-current in this potential range will contribute to speed up the final repolarization of the action potential and loss of available i_{K1}-current will slow down the last repolarization phase of the action potentials, which is actually observed. Thirdly i_{K1}-current controls to a large extent the resting potential in sheep Purkinje fibres and it's block, for instance with barium chloride, results in a depolarization of the membrane. Therefore i_{K1}-block may explain the observed depolarization under the influence of sotalol at high concentrations.

Delayed outward i_K-current was on the average inhibited only at 1000 µmol/l (+)- or (±)-sotalol to some extent. This result may be in contrast to the results of Carmeliet (1985)

who reported a substantial reduction of i_K-current by (+)- and (±)-sotalol in rabbit Purkinje fibres.

Parameters characterizing our i_K-current recordings at positive test potentials are in good agreement to published results (Noble and Tsien 1969). Typical recordings of i_{inst}- and i_K-current show i_{inst} to be 1.8 times larger than i_K (Fig. 4) compared to a value of 1.7 according to Noble and Tsien (1969, their Fig. 3). Time dependence of i_K-activation in our preparations revealed two time constants which were on the average τ_{fast} = 312 ms and τ_{slow} = 2.75 s (test potentials: 30 to 50 mV) compared to published values of τ_{fast} = 500 ms and τ_{slow} = 4 s (Noble and Tsien 1969; potential range −40 to 0 mV). Tail currents were small after long depolarizing test potentials (e.g. Fig. 4A) or at rather elevated potentials following the test pulse (e.g. Fig. 4C). This effect may be explained by an extracellular potassium accumulation during the test pulse resulting in an elevation of the potassium equilibrium potential and a reduction of driving force. Therefore tail currents were large at potentials of −20 to −30 mV after short activation pulses (e.g. Fig. 4B).

We looked for effects of (+)- or (±)-sotalol on i_K-current in 11 different preparations and found on the average only a minor reduction of i_K-current at high drug concentrations. Furthermore we used two different strategies to block i_{to}-current during the investigation of i_K-current in order to rule out a deterioration of i_K-current by this procedure. Either i_{to}-current was blocked by 4-aminopyridine (0.5−1 mmol/l) or we produced a voltage-dependent inactivation of i_{to}-current at holding potentials around −30 mV. Under either condition we observed on the average no marked reduction of i_K. Furthermore we tested the effects of (+)-sotalol on i_K-current under different frequencies of activation (e.g. Fig. 4C, 0.05 Hz and 0.25 Hz). We found no frequency-dependent effect of (+)-sotalol on i_K-current in the frequency-range tested.

Up to now one way of explaining the differences between our results and Carmeliet's (1985) may be that i_K-current in rabbit Purkinje fibres (IC_{50}: 10 μmol/l; Carmeliet 1985) is more sensitive to (+)- and (±)-sotalol than i_K-current in sheep Purkinje fibres [1000 μmol/l (+)- or (±)-sotalol: 84 ± 7% of reference i_K-amplitude]. This view is supported by the fact that rabbit Purkinje fibres are more sensitive to sotalol than sheep fibres if one regards the effects on APD. At 10 μmol/l sotalol Carmeliet (1985) reported the APD to be 141% of control in rabbit whereas we observed a value of 104% in sheep Purkinje fibres. Effects of 10 μmol/l sotalol in sheep are similar in magnitude to effects observed in dog (Purkinje fibre: 106% of control; ventricle: 102%; Strauss et al. 1970), guinea-pig ventricle (110% of control; Carmeliet 1985; Hafner et al. 1988) and in the one preparation of human ventricle (105%) we have tested so far.

The outward current i_{inst} was on the average not affected by (+)- and (±)-sotalol. This outward current appears under different notations as e.g. time-independent background current i_{bg} (McDonald and Trautwein 1978), outward rectifying K-current (Isenberg et al. 1983), time-independent current i_{ti} (Boyett et al. 1980) or it is attributed to the i_{to}-current (DiFrancesco and Noble 1985). In some current recordings a small and short initial peak appeared (e.g. Fig. 4B). This peak might represent a small remaining fraction of 4-aminopyridine-sensitive transient outward current or, more likely, the 4-aminopyridine insensitive Ca-activated i_{bo}-transient outward current (Coraboeuf and Carmeliet 1982). We tried to dissect this current in our determination of i_{inst}-current. The curve fitted to i_K-activation time course was extrapolated to time zero (test pulse onset). The difference between this extrapolated current value and current value at the holding potential was taken as a measure of i_{inst}-amplitude.

For clinical use nowadays only the racemic (±)-sotalol is available, which produces beta-blocking [(−)-isomer] as well as class III antiarrhythmic [(+)- and (−)-isomer] effects. In many reports of the class III-antiarrhythmic effects of (±)- and (+)-sotalol in humans sotalol plasma concentrations in the range of 2−4 μg/ml were employed, corresponding to 7−13 μmol/l (molecular weight of sotalol: 308.8 g/mol; no plasma-protein binding). Duration of monophasic ventricular action potential as well as QT-intervals corrected for heart rate (QT_c) were increased to about 110% of the respective control (e.g.: Edvardsson et al. 1980; Neuvonen et al. 1981; Echt et al. 1982; McComb et al. 1987; Schwartz et al. 1987). In the same concentration range (about 10 μmol/l) we observed AP-prolongation in sheep Purkinje fibres and in the one human ventricular muscle preparation we tested, whereas the maximal effect was achieved between 100 and 300 μmol/l. These effects correlated with a reduction of i_{to}-current and i_{K1}-current.

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