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AVE 0118, an Antiarrhythmic Drug with a Novel Action Mechanism: Block of I_{kur} and I_{to} Potassium Currents in Human Atrial Myocytes

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Abstract: The effects of the novel Kv1.5 channel blocker AVE 0118 on human atrial potassium currents (I_{Kur}, I_{to} and I_{K1}) were investigated in this study. We could demonstrate that AVE 0118 blocks I_{Kur} not voltage dependent with an IC_{50} of 37 (5;270) nmol L^{-1} at +40 mV. Compared with I_{Kur}, I_{to} peak current was only slightly altered. At 30 μmol L^{-1} current amplitude was reduced by 31±6%, I_{to} inactivation time constant decreased from 33.9±2.0 ms to 10.0±1.9 ms resulting in a 73 ±5% reduction of total potassium flow through I_{to}. IC_{50} of the dose response relation of the reduction of I_{to} net current was 300 (100;1000) nmol L^{-1}. There was only little effect of AVE 0118 on I_{K1}, current was reduced only 11±3% at a concentration of 10 μmol L^{-1} at a test-potential of −90 mV. AVE 0118 is an antiarrhythmic substance with a new drug action mechanism. Drugs with an attenuated effect on I_{kur} and a (compared to the ventricle) stronger delay of the repolarization on the atrial level are therefore a new and interesting tool for treatment of supraventricular arrhythmias like atrial fibrillation.

Key words: Kv1.5, I_{Kur}, I_{to}, Atrial fibrillation, AVE 0118

INTRODUCTION

Atrial fibrillation (AF) is the most frequent arrhythmia in men and has enormous clinical and economic implications (Hennersdorf *et al*., 2001). Although a variety of non-pharmacological approaches for the treatment of AF have been developed within the last years, pharmacological treatment is still the first choice for the majority of patients.

Current phannacological treatment is limited due to a low efficiency and a considerable proarrhythmic potential (Ruskin, 1989; Hohnloser and Singh, 1995; Tan, 1996; Camm *et al*., 1998). These effects, for example torsade de pointes, are mainly caused by the ventricular effects of these substances (Hohnloser, 1997).

The ultra rapid potassium current I_{Kur} and its underlying molecular correlate, the Kv 1.5 potassium channel (Wang *et al*., 1993) , is an interesting new target for drugs because in humans this channel is only expressed in the atrium (Konarzewska *et al*., 1995; Mays *et al*., 1995; Amos *et al*., 1996; Feng *et al*., 1997). Therefore, blocking of this channel should prolong the repolarization only on the atrial level (Courtemanche *et al*., 1998, *et al*., 1999).

In CHO cells it has been shown that the new antiarrhythmic drug AVE 0118 is a potent blocker of Kv 1.5. (Gogelein *et al*., 2004). At the moment it is not clear how ionic currents in the human atrium are affected by AVE 0118. The effects of AVE 0118 on I_{Kur}, I_{to} and I_{K1} in native human atrial cells were examined in this study.

MATERIALS AND METHODS

Cell isolation and solutions: Specimens of human right atrial appendages were obtained from hearts of 27 patients (67.7±8.7 years) undergoing cardiac surgery with extra corporal circulation. All patients were free from supraventricular arrhythmias, further characteristics are shown in Table 1.

The procedure for tissue procurement was approved by the ethics committee of the university of Tübingen, Germany.

The atrial tissue was explanted and transported to the laboratory as fast as possible in a cardioplegia solution containing (mmol L^{-1}) KH_2PO_4 50, $MgSO_4$ 8, HEPES 10,

Table 1: Patients' characteristics

n (male/female)	27 (23/4)
cardiac disease (CAD/ Valve disease)	(25/2)
ß-blocker	18 (67%)
Ca-channel blockers (non-dihydropyridines)	7 (26%)
Ca-channel blockers (dihydropyridines)	3 (11%)
ACE inhibitors	19 (70%)
AT 1 antagonists	2 (7%)
digitalis	1 (4%)
nitrates	16 (59%)
diuretics	9 (33%)

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adenosine 5, glucose 140, mannitol 100 and taurine 10 (pH adjusted to 7.35 using KOH). The following agents were used for the isolation of the myocardial cells:

Thirty microgram albumins were dissolved in a solution containing (mmol L^{-1}) NaCl 137, KH_2PO_4 5, $MgSO_4$ 1, glucose 10, HEPES 5 and taurine 10. For tissue digestion, approximately 15 μg of collagenase type 5 (sigma C9263) was added (= digestion solution 1). Five milliliter were taken separately (= digestion solution 2) and 5 μg of collagenase type 5, respectively 5 μg of protease type 24 (sigma P8038) was added to that solution. The atrial tissue was put in a flask containing digestion solution 1 for 30 min at 37°C . Next, atrial tissue was placed into 5 mL digestion solution 2 and after approximately 15 min the first supernatant was removed and the specimen was re-incubated in fresh digestion solution. To the cell suspension 5 mL of KB (Kraftbrühe with containing (mmol L^{-1}) KCl 20, KH_2PO_4 10, glucose 25, mannitole 40, l-glutamic-acid 70, β-hydroxybutyric acid 10, taurine 20, EGTA 10, albumins (0.1%), pH adjusted to 7.35 with KOH) was added to stop further digestion. The described procedure was repeated several times approximately after 15, 20, 25 and 30 min. Cell suspensions were stored at 4°C and warmed up to room temperature just before each experiment started.

For the experiments, only rod-shaped cells showing clear cross-striations were used. The cells were superfused in a chamber with tyrode solution (containing (mmol L^{-1}) NaCl 126, KCl 5.4, $MgCl_2$ 0.8, $CaCl_2$ 1, NaH_2PO_4 0.33, HEPES 10, glucose 5.5, pH adjusted to 7.35 with NaOH). Five minute was allowed for cell adhesion to the bottom of the chamber. During recording of I_{Kur} and I_{to}, the following chemicals were used in order to prevent contamination from other currents: $CdCl_2$ (200 μmol L^{-1} to inhibit I_{caL}), TEA (10 mmol L^{-1}, to inhibit I_K), $BaCl_2$ (1 mmol L^{-1}, to inhibit $I_{K,1}$) and atropine (1 μmol L^{-1}, to inhibit $I_{K,Ach}$). All chemicals were taken from Sigma-Aldrich. In previously published studies it was shown, that none of those drugs have an effect on I_{kur} and I_{to} (Wang *et al*., 1993, 1995). Experiments were done at room temperature, it was shown that the amplitude of I_{Kur} at 20°C is similar to that at 37°C (Wang *et al*., 1993).

During recording of I_{K1}, I_{CaL} was blocked with Nisoldipin (1 μmol L^{-1}) and $I_{K,r}$ with Dofetilide (1 μmol L^{-1}).

Voltage-clamp technique: Ionic currents were recorded using the whole-cell configuration of the patch-clamp-techmique. Borosilicate glass electrodes (outer diameter 1.5 mm) had resistances between 2.5 and 5 MΩ when connected to a patch clamp amplifier (Axopatch 200B, Axon Instruments, Foster City, CA).

Pipette solution contained the following substances (mmol L^{-1}) : GTP (lithium salt) 0.1, K-aspartate 110, KCL 20, $MgCl_2$ 1, Mg_2ATP 5, HEPES 10, EGTA 5, phosphocreatine 5. pH was adjusted to 7.35 using KOH.

After rupture of the cell membrane, pipette series resistance (R_S) was electrically compensated to minimize the capacitive surge on the current recording.

Cells with significant leak current were rejected, leak compensation was not applied.

Data analysis: To record I_{Kur} in the absence of contamination by I_{to}, a 1000 ms prepulse to +40 mV was used to inactivate I_{to} 10 ms before a depolarising test pulse (Wang *et al*., 1993, 1995; Li *et al*., 1996; Feng *et al*., 1998; Yue *et al*., 1999).

For the analysis of all concentration dependencies the logistic form of the Hill equation y = a/(1+10^(-n*(px-pK))) was used. Here, a denotes the extent of the effect (amplitude), n (= n_H) is the Hill coefficient, x the concentration of the compound under study and K the midpoint of the curve with px = -logx and pK = -logK (Bachmann *et al*., 2001).

The amplitude of I_{to} was measured as the difference between the peak of the transient outward current and the sustained current at the end of the pulse (Wang *et al*., 1995). To study the inactivation of I_{to}, we fitted the current decay at +30 mV with a monoexponential function to obtain the time constant τ. To evaluate total net current though I_{to}, the following method was used : a.) Fitting complete current decay at 30 mV with an exponential function of third order. b.) Using that function for calculating the integral from the beginning to the end of test pulse, which indicates the net current generated by I_{to} and I_{Kur}. c.) Subtraction of the net current generated by I_{Kur} (amplitude of the sustained component multiplied with the duration of the test pulse) to get I_{to} net current

To examine onset of I_{to} blockade, current amplitude at a given time after washing of AVE 0118 was divided by control current amplitude at the same time, the resulting value was subtracted from 1. By fitting the resulting curve with a monoexponential function, time constant τ could be used for description of the onset of I_{to} block. I_{K1} was recorded with a standard pulse protocol (Bosch *et al*., 1999).

Statistics: Single comparisons between base line and drug data were performed with student's t-test and a two-tailed probability of 5% was taken to indicate statistical significance. Non-linear curve fitting was performed using Clamp fit in pClamp (Axon Instruments).

RESULTS

Effects of AVE 0118 on I_{kur}.: Representative± current tracings in the absence and presence of 10 μmol L^{-1} AVE 0118 are shown in Fig. 1 A and B. Cells were depolarised

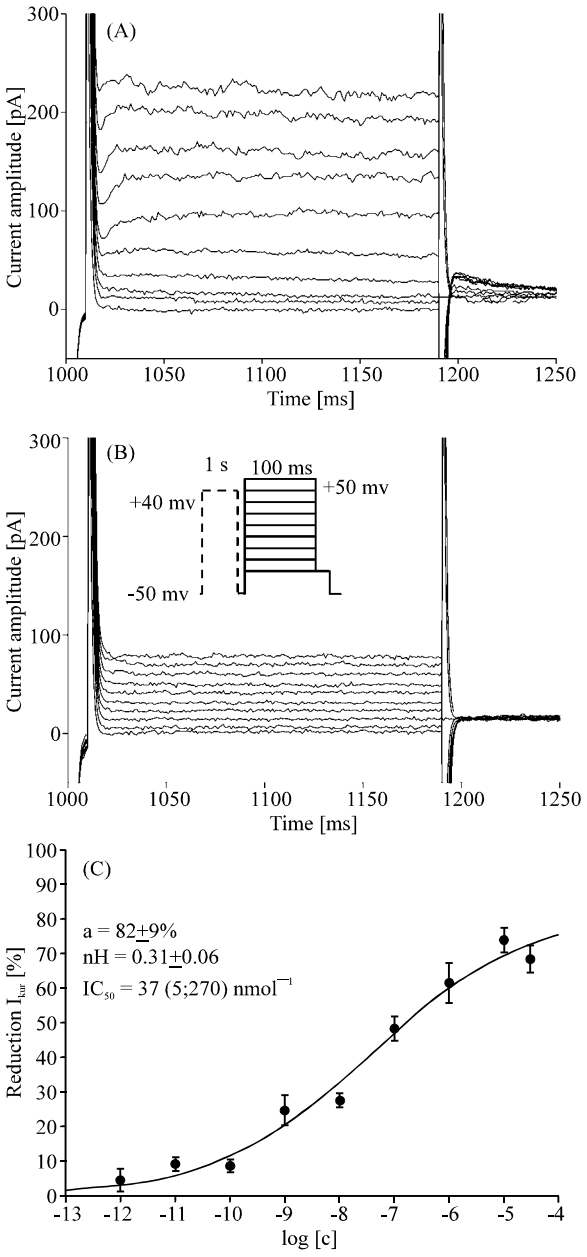

Fig. 1: The ultra rapid delayed rectifier potassium current in the human atrium (a) before and (b) after the application of 10 μmol L^{-1} AVE 0118 at room temperature. Currents were elicited by depolarising the cell from a holding potential of –50 mV to various test potentials between –30 mV and +50 mV 10 ms after a prepulse (not shown) to inactivate I_{to} and (c) Concentration-response curve of AVE 0118, exemplarily at +40 mV. n = 4-7 per data point. a = maximum block, nH = Hill coefficient, IC_{50} = concentration of half-maximal effect

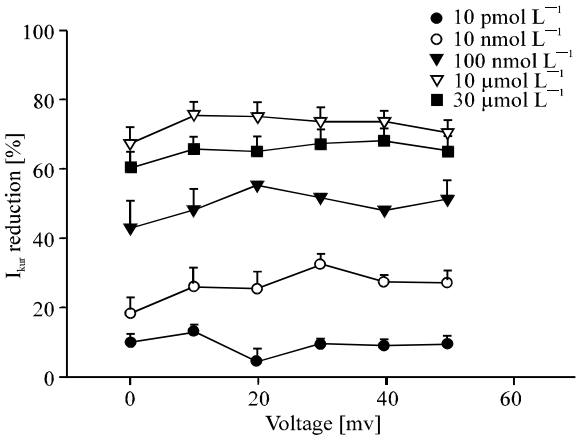

Fig. 2: Voltage dependence of I_{Kur} block, expressed as percent reduction of I_{Kur} relative to control at different test-pulse voltages. No significant voltage-dependency could be observed

from a holding potential of –50 mV for 1000 ms to inactivate I_{to}. After going back to holding potential for 10 ms, cells were again depolarised to various test potentials between –40 and +50 mV (Wang *et al*., 1993).

AVE 0118 produced a concentration dependent inhibition of the sustained outward current. A maximum reduction of ca. 70% of control current was achieved at a concentration of 10 μmol L^{-1}, higher concentrations did not aggravate blockade. Dose response relation– exemplarily at +40 mV- is shown in Fig. 1C. Using the Hill relation, IC_{50} averaged 37 (5;270) nmol L^{-1}. Hill factor was 0.31±0.06.

As shown in Fig. 2, no significant voltage dependency of the I_{Kur} block could be observed.

Effects of AVE 0118 on I_{to}.

I_{to} peak current: The response of I_{to} to 10 μmol L^{-1} AVE 0118 is shown in Fig. 3. Blockade reached statistical significance ($p<0.05$) at concentrations ≥10 μmol L^{-1}, but - in comparison to the I_{kur} block- I_{to} peak current amplitude was only slightly decreased: e.g., 30 μmol L^{-1} AVE 0118 reduced amplitude only by 31%.

At a concentration of 30 μmol L^{-1} AVE 0118 blockage of the peak current was not saturated, but higher concentrations were not tolerated by the myocytes (n = 5), therefore no dose-response relation could be determined.

Inactivation of I_{to}: After washin of AVE 0118 current inactivation increased (Fig. 3). Current inactivation was fit by a monoexponential function at a test potential of +50 mV. Resulting changes of the calculated time constant τ after washin of AVE 0118 at several concentrations were

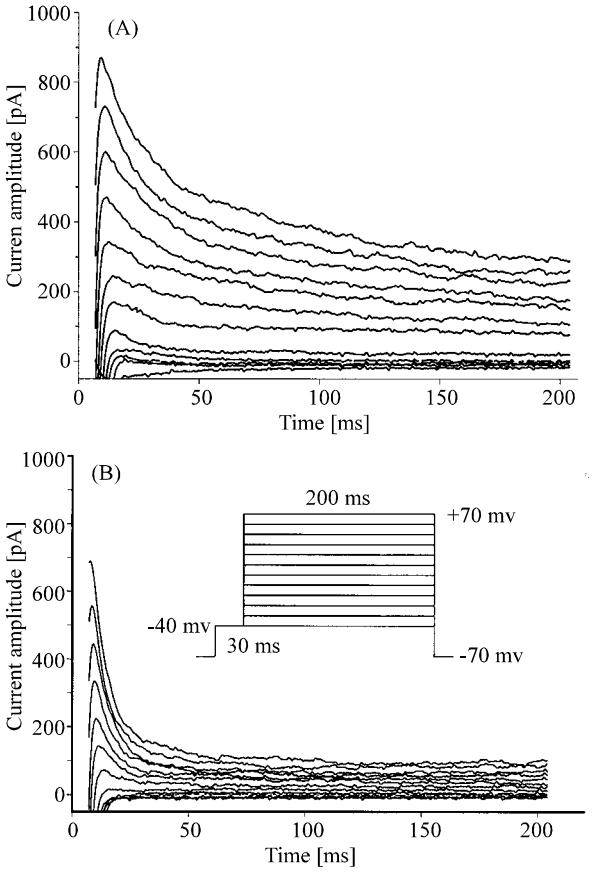

Fig 3: I_{to} (A) before and (B) after the application of 10 μmol L^{-1} AVE 0118. Currents were elicited by depolarising the cell from a holding potential of –70 mV to –40 mV for 30 ms to inactivate I_{Na}, followed by a 200 ms test pulse at various potentials between –40 mV and +70 mV. Note faster inactivation of the current after drug application

used to quantify the amount of change (Fig. 4). At the highest concentration tested (30 μmol L^{-1}), τ was reduced from 33.9±2.2 ms (control) to 10.0±1.9 ms (washin), which is a 70.89±4.38% reduction, but a steady state was not yet reached.

I_{to} net current: Acceleration of I_{to} inactivation results in a remarkable reduction of I_{to} net current. To evaluate this, AUC (area under curve) was calculated before and after washin of 10 μmol L^{-1} AVE 0118 at a test potential of +50 mV. Figure 5 shows the effects of AVE 0118 on I_{to} net current (represented by the AUC). At the highest concentration tested (30μmol L^{-1}) it was reduced by 72.63%±4.79%. Calculated EC_{50} was 0.3 (0.1;1) μmol L^{-1}, hill factor was 0.75±0.22.

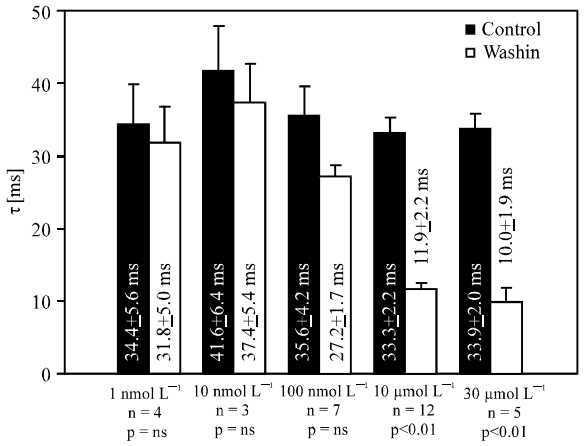

Fig. 4: Change of inactivation time constant τ of I_{to} at several concentrations of AVE 0118. n denotes the numbers of cells

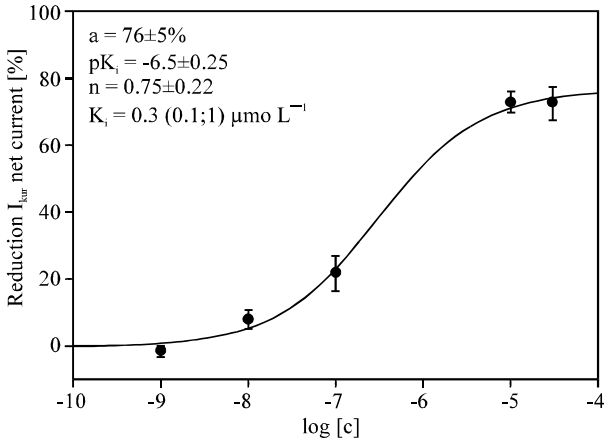

Fig. 5: Concentration dependency of the reduction of I_{to} net current. Net current was calculated as described in methods. At highest concentration of AVE 0118 tested blockage was already saturated. Therefore, we could calculate the pK_i, hill-factor (n) and maximum effect (a) using the logistic form of the hill equation described in methods. At each concentration from 4 to 12 cells were used, blockage was significant ($p<0.05$) at concentrations ≥10 nmol L^{-1} (paired t-test)

Onset of I_{to} blockade: To study the kinetics of the onset of I_{to} block at a test concentration of 10 μmol L^{-1} AVE 0118 at a test potential of +50 mV, the time constant τ for the onset of I_{to} block was calculated (see methods). The average τ was 7.40±0.60 ms. Fig. 6 shows average current amplitude before and after washin of AVE 0118 compared to the onset of I_{to} blockade.

Effects on I_{K1}: AVE 0118 had only a minor effect on I_{K1}. For example, at a test potential of –90 mV 10 µmol L^{-1} AVE 0118 reduced I_{K1} by only 11±3 % (n = 7, data not shown).

DISCUSSION

Effects on I_{kur}: Our results demonstrate that AVE 0118 is a potent blocker of I_{kur}. Surprisingly the IC_{50} for the human I_{Kur} blockage (e.g., at +40 mV 37 (5;270) nmol L^{-1}) is much lower than the IC_{50} in e.g., CHO cells (1.1±0.2 µmol L^{-1}(Gogelein *et al*., 2004)). In addition, dose response relation for I_{kur} blockage has a remarkable low hill factor (e.g., at +40 mV 0.31 (0.25;0.37)).

This phenomenon was already described by another selective Kv1.5 blocker of the same chemical substance class (Bachmann *et al*., 2001). The authors provided a possible explanation which can probably also apply to AVE 0118: they speculated that the substances might not bind directly to the channel but to a modifying beta subunit, which is expressed in human atrium but not CHO cells. But this explanation is merely notional at this time and more experiments are needed.

Effects on I_{to}: Compared with the effects on I_{kur}, I_{to} peak current reduction by AVE 0118 was less pronounced. Inactivation time constant of I_{to} after superfusion with AVE 0118 was remarkably reduced (which means that inactivation is faster). We observed a saturated block of the net current (fig. 6) at the highest concentration tested.

Potential mechanism of drug action: A similar decrease of inactivation time constant can be found in I_{to} block by flecainide, quindine and 4-AP (Wang *et al*., 1995). It was concluded that this was caused by an accelerated inactivation. But one cannot exclude that accelerated inactivation is "imitated" by the onset of the channel's block by a drug. Due to a rapid onset of the block compared to the opened channel, it leaves its open state more rapidly compared to spontaneous endogenous inactivation (Dukes and Morad, 1989). To evaluate this possibility, time dependence of the onset of I_{to} blockade was further studied. The time course could be described by a mono exponential function. The time constant τ at a concentration of 10 µmol L^{-1} AVE 0118 (7.4±0.6 ms) was in the same range as the inactivation time constant at the same concentration (11.89±2.15 ms).

Because there was no significant reduction of the current at the beginning of a test pulse and it took some time after channel opening until a reduction of I_{to} could be observed, channel opening is a prerequisite for onset of block.

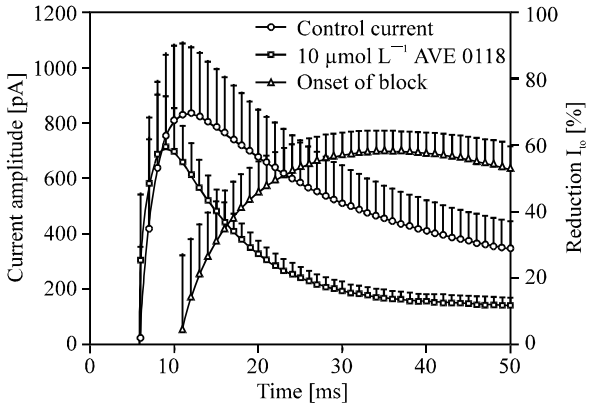

Fig. 6: Onset of I_{to} block. At each time ([ms], abscissa) average current amplitudes (left ordinate) of control current and of current inhibited by 10 µmol L^{-1} AVE 0118 as well as the percentage inhibition of I_{to} (right ordinate) is plotted. Time of the onset of the blockade was fitted with a mono exponential function, resulting time constant τ averaged 7.4 ± 0.6 ms. (n = 5)

The binding site for drugs for an open channel block is located intracellular or in the pore (Dukes and Morad, 1989), so that blocking mechanism could be responsible for block of I_{to} by AVE 0118.

Clinical implication: Highly selective blockade of Kv 1.5 should minimize the risk of ventricular proarrhythmogenity, a possible side effect of treatment of AF by currently available antiarrhythmic drugs. It has been shown that the left atrial effective refractory period of anaesthetized pigs is 30% prolonged by AVE 0118 (0.5 mg kg^{-1}) without any significant effect on QT time and inducibiltiy of supraventricular tachyarrhythmias by programmed atrial stimulation (400S2) is reduced by 100% (Knobloch *et al*., 2002).

Furthermore, atrial refractory period of goats is prolonged without any effects on QT interval (Blaauw *et al*., 2004). Surprisingly, percentage of AERP prolongation is even augmented in chronically remodelled atrium.

Kv 1.5 blockers like AVE 0118 are a new class of antiarrhythmic drugs that seem to have a great potential for treatment of supraventricular arrhythmias like AF with a high efficiency and a reduced proarrhythmogenic potential. However, clinical data is needed to continue the promising experimental results of Kv 1.5 block.

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