Can inhibition of I_{Kur} promote atrial fibrillation?

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Abstract

BACKGROUND—Block of ultra-rapid delayed rectified potassium current (I_{Kur}), present in atria, but not in ventricles, is thought to be a promising approach for atrial-specific therapy of atrial fibrillation (AF). However, it has been shown that I_{Kur} block may abbreviate atrial repolarization and that loss-of-function mutations in *KCNA5*, which encodes $K_v 1.5$ channels responsible for I_{Kur}, is associated with familial AF.

OBJECTIVE—Our objective in this study was to use low concentrations of 4-aminopyridine (4-AP, 10–50 µM), known to selectively block I_{Kur}, to assess the pro-and antiarrhythmic effects of I_{Kur} block in "healthy" and "remodeled" atria.

METHODS—Isolated canine coronary-perfused right atrial preparations were used. Acetylcholine or ischemia/reperfusion was utilized to acutely "remodel" the atria. Transmembrane action potentials and a pseudo-ECG were simultaneously recorded.

RESULTS—Normal ("healthy") atria typically displayed action potentials (AP) with a prominent plateau, whereas remodeled atria displayed triangular-shaped APs ("remodeled"). In "healthy" atria, in which AF could not be induced with programmed stimulation, 4-AP abbreviated action potential measured at 90% repolarization (APD_{90}) and effective refractory period (ERP), permitting the induction of AF in 4/12 preparations (33%). In "remodeled" atria, 4-AP produced little (50 µM) to no (10–25 µM) prolongation of APD_{90} or ERP and was either ineffective or poorly effective in terminating AF or preventing its induction.

CONCLUSIONS—Our findings suggest that block of I_{Kur} can provide the substrate for development of AF in "healthy" canine atria, presumably via abbreviation of APD and ERP.

Keywords

atrial fibrillation; antiarrhythmic agents; action potential

INTRODUCTION

Ultra-rapid delayed rectified potassium current (I_{Kur}), carried by $K_v 1.5$ channels encoded by the *KCNA5* gene, is present in atria, but not in the ventricles.[1–3] Block of I_{Kur} is thought to be a promising approach for atrial-specific therapy of atrial fibrillation (AF). However, a recent report has associated *KCNA5* loss-of-function mutations with familial AF.[4] It has been shown also that reduction of I_{Kur} abbreviates action potential duration (APD_{90}) in non-remodeled ("healthy") atrial cells, but prolongs APD_{90} in remodeled atrial cells (displaying a triangular-

shaped action potential).[5, 6] Knowing that abbreviation of atrial repolarization promotes AF, we hypothesized that I_{Kur} block could promote AF in "healthy" atria. We used low concentrations of 4-AP (10–50 µM), shown to be specific for I_{Kur} block,[2, 3] to assess the pro- and antiarrhythmic effects of I_{Kur} block in "healthy" vs. "remodeled" canine isolated coronary-perfused atria.

METHODS

Experiments were performed using isolated arterially-perfused canine right atrial preparations. The methods used for isolation and perfusion of these preparations have been described in previous publications.[5, 7] Briefly, right atria were dissected from hearts removed from anesthetized (sodium pentobarbital) adult mongrel dogs (20–25 kg). Unfolded atria with a rim of the right ventricle was cannulated and perfused through the ostium of the right coronary artery. Unperfused tissue was removed with a razor blade or scissors. The cut ventricular and atrial branches were ligated using silk thread. After these procedures (performed in cold cardioplegic solution, 4–8°C), the preparations were transferred to a temperature-controlled bath and arterially-perfused with Tyrode's solution by use of a roller pump. The composition of the Tyrode's solution was (in mM): NaCl 129, KCl 4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, and D-glucose 5.5, buffered with 95% O_2 and 5% CO_2 (37±0.5 °C, pH=7.35)

Transmembrane action potential (AP) recordings were obtained using floating glass microelectrodes (2.7 M KCl, 10–25 MΩ DC resistance). A pseudo-electrocardiogram (ECG) was recorded using two electrodes consisting of Ag/AgCl half cells placed in the Tyrode's solution bathing the preparation, 1.0 to 1.2 cm from opposite ends of the atrial preparations. Effective refractory period (ERP) was measured by delivering premature stimuli of twice diastolic threshold intensity after every 10th basic beat at a pacing CL of 500 ms.

"Healthy" and "Remodeled" atria

Arterially-perfused atrial preparations not subjected to any interventions were referred to as "Healthy" atria. Acute "remodeling" of atrial coronary perfused preparations was achieved with two experimental approaches known to abbreviate atrial APD and promote AF. In the first, acetylcholine (ACh, 0.5 µM) was used to abbreviate APD and ERP and mimic a "remodeled" atrium.[7, 8] The second approach involved a recently developed AF model in which isoproterenol (ISO, 0.1–0.2 µM) was added to the coronary perfusate after the atrial preparations are exposed to ischemia and reperfusion.[9, 10] Ischemia and reperfusion were produced by stopping (for 30–40 min) and reinitiating coronary flow with continuous superfusion flow. On the 15–30th minutes after the start of reperfusion, ISO was added to the coronary perfusate, which reproducibly generated non-sustained AF in the ischemia/reperfusion-damaged atria.[9, 10] Note that β-adrenergic stimulation only mildly promotes AF in "healthy" atria.[9, 11]

Experimental Protocols

The equilibration period for the preparations was 30–40 min. The concentration of 4-aminopyridine (4-AP, 10–50 µM in "healthy" atria and 10–200 µM in "remodeled" atria) was increased in a step-wise manner (in ≥ 20 min after the start of each concentration). APs were recorded from the endocardial crista terminalis (CT) and pectinate muscle (PM). Arrhythmogenicity was tested using programmed electrical stimulation (PES) protocols at a CL of 500 ms (a single premature beat with a twice-threshold stimulus intensity) at three locations (two in PM and one in CT) under baseline conditions and in the presence of each concentration of 4-AP. In some experiments involving ACh, persistent AF was induced by a rapid pacing protocol (CL = 100–50 ms for 3–5 seconds).

Drugs

4-AP, ACh, and ISO (all SIGMA, MO) were dissolved in distilled water and prepared fresh as a stock of 1–100 mM before each experiment.

Statistics

Statistical analysis was performed using paired or unpaired t test and one way repeated measures or multiple comparison analysis of variance (ANOVA) followed by Bonferroni's test, as appropriate. All data are expressed as mean ± SD.

RESULTS

In "healthy" coronary-perfused right atrial preparations, block of I_{Kur} with low concentrations of 4-AP significantly elevated the plateau level and prolonged the early repolarization phase (APD_{20} from 5±3 to 42±19 ms, p<0.001; in CT; 25 µM; n=10), but abbreviated APD_{90} and ERP (Fig. 1). The magnitude of phase 1 of the action potential was reduced from 30±6 mV in control to 22±5, 17±4, and 8±3 mV in the presence of 10, 25, 50 µM of 4-AP, respectively (all p<0.05 vs. control; CT, n=12 for each; CL = 500 ms; Fig. 1). Plateau voltage (dome) was significantly elevated from −4±4 mV in control to +3±5, +10±3, and +15±6 mV after 10, 25, and 50 µM of 4-AP, respectively (all p<0.05 vs. control; CT, n=12 for each; CL = 500 ms).

Under baseline conditions, 2 out of 14 "healthy" atria developed short AF (<1 sec) in response to PES. These two preparations were removed from further study. In the presence of low concentrations of 4-AP (10–50 µM), AF could be induced in up to 33% of "healthy" atrial preparations, which did not develop AF in the absence of 4-AP (Fig. 2 and Table I). All episodes of AF were non-sustained (≤3 sec). Spontaneous arrhythmias were not observed.

In contrast to "healthy" atria, block of I_{Kur} using 10 and 25 µM 4-AP produced little to no change in APD_{90} or ERP in "remodeled" atria (Fig. 3). Higher concentrations of 4-AP (50 and 100 µM) prolonged APD_{90} and ERP in the "remodeled" atrial preparations (Fig. 4).

4-AP at concentrations of 10–25 µM was not able to prevent or terminate AF and at a concentration of 50 µM exerted only mild antiarrhythmic actions in both experimental AF models used in our study (Table II and III). Higher concentrations of 4-AP (100–200 µM) were more effective in preventing AF initiation and termination of persistent AF, but still showed limited antiarrhythmic efficacy in our experimental AF models (Table II and III).

DISCUSSION

The main findings of our study are that block of I_{Kur} with low concentrations of 4-AP abbreviates APD_{90} and ERP and promotes AF in "healthy" canine coronary-perfused atria.

Ion channel specificity of 4-AP

Within the range of concentrations used in the present study to block I_{Kur} (10–50 µM), 4-AP is thought to exert little or no inhibition of I_{to} and not to affect other currents.[2, 3] When measured in isolated atrial myocytes, 4-AP blocks I_{Kur} and I_{to} with an IC_{50} of 5 and 471 µM in dogs (potency ratio = 94),[2] and 8 and 1000 µM in human (potency ratio = 125).[3] Apparently, all I_{Kur} blockers inhibit I_{to}. The I_{to}/I_{Kur} blocking potency ratios for AVE0118, AVE1231, DPO-1, AZD7009, RSD1235, and NIP-141 are 3.1, 1.6, 8.0, 0.9, 2.3, and 3.1, respectively (data in most cases obtained from heterologous expression systems such as CHO or HEK cells).[12–17] Apart from I_{to}, most of these I_{Kur} blockers also inhibit $I_{K(ACh)}$, and/or I_{Kr}, and/or I_{Na} with affinities comparable to that of I_{Kur} block. Thus, 4-AP at low concentrations is a more selective I_{Kur} blocker than the above-listed agents.

In mathematical models of canine or human "healthy" atrial action potential, 80–90% percent block of I_{Kur} results in about 20–25% reduction of phase 1 magnitude.[6, 18, 19] The large reduction of the phase 1magnitude induced by 50 µM of 4-AP (by 71%; see Fig. 1) suggests that in the coronary-perfused canine atria 50 µM 4-AP may also block a substantial amount of I_{to}. A relatively selective I_{Kur} inhibition appears to be achieved with ≤25 µM of 4-AP (which causes a 43% reduction in phase 1magnitude). It is noteworthy that the pro-arrhythmic potency of 25 and 50 µM 4-AP was comparable (Table I).

Atrial-selective approaches for management of AF

A number of antiarrhythmic agents have been shown to be effective in terminating and/or preventing AF in the clinic. Most of these agents have as a primary action the ability to reduce I_{Na} (e.g., propafenone and flecainide) or I_{Kr} (e.g., dofetilide) or to block multiple ion channels (e.g., amiodarone). An important limitation of these antiarrhythmic agents is their potential ventricular pro-arrhythmic actions and/or organ toxicity at therapeutically effective doses.[20, 21]

As a consequence, much of the focus in recent years has been on development of atrial-selective drugs that take advantage of the presence of I_{Kur} channels in atrial but not in ventricular cells.[1–3] A number of studies have demonstrated that agents capable of blocking I_{Kur} (AVE0118, AVE1231, S9947, S20951, ISQ1, DPO-1, RSD1235; AZD7009; NIP141, NIP-142) selectively prolongs atrial ERP both in electrically-remodeled and non-remodeled atria *in vivo* and *in vitro*.[13, 22–2417, 25–27]

It is well appreciated that the effect of I_{Kur} inhibition on $APD_{70–90}$ is dependent on the baseline AP morphology.[6] I_{Kur} block has been reported to prolong $APD_{70–90}$ in atrial cells displaying a triangular AP morphology (typical for remodeled atria) and abbreviate $APD_{70–90}$ in atrial cells having a plateau AP morphology (typical for non-remodeled atria)[6] (as observed in the current study as well). Such APD_{90} abbreviation is explained by elevation of AP plateau voltage (see fig. 1), which leads to augmentation of I_{Kr} and I_{Ks}.[5, 6] I_{Kur} block-induced ERP prolongation in electrically-remodeled atria is consistent with $APD_{70–90}$ prolongation. On the other hand, I_{Kur} block-induced ERP prolongation in non-remodeled atria is not readily explained by $APD_{70–90}$ abbreviation,[5, 6] and may be attributable to concomitant block of sodium channel current. I_{Na} blockers are known for their ability to prolong ERP without prolonging APD. This effect of I_{Na} is due to the development of post-repolarization refractoriness (PRR), secondary to the depression of excitability.[10, 28] Atrial-selective ERP prolongation attributable to sodium channel blockade, has been reported secondary to development of PRR preferentially in atria.[10, 29]

Interestingly, atrial selective agents AZD7009 and RSD1235 (or vernakalant) have been shown to potently block I_{Na}, along with I_{Kur}, I_{to}, and I_{Kr}.[16, 25, 26, 30] The atrial selectivity of these agents to prolong ERP is largely attributed to their ability to block I_{Kur}. However, AZD7009 also depresses sodium channel-dependent parameters (conduction velocity and diastolic threshold of excitation) in an atrial selective manner.[26] AZD7009 prolongs atrial ERP much more than APD_{90}, leading to capture failure; due to development of PRR.[30] These observations suggest that atrial-selective or predominant sodium channel block may contribute prominently to the atrial-selective ERP prolongation of AZD7009. ISQ-1 may also block I_{Na}, since this agent slows down conduction velocity in atria *in vivo*.[31] Note that with the exception of AZD7009,[26] comparison of the effects of I_{Kur} blockers on I_{Na} or sodium channel-dependent parameters in atria and ventricles has not been conducted.

There is an apparent contradiction between our data and those of others[6, 32] showing that 4-AP abbreviates atrial APD_{90} in healthy atrial preparations *in vitro* with data reported by Nattel et al[33] demonstrating that 4-AP at a plasma concentration range of 25–50 µM significantly

increases ERP in healthy canine atria *in vivo*. This discrepancy may be due to the fact that Nattel et al[33] recorded ERP at relatively rapid pacing rates (300–400 ms CLs), at which AP shape tends to become triangular. Under these conditions, 4-AP may prolong late repolarization and thus ERP. The *in vivo* vs. *in vitro* differences may be due to the effect of 4-AP on autonomic tone *in vivo*.[34]

Inhibition of I_{Kur} promotes non-sustained AF in healthy atria

Most experimental models of AF, as well as clinical cases of AF, are associated with an abbreviation of atrial repolarization.[7, 8, 35] It is therefore no surprise that abbreviation of APD_{90} and ERP by 4-AP in "healthy" atria promoted AF in our study. The extent of APD and ERP abbreviation induced by 4-AP is much smaller that that induced by ACh or electrical remodeling. This may account for the relatively low incidence of AF and its brief duration in our experiments. Our findings relative to the ability of I_{Kur} inhibition to promote AF are consistent with a recent report demonstrating an association of *KCNA5* loss-of-function mutations with familial AF.[4]

Can I_{Kur} block alone suppress AF?

At concentrations that terminate AF, I_{Kur} blockers relatively potently inhibit I_{to} and/or I_{KACh} (e.g., AVE0118 or NIP-142)[22, 27] and/or I_{Na} (e.g., AZD7009 and vernakalant).[16, 26, 36] Our experimental findings suggest that "pure" I_{Kur} block may be incapable of effectively suppressing AF. Note the IC_{50} of 4-AP block of atrial I_{to} is one third of that of ventricular I_{to}.[3, 33] If it is the case with the other I_{Kur}/I_{to} blockers, then I_{to} block may importantly contribute to the atrial-selectivity of I_{Kur} blockers.

A possible reason for the failure of "pure" I_{Kur} block to terminate AF in our experimental models may be the fact that I_{Kur} density is reduced at rapid activation rates,[37, 38] so that at rates encountered during AF I_{Kur} may already be greatly reduced without much left to block.

It is commonly believed that anti-AF actions of potassium channel blockers are mediated through the prolongation of APD_{90}/ERP. A recent mathematical modeling study suggests that I_{Kur} and/or I_{to} blockade may terminate AF by prolonging APD at the level of the plateau, but not the terminal phase of the action potential, leading to random tip meander and wavebreak, resulting in rotor termination.[39]

Study limitations

Our experiments were performed in isolated atria coronary-perfused with Tyrode's solution. The presence of autonomic influences and other factors present *in vivo* may modulate the effect of I_{Kur} inhibition, resulting in outcomes different from those observed in the present study. I_{Kur} is known to be modulated by β-and α-adrenergic sympathetic activity.[40] I_{Kur} blockers, including 4-AP, are known to increase atrial contractility (i.e., intracellular calcium activity), apparently secondary to elevation of plateau voltages and augmentation of I_{Ca} and reverse mode of sodium-calcium exchange.[41] It is not known whether these influences secondary to I_{Kur} block, or possibly 4-AP directly, contribute to the arrhythmogenesis observed. If due to I_{Kur} block, this effect should be relevant to all I_{Kur} blockers.

Our results obtained in "acutely remodeled" atrial preparations, lacking a number of principal electrical ($I_{Ca,L}$, I_{to}, I_{K1}, etc) and structural changes observed in chronically remodeled atria,[35] should be interpreted with due caution. In that there are important inter-atrial electrophysiological differences, the data obtained from the right atrium in the present study may not be directly applicable to the left atrium.

CONCLUSIONS

Our findings suggest that inhibition of I_{Kur} can create the substrate for development of AF in "healthy" canine atria, via an abbreviation of ERP. These observations advance our understanding of why *KCNA5* loss-of-function mutations are associated with the development of AF.[4]

ABBREVIATIONS LIST

AF, atrial fibrillation; AP, transmembrane action potential; APD, action potential duration; ERP, effective refractory period; I_{Kur}, ultra-rapid delayed rectified potassium current; I_{to}, transient outward potassium current; PES, programmed electrical stimulation; PRR, post-repolarization refractoriness; RMP, resting membrane potential.

ACKNOWLEDGMENTS

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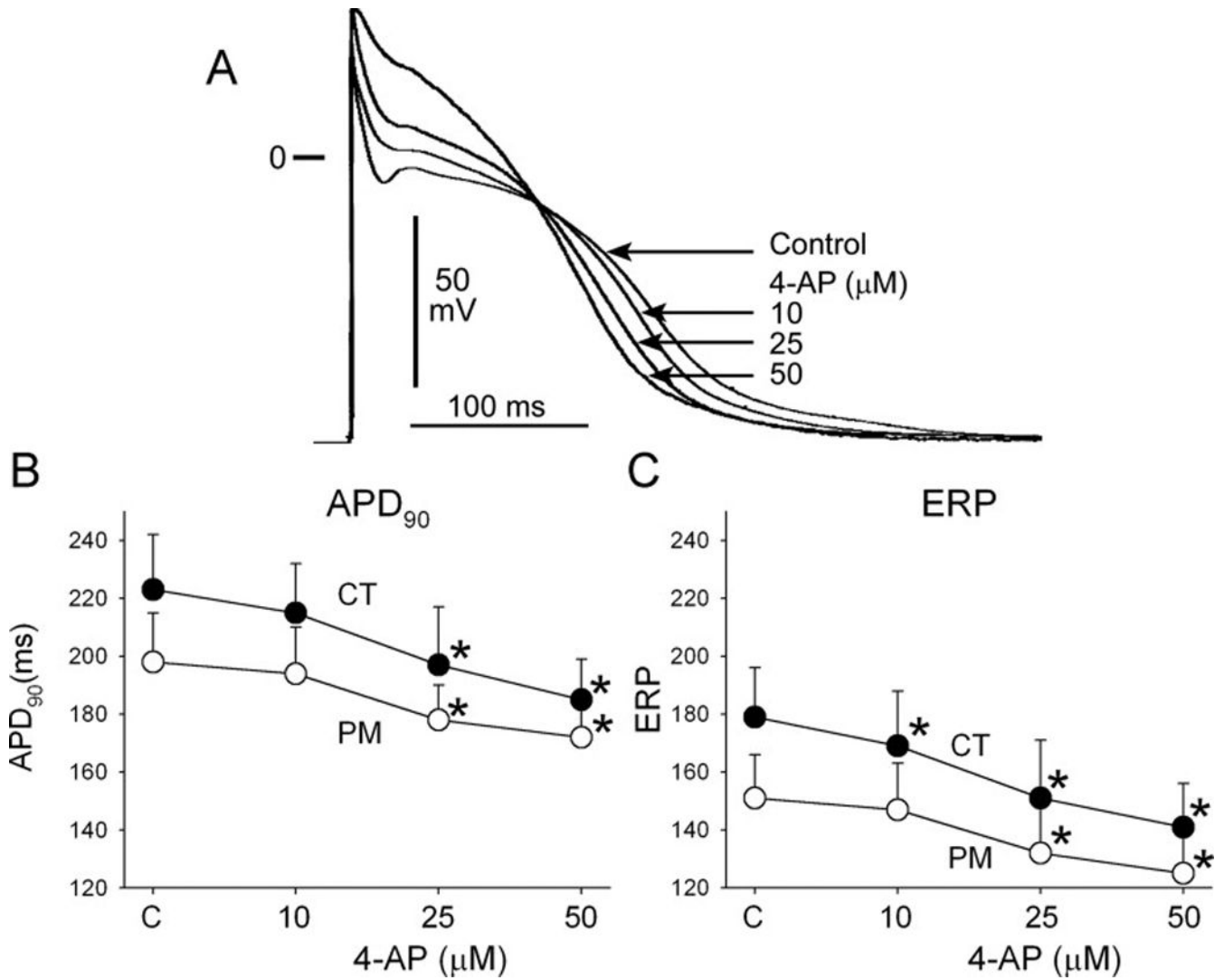
Figure 1.
Inhibition of I_{Kur} using low concentrations of 4-aminopyridne (4-AP) abbreviates APD_{90} and ERP in "healthy" atrial preparations. Shown are superimposed action potentials recorded from the crista terminalis (CT) under control conditions (C) and after the addition of 4-AP (**A**). Graphs plot summary data of APD_{90} (**B**) and ERP (**C**) recorded from CT and pectinate muscle (PM) sites as a function of 4-AP concentration. CL = 500 ms. n=7–12. * − p<0.05 vs. respective control. Note that atrial ERP corresponds to the level of APD_{75}.
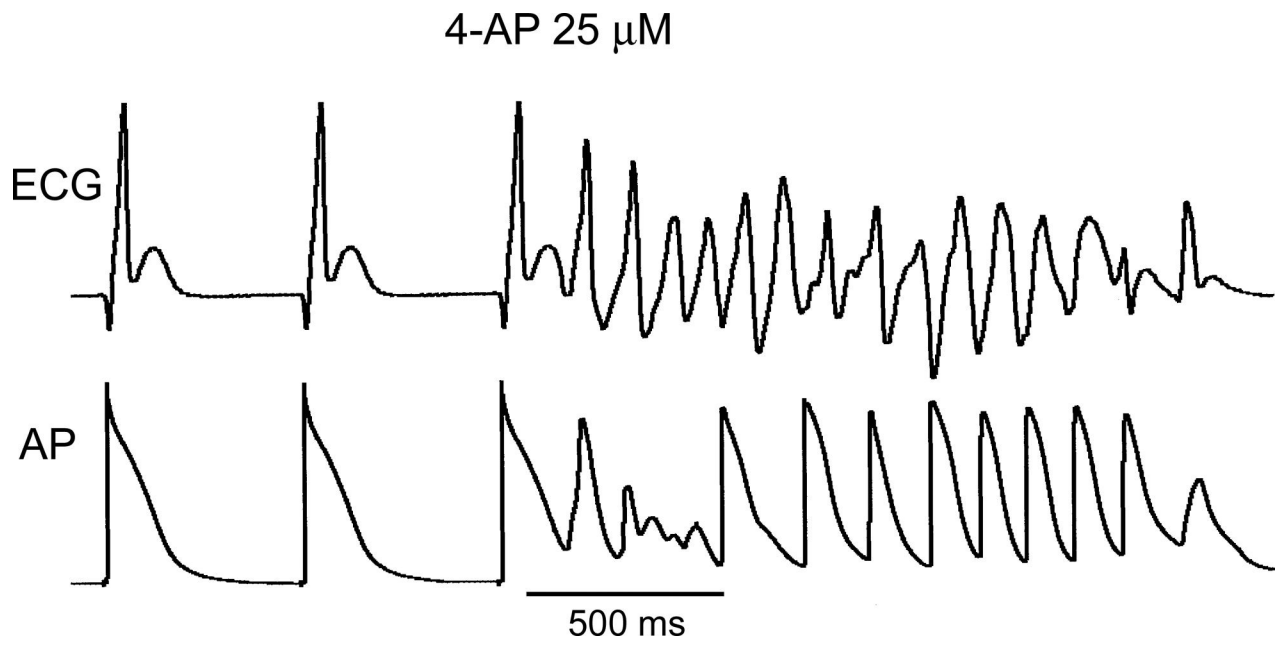
4-AP 25 µM

Figure 2.
Non-sustained AF induced by a single premature beat (S_1–S_2 = 115 ms) in the presence of 25 µM 4-AP in a "healthy" atrial preparation.
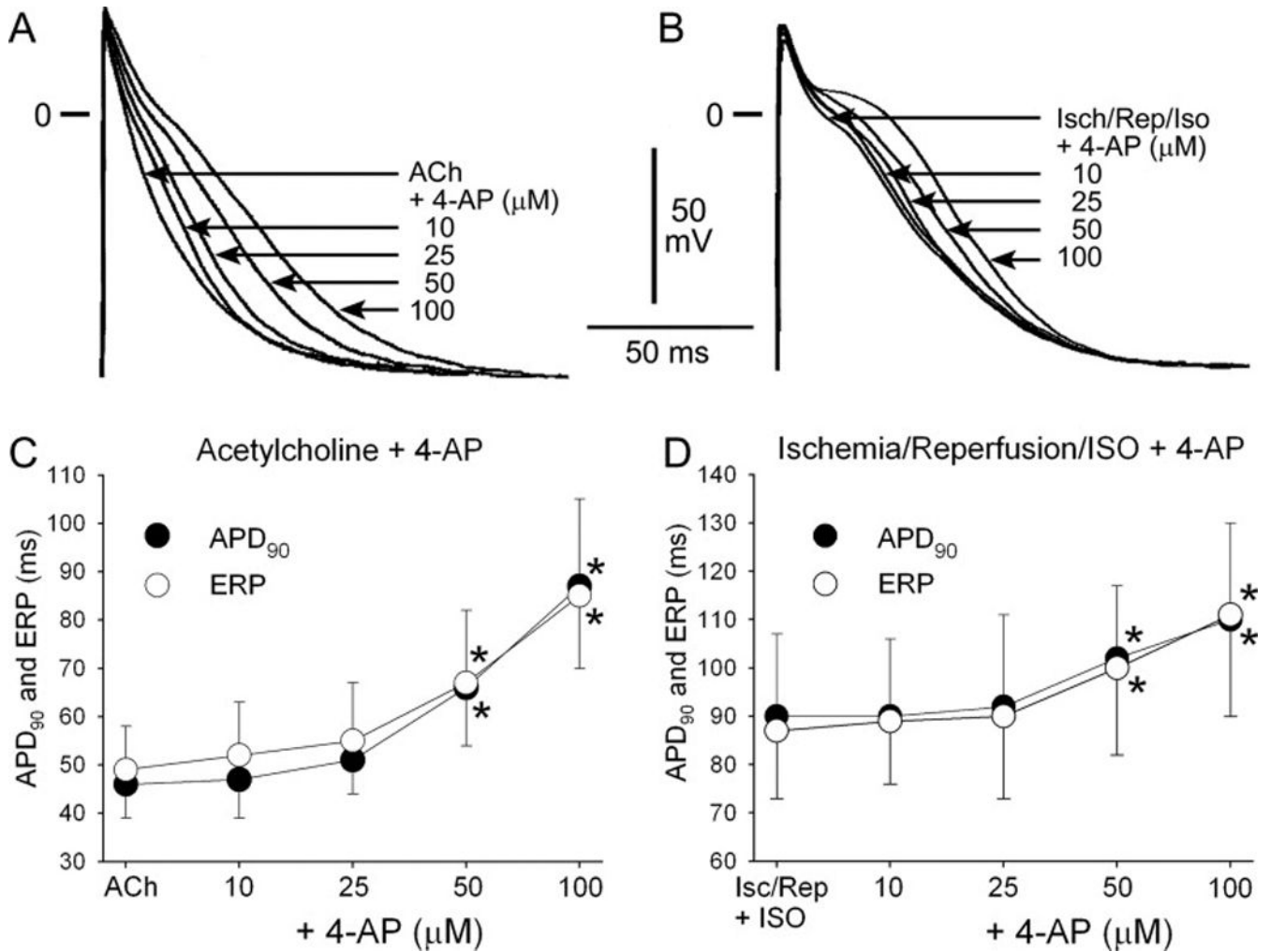
Figure 3.
Effects of 4-AP on APD_{90} and ERP in "acutely remodeled" atrial preparations. Shown are superimposed action potentials obtained from atria pre-treated with acetylcholine (ACh, **A**) and from atria exposed to ischemia and reperfusion + isoproterenol (Isch/Rep/Iso, **B**) before and after addition of 4-AP. **C–D**: Summary APD_{90} and ERP data from pectinate muscle. n=7–12. CL = 500 ms. * − p<0.05 vs. respective control.
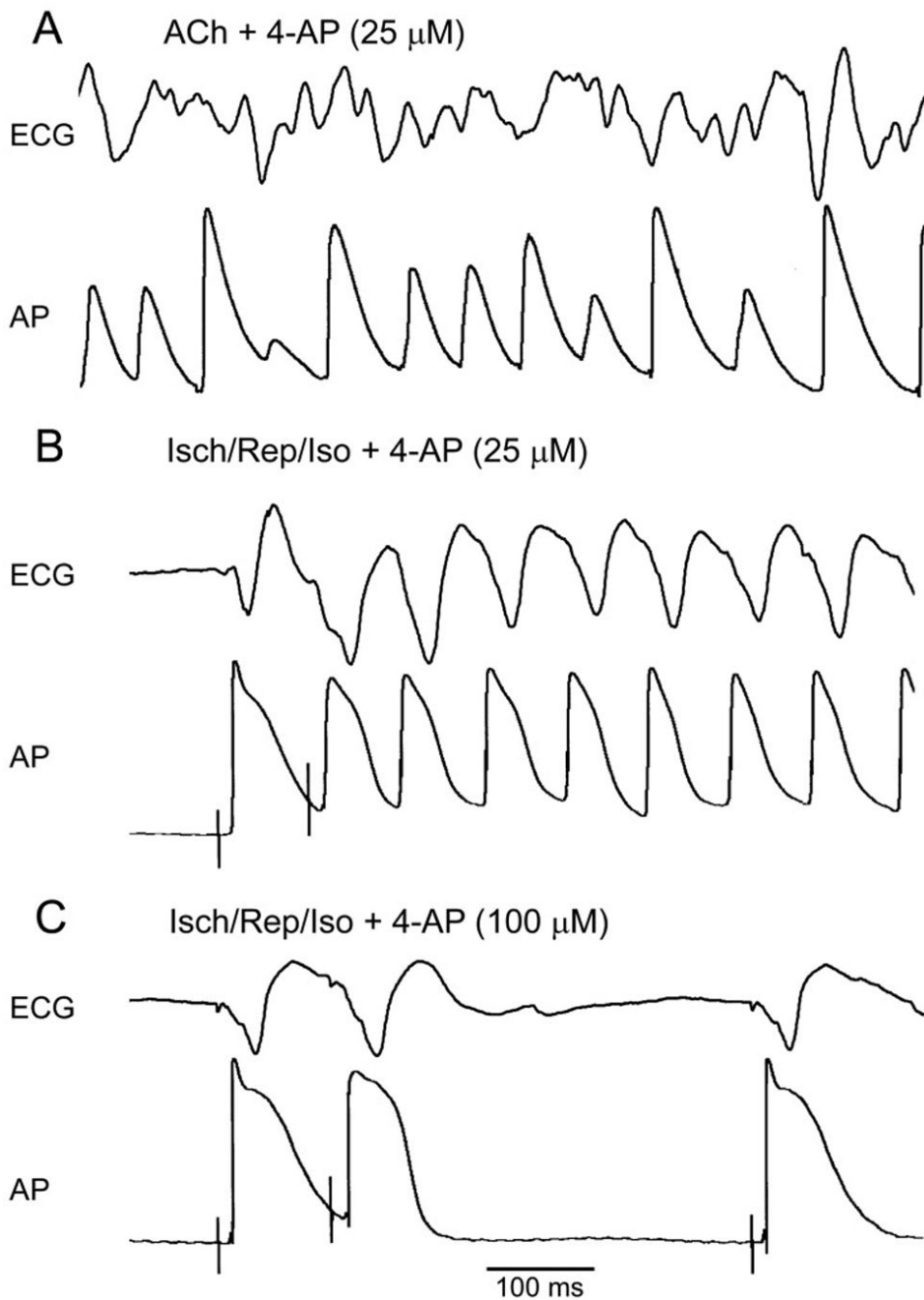
Figure 4.
Concentrations of 4-AP that selectively block I_{Kur} fail to prevent or terminate atrial fibrillation in "remodeled" atria. Shown are simultaneously recorded ECG and AP traces. **A**: 25 µM 4-AP fails to terminate persistent AF induced in the presence of acetylcholine (ACh, 0.5 µM). **B**: 25 µM 4-AP fails to prevent the induction of non-sustained AF by a single premature beat (S_1–S_2 = 85 ms, which is ERP) in ischemia/reperfusion+isoproterenol AF model (Isch/Rep/Iso). **C**: An increase of 4-AP concentration to 100 µM prolongs ERP and prevents the initiation of the arrhythmia by premature stimulation (S_1–S_2 = 110 ms, which is ERP).

Table I

Incidence of PES-induced non-sustained AF in "healthy" atrial preparations in the presence of low concentration of 4-AP.

Control	4-AP 10 μM	4-AP 25 μM	4-AP 50 μM
0/12 (0%)[*]	1/12 (8%)	3/12 (25%)	4/12 (33%)

[*] PES induced non-sustained arrhythmias (≤ 1 sec) in 2 out of 14 atrial preparations under control conditions. These two preparations were excluded from further study.

Table II

Incidence of acetylcholine (ACh) -mediated AF and the ability of 4-AP to prevent the initiation of AF or terminate persistent AF in atrial preparations.

	Ach (0.5 µM)	ACh + 4-AP (10–25 µM)	ACh + 4-AP (50 µM)	ACh + 4-AP (100–200 µM)
Incidence of AF initiation	100% (10/10)	100% (4/4)	75% (3/4)	50% (3/6)
Termination of persistent AF	0% (0/10)	0% (0/4)	0% (0/5)	33% (2/6)

Experiments to prevent AF and terminate AF were performed in different preparations. Incidence of AF initiation: 4-AP was added to ACh-containing Tyrode's solution and AF induction was attempted using PES. Termination of persistent AF: Persistent AF was induced in the exposure to Ach and 4-AP was added 5–7 min after the initiation of AF.

Table III

Incidence of PES-induced non-sustained AF with and without 4-AP in ischemia/reperfusion + isoproterenol model of AF in atrial preparations.

Ischemia/Reperfusion + isoproterenol	+ 4-AP (10–25 µM)	+ 4-AP (50 µM)	+ 4-AP (100–200 µM)
100% (5/5)	100% (5/5)	80% (4/5)	40% (2/5)