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Original Paper

Pharmacological Profile of the Bradycardic Agent Ivabradine on Human Cardiac Ion Channels

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Key Words
Ivabradine • S16257 • Human • Cardiac ion channels • Induced pluripotent stem cells

Abstract
Background/Aims: Ivabradine lowers the heart rate by inhibition of hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels mediating the 'funny' pacemaker current I_f in the sinoatrial node. It is clinically approved for the treatment of heart failure and angina pectoris. Due to its proposed high selectivity for I_f administration of ivabradine is not associated with the side effects commonly observed following the application of other heart rate lowering agents. Recent evidence, however, has shown significant affinity of ivabradine towards $K_v11.1$ (ether-a-go-go related gene, ERG) potassium channels. Despite the inhibition of $K_v11.1$ channels by ivabradine, cardiac action potential (AP) duration and heart rate corrected QT interval (QT_c) of the human electrocardiogram (ECG) were not prolonged. We thus surmised that compensatory mechanisms might counteract the drug's inhibitory action on $K_v11.1$. ***Methods:*** The effects of ivabradine on human $K_v11.1$ and $K_v7.1$ potassium, $Ca_v1.2$ calcium, and $Na_v1.5$ sodium channels, heterologously expressed in tsA-201 cells, were studied in the voltage-clamp mode of the whole cell patch clamp technique. In addition, changes in action potential parameters of human induced pluripotent stem cell (iPSC) derived cardiomyocytes upon application of ivabradine were studied with current-clamp experiments. ***Results:*** Here we show that ivabradine exhibits significant affinity towards cardiac ion channels other than HCN. We demonstrate for the first time inhibition of human voltage-gated $Na_v1.5$ sodium channels at therapeutically relevant concentrations. Within this study we also confirm recent findings of human $K_v11.1$ inhibition by low µM concentrations of ivabradine and observed no prolongation of ventricular-like APs in cardiomyocytes derived from iPSCs. ***Conclusion:*** Our results provide an explanation why ivabradine, despite its affinity for $K_v11.1$ channels, does not prolong the cardiac AP and QT_c interval. Furthermore, our results suggest the inhibition of voltage-gated $Na_v1.5$ sodium channels to underlie the recent observations of slowed

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atrioventricular conduction by increased atrial-His bundle intervals upon administration of ivabradine.

Introduction

The bradycardic agent ivabradine is clinically approved for the treatment of heart failure (HF) and stable angina pectoris [1, 2]. The heart-rate lowering action of ivabradine was proposed to rely on the inhibition of hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels [3–5]. HCN channels are strongly expressed in cells of the sinoatrial (SA) node and mediate the "funny" pacemaker current If. Inhibition of these channels slows diastolic depolarization and delays the generation of subsequent action potentials (APs).

In contrast to conventional treatment with beta blockers and calcium channel inhibitors, the administration of ivabradine is not associated with hypotension and negative inotropic side effects [1, 6,7]. This is attributed to the drug's high selectivity towards HCN channels. Consequently, the drug exhibits a good overall cardiac safety profile [8, 9]. In particular, application of ivabradine induced only negligible if any prolongation of the cardiac AP in various animal models [9–11] and did not alter the heart rate corrected QT interval (QT_c) of the human ECG [12]. Nevertheless, ivabradine was added to the list of drugs with an inherent risk for torsade-de-pointes (TdP) arrhythmias when combined with QT interval prolonging drugs, diuretics, or in the context of altered electrolyte levels [8]. Recently it was noted that ivabradine has the propensity to inhibit the rapid delayed rectifying potassium (K^+) current IK_r both in rabbit ventricular cardiomyocytes [10] and when respective $K_v11.1$ channels were studied in a heterologous expression system [9, 13]. The obtained IC_{50} values for current inhibition were comparable to the concentration range responsible for the action of ivabradine on HCN channels. Thus, ivabradine exhibits significant affinity towards $K_v11.1$ (ether-a-go-go-related gene channel, ERG) channels [8, 9,13], but does not per se prolong the cardiac AP [10, 11]).

Such failure of a drug to prolong the AP despite potent block of $K_v11.1$ could result from simultaneous compensatory interaction with other ion channels. Thus, multichannel blockers such as amiodarone, ranolazine and verapamil rarely cause TdP arrhythmias despite blocking $K_v11.1$ channels [14]. This prompted us to hypothesise that a compensatory mechanism could counteract the expected AP prolonging effect of $K_v11.1$ channel inhibition by ivabradine. One possibility to counteract $K_v11.1$ inhibition could be a potentiation of $K_v7.1$ channels, which mediate the K^+ current IK_s. The effect of ivabradine on these channels, which provide the second major conductance driving late repolarisation, however, haven't been studied as yet. Notably, apart from the inhibition of $K_v11.1$ channels by ivabradine, none of the mentioned evidence was derived from human tissue or human channel isoforms. Inhibition of voltage-gated sodium (Na^+) and/or calcium (Ca^{2+}) channels represents another potential compensatory mechanism, which would lead to a shortening of the cardiac AP. Some evidence in this regard has emerged from previous studies on animal models. In cells from rabbit SA node T-type calcium channels were not affected, but L-type calcium channels were inhibited by about 20% in the presence of 10 µM ivabradine [3]. In dog cardiac Purkinje cells and papillary muscle from guinea pig it was noted that maximal AP upstroke velocity (V_{max}) was significantly slowed in the presence of 1 and 10 µM ivabradine [10, 11], suggesting an inhibition of voltage-gated sodium channels by the drug in these preparations.

The notion that ivabradine may inhibit depolarizing currents is also supported by the finding that AV-nodal conduction is slowed by ivabradine alone [15] or in combination with the sodium channel blocker ranolazine [16].

As a matter of fact there is growing interest in the potential use of ivabradine as a rate-controlling agent in atrial fibrillation. Hence the exploration of the molecular underpinnings of the effect of ivabradine on AV-nodal conduction may be of substantial clinical relevance [17–21]. Although in silico studies suggest that block of HCN channels per se may result in slowing of AV-nodal conduction [22, 23], it has been suggested that this effect of ivabradine

may result from additional block of voltage-gated Ca^{2+} and/or K^+ channels [24]. In addition to voltage-gated Ca^{2+} channels, voltage-gated Na^+ channels may also contribute to AV-nodal conduction (see below). Therefore, we explored the effect of ivabradine both on voltage-gated Ca^{2+} and on voltage-gated Na^+ channels.

In summary, both the lack of increase of AP duration by ivabradine despite significant IK_r blocking properties, and the slowing of AV-nodal conduction could be a result of block of either voltage-gated calcium or sodium channels. Within the present study we therefore investigated the effect of ivabradine on human voltage-gated ion channels responsible for shaping cardiac repolarisation including human $K_V11.1$ (hERG), $K_v7.1$ (mediating IK_s current), $Ca_v1.2$ (L-type calcium current), and $Na_v1.5$. In addition, we studied the effect of ivabradine on APs using cardiomyocytes derived from human induced pluripotent stem cells.

We show that ivabradine exhibits significant affinity towards cardiac ion channels other than HCN. In particular, we demonstrate a pronounced inhibition of human voltage-gated $Na_v1.5$ sodium channels in the presence of low μM concentrations of ivabradine.

Materials and Methods

Cell lines and culturing

Heterologous expression was performed in tsA-201 cells, a transformed human embryonic kidney 293 (HEK293) cell line expressing an SV40 T-antigen, known for its efficient expression of recombinant proteins. As with HEK293 cells, tsA-201 cells do not express endogenous sodium or calcium currents and only a very small component of outward K^+ currents at depolarised potentials. tsA-201 cells (American Type Culture Collection, Manassas, VA) were propagated in Dulbecco's modified Eagle's medium (Invitrogen, Vienna, Austria) containing 10% fetal bovine serum and incubated at 37°C in a humidified incubator with 5% CO2. Human induced pluripotent stem cell derived cardiomyocytes (hiPS-CM) were purchased from Cellular Dynamics, International (USA), and cultured according to the manufacturer's protocol.

Heterologous transfection

For expression of human $K_v11.1$ (hERG) channels, cells were transfected with pcDNA3 plasmid containing the canonical coding sequence for the human cardiac ERG K^+ channel (Kcnh2, $K_v11.1$, UniProt Q12809) (0.7 μg/3.5 cm dish). Co-transfection with pEGFP-C1-plasmid (0.02 μg) encoding green fluorescent protein allowed the identification of successfully transfected cells. For expression of $K_v7.1$ channels, cells were transfected with pcDNA3 plasmid containing the canonical coding sequence for the human $K_v7.1$ (KCNQ1) and the accessory human subunit encoded by human KCNE1 at a 1:1 stoichiometry. For expression of $Ca_v1.2$ channels, the pore forming human alpha subunit $hCa_v1.2$ α1 (77-pcDNA3) was co-expressed together with the auxiliary subunits cloned from rat, $Ca_vβ3$ and $Ca_vα2δ1$, as previously described [25]. For expression of $Na_v1.5$ channels, cells were transfected with 1.6 μg/3.5 cm dish of pGEM3-plasmid containing the coding sequence for a GFP-tagged human cardiac $Na_v1.5$.

Patch Clamp Technique

Ionic currents were recorded from tsA-201 cells 24–48 hours after transfection at room temperature (22 +- 2°C) using an Axoclamp 200B patch-clamp amplifier (Axon Instruments, Union City, CA). Pipettes were formed from aluminosilicate glass (A120-77-10; Science Products, Hofheim, Germany) with a P-97 horizontal puller (Sutter Instruments, Novato, CA) and had resistances between 1 and 2 MΩ when filled with the respective pipette solutions. Data acquisition was performed with pClamp 11.0 software (Axon Instruments) through a 16-bit A-D/D-A interface (Digidata 1440; Axon Instruments). Data were analysed with Clampfit 10.2 (Axon Instruments) and Prism 5.01 (GraphPad Software, San Diego, CA) software. Rapid solution exchange was performed by a DAD-8-VC superfusion system (ALA Scientific Instruments, Westbury, NY). If not stated otherwise all recordings were obtained in the whole cell configuration of the patch-clamp technique. However, to prevent run-down of K_v7 currents [26] the perforated patch clamp technique was performed. To this end, glass capillaries were front filled by dipping them into pipette solution for a few seconds. Thereafter the same capillaries were backfilled with the same solution including 200 μg ml^{-1} amphotericin B (in 0.8% DMSO). Currents were recorded after 20–30 minutes when series resistance had

stabilised below 12 MOhm. Action potentials (APs) were recorded from ''ventricular-like'' hiPS-CM in the current-clamp mode of the whole cell patch clamp technique as in our previous study [27] using both an Axoclamp 200B as well as an Axoclamp 700B amplifier. APs were elicited at 0.5 Hz by rectangular current pulses of 4 ms duration at 125 % threshold level. Cardiomyocytes were classified as ventricular-like if their APs showed a distinct ''shoulder'' (plateau or flat repolarization phase) prior to a final steep phase of repolarization. As a shoulder we classified cells that showed a depolarisation above 0 mV for more than 100 ms and which exhibited a "clear" plateau, i.e. a time period of at least 50 ms in duration with less than an overall drop of 20 mV in membrane potential. Although we have not determined the distribution of such ventricular-like APs among the entire population of patched cells, we estimate the percentage of ventricular-like cells to be at least 20%.

Electrophysiological solutions

Solutions to record K^+ currents; pipette solution contained 130 mM KCl, 5 mM MgCl2, 5 mM K2-ATP, 5 mM EGTA, and 10 mM HEPES (pH 5 7.2, KOH), and bath solution consisted of 137 mM NaCl, 4 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 10 mM glucose, and 10 mM HEPES (pH 5 7.4, NaOH). A pH of 6.8 and 8.2 was adjusted with HCl and NaOH, respectively. Barium was used as charge carrier in experiments to measure $Ca_v1.2$ calcium channels. Formulation of the recording solutions were as follows, pipette solution: 145 mM Cs-aspartate, 2 mM MgCl2, 10 mM HEPES, 0.1 mM Cs-EGTA, 2 mM Mg-ATP, pH = 7.4 adjusted with CsOH, and bath solution: 10 mM BaCl2, 145 mM TEA-Cl, 10 mM HEPES, pH = 7.4 adjusted with TEA-OH. To measure action potentials the pipette solution contained 10 mM NaCl, 140 mM KCl, 2 mM EGTA, 1 mM MgCl2, 0.1 mM Na-GTP, 5 mM Mg-ATP, 10 mM Hepes, and pH = 7.2 adjusted with KOH, while the cells were bathed in 140 mM NaCl, 4 mM KCl, 2 mM CaCl2, 2 mM MgCl2, 5 mM HEPES, 5 mM glucose, and pH = 7.4 adjusted with NaOH.

To measure sodium currents the pipette solution contained 105 mM CsF, 10 mM NaCl, 10 mM EGTA, 10 mM HEPES, pH = 7.3 adjusted with CsOH. Recordings of hNav1.5 sodium channels expressed in tsA-201 cells were made in a bath solution that consisted of 140 mM NaCl, 2.5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 10 mM HEPES, pH = 7.4 adjusted with NaOH.

Ivabradine

Ivabradine was purchased from Sigma Aldrich (SML0281). The drug is the S-stereoisomer, (+)-S16257, of the parent racemate, (+-)-S15544. Ivabradine was dissolved in dimethyl sulfoxide at a stock concentration of 100 mM and stored in aliquots at -20°C. All solutions were prepared freshly on the day of experiment by diluting the ivabradine stock to the respective concentrations as given within this study.

Curve fitting

Normalized concentration response relations were fit with a Hill equation: Inorm = Bottom + (Top-Bottom)/(1+ (IC_{50}/[iva])^n_H). Inorm is the normalised current during drug exposure in relation to the current during drug-free conditions, Bottom and Top refer to the minimal and maximal current level, IC_{50} is the concentration at 50% current inhibition, and [iva] is the concentration of ivabradine. The Hill slope n_H was set to 1, assuming a 1:1 binding stoichiometry.

Results

Ivabradine inhibits human $K_v11.1$ channels at low µM concentrations

Recently, inhibition of delayed rectifying potassium current IK_r was reported in rabbit ventricular myocytes with low µM concentrations of ivabradine [10]; its molecular correlate, the ether-a-go-go-related gene channel (ERG, $K_v11.1$), was inhibited with similar potency [9, 13]. Within our aim to determine the effects of ivabradine on major human ventricular voltage-gated ion channels, we first repeated to test for the effect of ivabradine on human $K_v11.1$ channels (Fig. 1). To this end we expressed human $K_v11.1$ channels in tsA-201 cells. Potassium currents through these channels were activated from a holding potential of -80 mV by rectangular voltage steps and subsequently deactivated by a step to -50 mV (Fig. 1a, b). The effect of ivabradine was then tested for different concentrations on the tail current

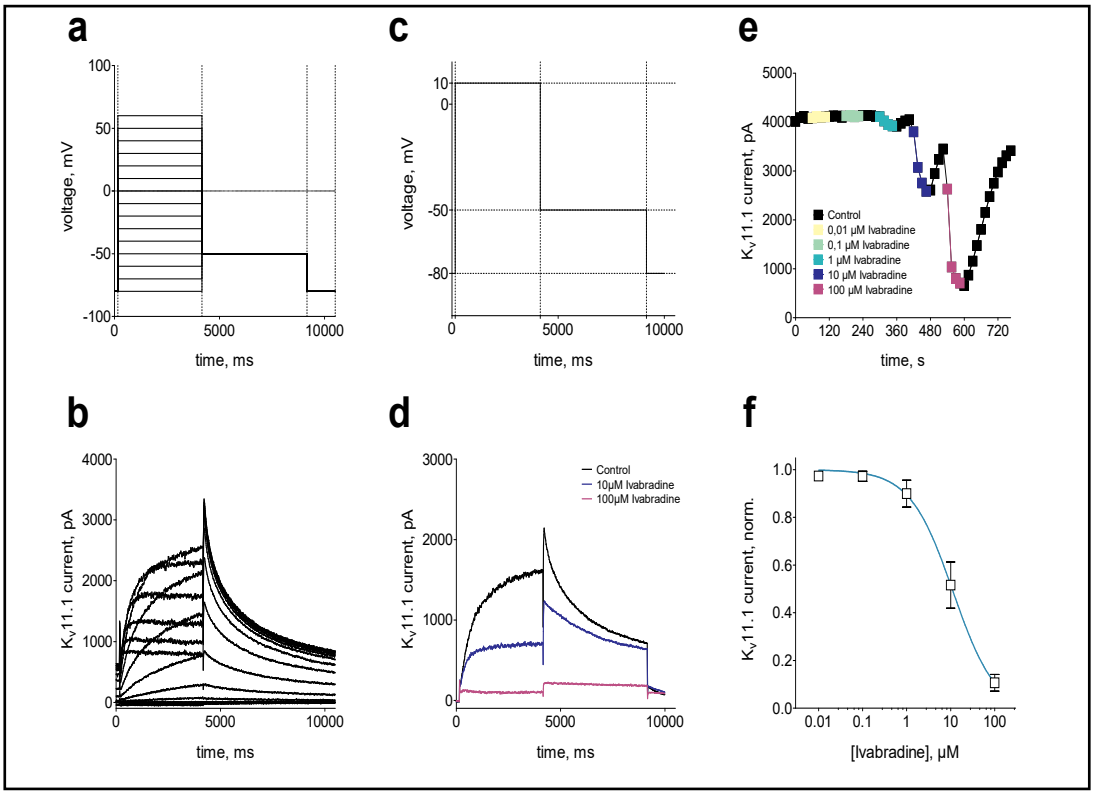

Fig. 1. Effect of ivabradine on heterologously expressed human K_v11.1 channels. (a) Voltage-clamp protocol to derive current voltage relationships. (b) Typical outward potassium currents as elicited by the voltage-clamp protocol shown in (a) for recombinant K_v11.1 channels expressed in tsA-201 cells. (c) Two-step voltage-clamp protocol (+10 mV, -50 mV) applied every 15s to first activate and then deactivate K_v11.1 channels. (d) Typical outward potassium currents for the voltage-clamp protocol shown in (c) under control conditions and in the presence of 10 and 100 µM of ivabradine. The peak of tail currents at -50 mV was monitored to test for the effect of different ivabradine concentrations. (e) K_v11.1 tail current amplitude as elicited by the voltage-clamp protocol in (c, d) under control conditions and in the presence of ascending concentrations of ivabradine. (f) Summary of normalized steady-state K_v11.1 current amplitudes (mean +- SEM, n = 7) for all ivabradine concentrations tested. A Non-linear fit with a Hill equation (petrol) resulted in an IC_{50} value of 11+-2 µM.

amplitudes measured at -50 mV after maximal activation at +10 mV (Fig. 1c, d). Wash-in of ivabradine resulted in visible inhibition of the tail current for concentrations exceeding 0.1 µM and in a maximal inhibition at about 100 µM (Fig. 1e). A summary of relative K_v11.1 tail current inhibition for all tested ivabradine concentrations is shown in Fig. 1f and revealed an IC_{50} of 11 +- 2 µM. These results confirm previous findings in that ivabradine inhibits K_v11.1 potassium current at low µM concentrations.

Human K_v7.1 channels are not affected by ivabradine

Human ventricular repolarisation is dominated by the efflux of potassium through K_v11.1 and K_v7.1 channels, mediating the fast and slow rectifying potassium currents, IK_r and IK_s, respectively. Having determined the effect of ivabradine on K_v11.1, we next wanted to investigate the drug's effect on human K_v7.1 channels. To this end we heterologously co-expressed the pore-forming KCNQ1 subunit together with KCNE1, mimicking the suggested in situ channel complex. A two step voltage-clamp protocol was used to activate K_v7.1 channels and elicit respective potassium outward currents (Fig. 2a, b). The effect of ivabradine was tested on peak K_v7.1 current amplitudes elicited at +20 mV (Fig. 2c, d). As can be seen from Fig. 2e, none of the employed ivabradine concentrations affected currents through K_v7.1. A

summary of all recordings revelead no significant effect of $K_v7.1$ up to 100 µM, the highest concentration tested.

Partial inhibition of human $Ca_v1.2$ calcium channels by ivabradine

Previous reports [9, 13] and the results of the present work (Fig. 1) demonstrated substantial inhibition of $K_v11.1$ by ivabradine at low µM concentrations. Inhibiton of $K_v11.1$ channels is associated with a prolongation of the human ventricular AP and an increase of the QT interval in the ECG. However, in animal models no or only a very modest prolongation of the AP was observed when challenged with ivabradine concentrations of up to 10 µM [9–11]. This prompted us to hypothesise that inhibition of calcium channels may compensate for the inhibition of $K_v11.1$ and restore AP duration in the presence of ivabradine. In SA cells derived from rabbit hearts, ivabradine indeed inhibited L-type Ca^{2+} currents by about 20% at 10 µM [3]. Therefore we asked if $Ca_v1.2$, the predominant Ca^{2+}-channel expressed in the human ventricle, would be affected by ivabradine.

To this end we co-expressed the pore forming alpha1C-subunit together with the auxiliary beta and alpha2delta-subunit heterologously in tsA-201 cells. Inward currents through these channels were activated by depolarising voltage-steps from a resting potential of -80 mV (Fig. 3a, b). To test for the effect of ivabradine, currents through $Ca_v1.2$ channels

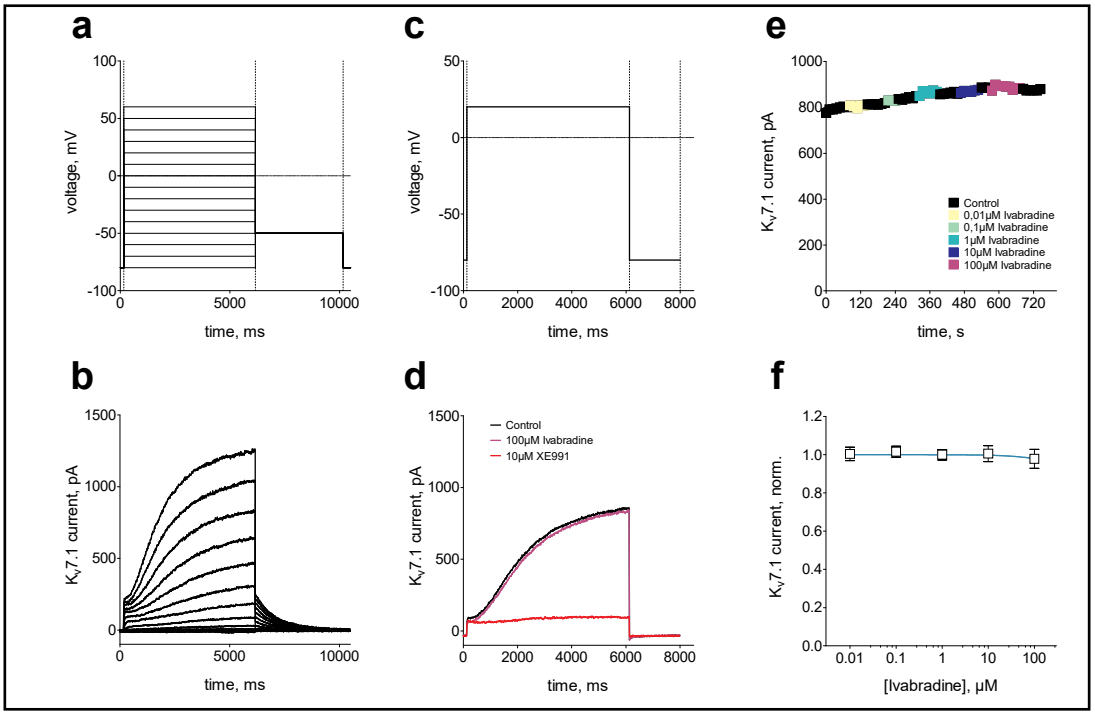

Fig. 2. Effect of ivabradine on heterologously expressed human $K_v7.1$ channels. (a) Voltage-clamp protocol to derive current voltage relationships. (b) Typical outward potassium currents as elicited by the voltage-clamp protocol shown in (a) for recombinant $K_v7.1$ channels expressed in tsA-201 cells. (c) A voltage-step to +20 mV was applied every 12 s to activate $K_v7.1$ channels. (d) Typical outward potassium currents for the voltage-clamp protocol shown in (c) under control conditions and in the presence of 10 and 100 µM of ivabradine. The peak of $K_v7.1$ current amplitude at the end of the +20 mV voltage-step was monitored to test for the effect of different ivabradine concentrations. A saturating concentration (10 µM) of XE-991, a selective inhbitor of $K_v7.1$ channels, was applied at the end in some experiments. (e) $K_v7.1$ current amplitude as elicited by the voltage-clamp protocol in (c, d) under control conditions and in the presence of ascending concentrations of ivabradine. (f) Summary of normalized steady-state $K_v7.1$ current amplitudes (mean +-SEM, n = 7) for all ivabradine concentrations tested. No significant current inhibitions was observed for ivabradine concentrations up to 100 µM.

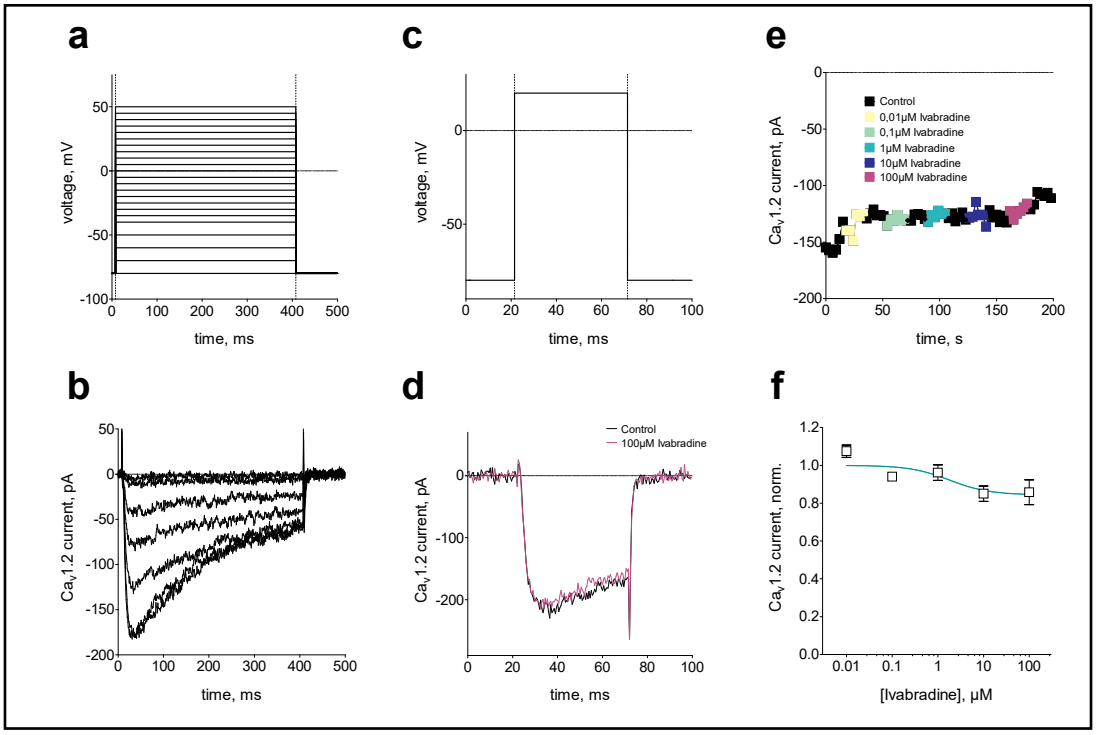

Fig. 3. Effect of ivabradine on heterologously expressed human $Ca_v1.2$ channels. (a) Voltage-clamp protocol to derive current voltage relationships. (b) Typical inward currents through recombinant $Ca_v1.2$ channels expressed in tsA-201 cells as elicited by the voltage-clamp protocol shown in (a) for selected voltage steps (-25, -15, -5, 0, 5, 10, and 15 mV). Barium was used as charge carrier (see Methods). (c) Voltage clamp protocol to elicit maximal calcium current amplitude was applied every 3 s. (d) Typical calcium current for the voltage-clamp protocol shown in (c) under control conditions and in the presence of 100 µM of ivabradine. (e) Maximal $Ca_v1.2$ current amplitude as elicited by the voltage-step to +20 mV (c, d) under control conditions and in the presence of ascending concentrations of ivabradine. (f) Summary of normalized steady-state $Ca_v1.2$ current amplitudes (mean +- SEM, n = 13-15) for all ivabradine concentrations tested. A Non-linear fit with a Hill equation (line) was statistically not significant using and extra sum of square F-test.

were repetitively activated by a voltage-step to +20 mV (Fig. 3c, d). Only a trend of inhibition could be observed upon application of ivabradine (Fig. 3e, f), which, however, was not statistically significant. Thus, under our experimental conditions human $Ca_v1.2$ calcium channels were not inhibited by ivabradine.

Ivabradine inhibits human $Na_v1.5$ channels at low µM concentrations

Previous reports in dog Purkinje fibres and guinea pig ventricular papillary muscle found a decrease in V_{max}, the action potential upstroke velocity, upon application of ivabradine [10, 11]. We therefore wanted to test the effect of ivabradine on human $Na_v1.5$ sodium channels.

Human $Na_v1.5$ channels were heterologously expressed in tsA-201 cells. Sodium currents through these channels were activated from a holding potential of -100 mV by rectangular voltage-steps of 25 ms duration (Fig. 4a, b). To test for the effect of ivabradine, sodium currents were repetitively activated at a rate of 1 Hz by a 25 ms voltage-step to -10 mV (Fig. 4c, d). Maximal current amplitude was monitored over time and ascending concentrations of ivabradine were applied for 2 minutes each (Fig. 4e). $Na_v1.5$ sodium currents were significantly inhibited by ivabradine. The half inhibitory conentration amounted to IC_{50} = 30 +- 3 µM (mean +- SEM, n = 3-13; Fig. 4f). The observed ivabradine block was not affected by a potential systematic current rundown (Supplementary Fig. 1a), but washout of 100 µM of the drug appeared slow and irreversible to some degree (Supplementary Fig. 1b-d - for all supplemental material see www.cellphysiolbiochem.com).

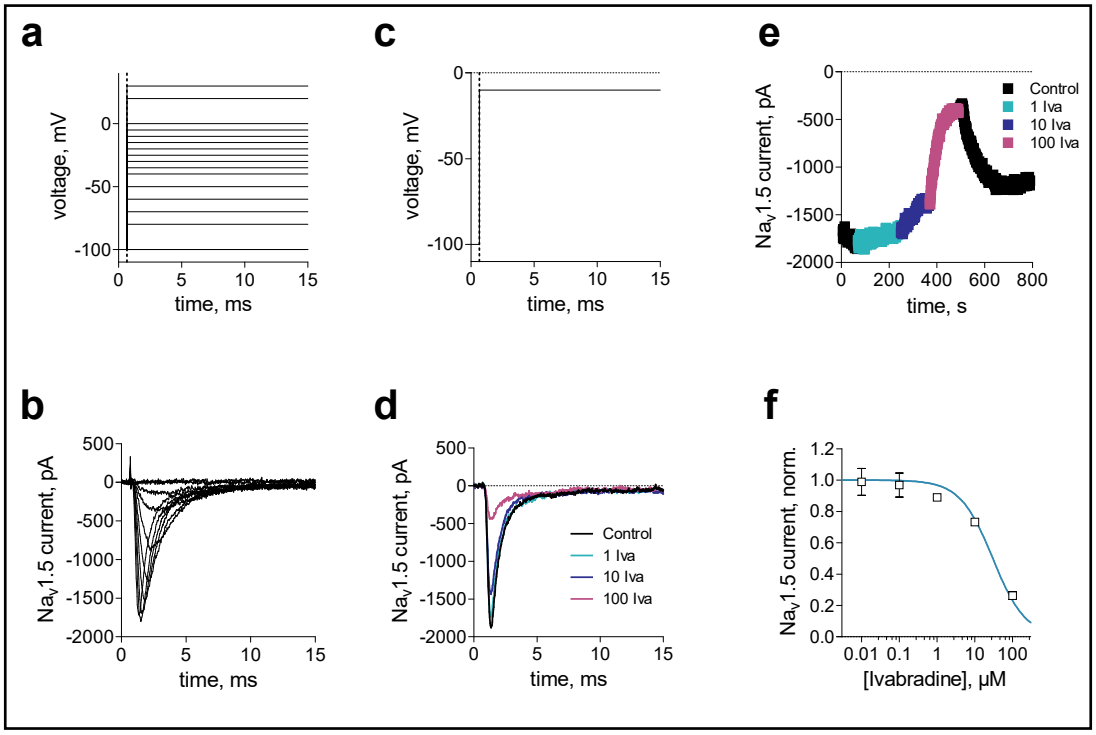

Fig. 4. Effect of ivabradine on heterologously expressed human Na$_v$1.5 channels. (a) Voltage-clamp protocol to obtain current voltage relationships. (b) Typical inward currents through recombinant Na$_v$1.5 channels expressed in tsA-201 cells as elicited by the voltage-clamp protocol shown in (a) for selected voltage steps (-60,-50,-40,-35,-30,-25, -20,-15, -10, and 0 mV). (c) Voltage clamp protocol to elicit maximal sodium current amplitude. (d) Typical sodium current for the voltage-clamp protocol shown in (c) under control conditions and in the presence of 1, 10, and 100 µM of ivabradine. (e) Maximal Na$_v$1.5 current amplitude as elicited repetitively at 1 Hz by a voltage-step to -10 mV as shown in (c, d) under control conditions and in the presence of ivabradine. (f) Summary of normalized steady-state Na$_v$1.5 current amplitudes for all ivabradine concentrations tested. The line represents a non-linear fit with a Hill equation.

Ivabradine does not prolong the human cardiac action potential

Recent studies reported no or only very minor changes in the AP duration upon application of ivabradine in various animal models [9–11], in a human right ventricular papillary muscle preparation [10], and in human induced pluripotent stem cells (iPSC)-derived engineered heart tissue [28].

Here we wanted to test the synergistic effect of sodium, calcium and potassium channel inhibition on a human model of the cardiac AP. To this end we used single cardiomyocytes derived from iPSCs. Action potentials were elicited by a 4 ms current injection at a rate of 1 Hz. Only cells with 'ventricular-like' APs were selected for experiments. After the AP had stabilized ivabradine was applied at concentrations of 1 and 10 µM for 1 min each (Fig. 5a). No change in action potential duration measured at 90% repolarization was observed (APD90, Fig. 5b). However, in accordance with the inhibition of Nav1.5 by ivabradine, we observed a reduction in the AP depolarisation level (Fig. 5c) and a slowing of the AP upstroke velocity (Fig. 5d).

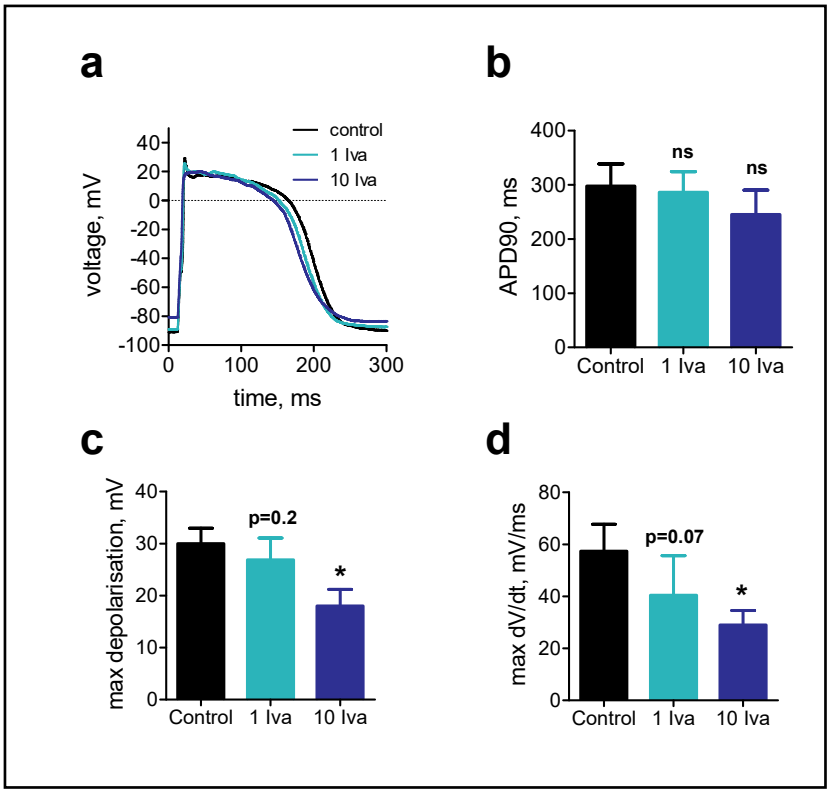

Fig. 5. Effect of ivabradine on human APs from iPSC derived cardiomyocytes. (a) Example of ventricular-like action potentials as obtained from current clamp measurements in iPS cell derived human cardiomyocytes under control conditions and after 1 minute equilibration with either 1 or 10 µM of ivabradine (1 Iva, and 10 Iva, respectively). (b) A summary of the AP duration measured at 90% repolarisation (APD90) as derived from experiments shown in (a). (c) Summary of the maximal depolarisation value reached by the AP. (d) Summary of the maximal upstroke velocity as derived by taking the maximum of the first time derivative, max dV/dt. All data are displayed as mean +- SEM (n = 8 and 7, for 1 and 10 Iva, respectively). Statistical significance for 1 and 10 Iva versus control was tested with a paired Student's t-test; ns, not significant.

Discussion

Within the present work we show significant inhibitory action of ivabradine on human cardiac ion channels other than HCN. We could confirm recent findings of the inhibition of human K_v11.1 (hERG) channels by low µM concentrations of ivabradine (Fig. 1). In order to find an explanation for the fact that, despite inhibition of K_v11.1 channels, AP duration is not affected, we investigated K_v7.1 channels, which constitute the second major potassium conductance driving late ventricular repolarisation. By showing that ivabradine, even at high µM concentrations, had no effect on K_v7.1 (Fig. 2), we could rule out the possibility that a potentiation of these channels counteract the inhibition of K_v11.1 channels. Next, we surmised that depolarising sodium and/or calcium currents could represent the sought-after compensatory mechanism. For human Ca_v1.2 calcium channels we found a trend of inhibition by ivabradine which, however, was not statistically significant (Fig. 3). No, or only a small effect of ivabradine, on voltage-gated calcium channels is consistent with previous findings in SA cells derived from rabbit hearts [3]. More importantly, however, when we next investigated the effect of ivabradine on human Na_v1.5 voltage-gated sodium channels, we found that these channels are considerably inhibited at low µM concentrations (Fig. 4).

We determined an IC_{50} value of 30 µM for the inhibition of human Na_v1.5 by ivabradine under our experimental conditions. This is an order of magnitude higher than the IC_{50} values reported for HCN channel inhibition [5, 29]. There are, however, a few points that need to be considered. First, sodium currents measured within this study were elicited by voltage-steps of relatively brief duration (25 ms), while during the rest of the protocol time voltage was clamped at -100 mV. Under these conditions, most channels are in the closed state for the

predominant time and open and inactivate only briefly during the time of depolarisation. The employed protocol thus favours open-channel block and leaves only very limited time for a binding of inactivated channel states. The human AP, which is an order of magnitude longer, would allow significantly more channels to enter the inactivated states. Taking into account that classical sodium channel inhibitors from the group of local anaesthetics, e.g. lidocaine, preferentially target the inactivated states [30, 31], it is reasonable to assume that this is also true for ivabradine. Second, our measurements were performed at room temperature for which channel gating and drug equilibration occurs at a much slower rate compared to physiological temperatures of 37 °C. Third, we did not study the rate-dependence or voltage-dependence of sodium channel inhibition by ivabradine. Here we only report the IC_{50} value for a voltage-clamp protocol consisting of 25 ms voltage steps to -10 mV applied at a rate of 1 Hz. The inherent voltage-dependence of a drug that supposedly binds in a state-dependent manner together with the fact that ivabradine is estimated to be 99% charged at physiological pH (tertiary amine with a pK_a of 9.4), would suggest a strong influence of membrane voltage on the observed IC_{50} value. Thus, our reported IC_{50} value should be seen as an upper estimate for $Na_v1.5$ inhibition under more physiological conditions. Clearly, future studies should investigate the state-dependence, temperature-dependence and use-dependence of $Na_v1.5$ inhibition by ivabradine in order to gain a better understanding of the physiological consequences associated with this finding.

Previous work has identified a slowing of AV conduction upon application of the I_f inhibitor zatebradine. Application of the drug induced a prolongation of the atrial-His bundle (AH) interval in a canine model of disrupted sinoatrial node function [32]. Recently, a similar finding was observed with ivabradine: slowed AV-node conduction in a guinea pig model [15]. The authors claimed that HCN channels, in particular HCN4, are expressed in AV tissue, and that inhibition of these channels would slow AV conduction. Our results provide the basis for an alternative interpretation. The inhibition of sodium channels, eventually in synergy with a small reduction in calcium current, could equally likely be responsible for the observed reduction in AV conduction speed.

There are several arguments that support the notion that sodium channel block may contribute to the ivabradine-induced suppression of conduction through the AV-node. First, Na_v 1.5 channels are expressed in the human AV-node, although to a lesser degree than in atrial and ventricular muscle [33]. Human channelopathies due to loss-of-function mutations in voltage-gated sodium channels are associated with prolonged conduction through the AV-node (for review see [34]). This phenotype is also reproduced in respective murine models of human cardiac conduction diseases (e.g. [35]). Increased AV-nodal conduction times have been reported after administration of the Na^+ channel blockers lidocaine and flecainide [36–38], and AV-block has been reported in patients treated with sodium channel blockers [39, 40]. In isolated guinea pig hearts ivabradine produced a strong rate-dependent prolongation of the AH-interval consistent with a drug-induced prolongation of AV-nodal conduction [15]. Interestingly, although the authors concluded that the HV-interval remained unchanged by ivabradine, close inspection of their original Fig. 6B (in [15]) strongly suggests a ~20% increase of the HV interval at a cycle length of 160 ms by 3 µM ivabradine. On the other hand, the same authors did not observe any effect of 0.1 mg/kg iv. ivabradine in the intact porcine heart at a cycle length of 333 ms. These changes may reflect a highly rate-dependent Na^+ channel block by ivabradine. Interestingly, voltage-gated sodium channels are involved in the pacemaker function of the sinus node [41, 42]. Hence, the sodium channel blocking activity of ivabradine may also contribute to its bradycardic action.

On the other hand a slowing of AV-nodal conduction has also been observed in a murine model of inducible cardiac-specific knockout of the pacemaker channel gene Hcn4 [43] implying that HCN channels themselves may be involved in signal conduction through the AV node. This notion is supported by in-silico studies using a theoretical model of AV-nodal conduction [22, 23]. Hence, both block of HCN channels and block of voltage-gated sodium channels may contribute to slowing of conduction through the AV node by ivabradine.

The present work presents a potential explanation why ivabradine despite its affinity for $K_v11.1$ (hERG) potassium channels does not lead to a prolongation of the cardiac AP and concomitant increase of the QT_c interval in human ECG recordings. Furthermore, our results provide an alternative explanation for the recent observations of increased atrial-His bundle intervals and thus slowed atrial-ventricular conduction upon administration of ivabradine [15].

Finally, the possibility of $Na_v1.5$ channel inhibition at clinically relevant ivabradine concentrations clearly demands further investigations.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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