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Differential effects of ivabradine and ryanodine on pacemaker activity in canine sinus node and Purkinje fibers

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Abstract

Introduction—It is generally accepted that at least two major mechanisms contribute to sinus node (SN) pacemaking: a membrane voltage (mainly I_f) clock and a calcium (Ca) clock (localized submembrane sarcoplasmic reticulum Ca^{2+} release during late diastolic depolarization). The aim of this study was to compare the contributions of each mechanism to pacemaker activity in SN and Purkinje fibers (PF) exhibiting normal or abnormal automaticity.

Methods and Results—Conventional microelectrodes were used to record action potentials in isolated spontaneously beating canine SN and free running PF in control and in the presence of 0.1 μM isoproterenol. Ryanodine (0.1–3 μM) and ivabradine (3μM) were used to inhibit sarcoplasmic reticulum Ca^{2+} release or I_f, respectively. To induce automaticity at low membrane potentials, PF were superfused with $BaCl_2$. In SN, ivabradine reduced the rate whereas ryanodine had no effect. Isoproterenol significantly accelerated automatic rate which was decreased by ivabradine and ryanodine. In normally polarized PFs, ryanodine had no effects on the automatic rate in the absence or presence of isoproterenol whereas ivabradine inhibited both control and isoproterenol-accelerated automaticity. In PF depolarized with $BaCl_2$, ivabradine decreased $BaCl_2$-induced automatic rate while ryanodine had no effect.

Conclusion—In canine SN,I_f contributes to both basal automaticity and β-adrenergic-induced rate acceleration while the ryanodine-inhibited Ca clock appears more involved in β-adrenergic regulation of pacemaker rate. In PF, normal automaticity depends mainly on I_f. Inhibition of basal potassium conductance results in high automatic rates at depolarized membrane potentials with SN-like responses to inhibition of membrane and Ca clocks.

Keywords

Sinus node; Purkinje fiber; Pacemaker; β-Adrenergic receptor stimulation; Ivabradine; Ryanodine

Introduction

Ivabradine is the first selective inhibitor of pacemaker current (I_f) in the sinus node (SN) approved for clinical application.[1] Clinical trials have demonstrated that ivabradine has anti-anginal and anti-ischemic efficacy at least as good as that of conventional therapies, but in contrast to β-adrenoreceptor blockers and calcium channel blockers, has no negative inotropic properties.[1-3] Consequently, ivabradine-induced reduction in heart rate is accompanied by an improvement of ejection fraction.[4, 5] Ivabradine decreases SN rate by a

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Ivabradine was a gift from Servier. No other disclosures.

concentration-dependent inhibition of I_f.[6] However, the effects of ivabradine on secondary pacemakers have not been studied, although ivabradine slows the rate of automaticity in rabbit pulmonary vein cardiomyocytes.[7] Therefore, the first aim of this study was to compare the effects of ivabradine in the primary and secondary pacemakers of the same species – canine SN and Purkinje fibers (PF).

It is generally accepted that at least two major mechanisms contribute to SN pacemaking: a voltage (or membrane) clock and a Ca^{2+} clock.[8] The voltage clock to which the pacemaker current I_f has been considered a major contributor, initiates diastolic depolarization.[6] Studying ivabradine effects (the first aim) allows comparison of the contribution of the voltage clock to primary and secondary pacemaking. The Ca^{2+}-clock depends on localized Ca^{2+} release from the sarcoplasmic reticulum during late diastole.[9] Local Ca^{2+} releases activate the Na^+/Ca^{2+} exchanger (NCX) current, causing an exponential increase in the rate of diastolic depolarization to drive the membrane potential to the threshold of the action potential upstroke.[10] The contribution of the Ca^{2+} clock to spontaneous activity in secondary pacemakers is not very clear. Consequently, the second aim of our study was to compare the effects of ryanodine (inhibitor of Ca^{2+} release from sarcoplasmic reticulum) in canine SN and in PF. Because both clocks are responsive to β adrenergic receptor stimulation,[11, 12] the experiments were performed in the absence and presence of β adrenergic activation. We hypothesized that the relative contributions of voltage and Ca^{2+} clocks to pacemaker activity are different in primary and secondary pacemakers.

Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Heath (NIH Publication No. 85–23, revised 1996). All protocols were approved by the Columbia University Institutional Animal Care and Use Committee. Mongrel dogs weighing 22–25 kg were anesthetized with thiopental (17 mg/kg IV), intubated, and ventilated with isoflurane 9(1.5% to 3.0%). The hearts with at least 1 cm of each vena cava attached were removed through a left lateral thoracotomy and immersed in cold Tyrode's solution equilibrated with 95% O_2 - 5% CO_2 and containing (in mmol/L): NaCl 131, $NaHCO_3$ 18, KCl 4, $CaCl_2$ 2.7, $MgCl_2$ 0.5, NaH_2PO_4 1.8, and dextrose 5.5. A piece of atrial tissue containing SN (~10×15 mm) was cut from the junction of the superior vena cava and free wall of the right atrium and placed in a tissue bath epicardial surface up.[13] To expose the SN to solution and facilitate microelectrode impalements the epicardial tissue layer of 0.3–0.5 mm thickness was removed. The preparations beat spontaneously and multiple microelectrode impalements were made to localize the cells in the center of the nodal region (smooth transition between diastolic depolarization and action potential upstroke), the cells located at the periphery of the SN (more abrupt transition), and atrial cells. The atrial tissue was cut off and the final dimension of preparations was ~2–3×5 mm approximately corresponding to the central region of canine SN.[14]

Free-running Purkinje fibers (PFs) were dissected from both ventricles. All preparations were placed in a 4–mL chamber, superfused with Tyrode's solution (37°C, ph 7.3 to 7.4) at 12 mL/min and allowed to beat spontaneously. Conventional microelectrode techniques were used to record transmembrane potentials.

Validation of SN preparation

Microelectrode recordings of transmembrane potentials from canine SN cells have previously been made using arterially-perfused right atrial preparations.[15, 16] Obtaining stable recordings was hampered because it was difficult to penetrate the several layers of epicardial cells covering the SN with microelectrodes, and electrodes were often dislodged and/or recordings distorted by mechanical displacement of the microelectrode. As a result,

few identical action potentials from the same cell could be recorded consecutively. At present, canine perfused right atrial preparations are being used for optical recordings of SN cell action potentials.[14, 17] The method allows recording of the time course of transmembrane voltage changes but not the actual voltages. However, recordings of absolute values of transmembrane potential are important for understanding the mechanisms of pacemaking and for interpreting the effects of pharmacological interventions. In addition, the maximum diastolic potential (MDP) is one of the major parameters that differentiate between primary and secondary pacemakers. Therefore, in the present study, to have a stable and long-lasting transmembrane potential recording, we developed an isolated superfused canine SN preparation. Because this preparation has not been used previously, control series of 5 experiments were performed to test its stability. Transmembrane potentials were continuously recorded during 3.5 hours beginning at 30 minutes after isolation of preparations. There were no significant changes between the start and end of registration either in rate (111±5 vs 107±6 beats/min, respectively, P>0.05) or MDP (-61±2 vs –62±2 mV, respectively, P>0.05). These values of rate and MDP are similar to those recorded in perfused right atrial preparations.[15, 16] The total durations of the protocols in the present study (<3 hours) were within the time frame during which the preparations were stable. It is well known that neural input and pulse within the node artery modulate the rate of SN.[18] Interestingly, despite of the absence of these factors the control rate of isolated SN preparations used in this study varied between 80 and 151 beats/min which is very similar to heart rate in adult dogs which varies between 70 and 160 beats/min.[19] This suggests that the preparations can be used for studying the basic mechanisms underlying SN pacemaking.

Automaticity in PF induced by $BaCl_2$ depolarization

After 1 hour of equilibration in control solution, fibers were superfused for 20 minutes with solution containing 0.15 mmol/L of $BaCl_2$. If automaticity at low membrane potentials did not appear, the $BaCl_2$ concentration was increased in steps of 0.05 mmol/L and at 20 minute intervals until stable automaticity was evident. Such stability is characterized by a low maximum diastolic potential, phase 4 depolarization, and action potentials with slow upstrokes and low overshoots.[20, 21]

Isoproterenol and ryanodine were purchased from Sigma Chemical Co. (USA). Ivabradine was a gift from Servier. Ivabradine was used in a concentration of 3×10^{-6} mol/L (the maximum concentration of the compound having specificity for I_f.[22] To functionally disable ryanodine receptors we used 10^{-7} and 3×10^{-6} mol/L ryanodine. These ryanodine concentrations lock ryanodine receptors in an open subconductance state, leading to depletion of SR Ca^{2+} content.[23] The effects of both compounds are reported at 30 minutes of superfusion.

Data are expressed as mean+SEM. Continuous parameters were analyzed with one- or two-way ANOVA for repeated measures. $P<0.05$ was considered significant.

Results

Effects of ivabradine and ryanodine on automaticity at normal membrane potentials in the SN and PF

Figures 1A and 1B show representative action potential traces recorded respectively from one SN and one PF preparation in control and in the presence of ivabradine. In control, the SN preparation beating rate was 125 bpm and the shape of the action potential was typical of pacemaker cells. MDP was –62 mV and there was a smooth transition between phase 4 and phase 0. Thirty minutes of superfusion with ivabradine resulted in slowing of the rate to 110 bpm and no change in MDP. In contrast to the SN preparation, ivabradine completely

terminated automaticity in PF. Summary data (Figure 1, C through F) show that ivabradine significantly decreased spontaneous rate in both types of preparations with more prominent effects on PF. No changes in MDP occurred.

The effects of ryanodine on SN and PF are shown in Figure 2. Neither ryanodine concentration altered SN automaticity: and there were no changes in rate or MDP (A, C and E). In PF, the low ryanodine concentration had no effects on automaticity and on MDP (B, middle trace, D and F), whereas 3×10^{-6} mol/L induced repetitive self-initiating and self-terminating episodes of impulse initiation at depolarized membrane potentials (B, bottom trace). The duration of episodes was about one minute. This pattern of activity started 12–15 minutes after beginning the ryanodine superfusion and lasted during the entire observation period.

Effects of ivabradine and ryanodine on SN and PF automaticity in the presence of isoproterenol

Isoproterenol significantly accelerated rate and hyperpolarized MDP in SN preparations (Figure 3, A, C and E). Ivabradine applied in addition to isoproterenol decreased rate and further hyperpolarized MDP. Qualitatively similar effects of isoproterenol were seen in PF: automatic rate increased and MDP hyperpolarized (Figure 3, B, D and F). In the presence of isoproterenol, ivabradine almost completely eliminated the acceleration in rate and had no effects on MDP.

Ryanodine significantly inhibited isoproterenol-accelerated SN automaticity while having no effect on isoproterenol-hyperpolarized MDP (Figure 4, A, C and E). Interestingly, the maximum ryanodine effect was achieved at the low (10^{-7} mol/L) concentration; increasing concentration to 3×10^{-6} mol/L did not further affect rate. In PF, in the presence of isoproterenol, the low ryanodine concentration did not change rate, whereas at 3×10^{-6} mol/L it induced repetitive self-initiating and self-terminating episodes of automaticity at depolarized membrane potentials (Figure 4, B D and F). These effects of ryanodine were similar to those seen in the absence of isoproterenol (compare bottom traces in panel B in Figures 2 and 4). In addition, the low ryanodine concentration eliminated the hyperpolarization of MDP induced by isoproterenol (Figure 4F).

Effects of ivabradine and ryanodine on abnormal automaticity in PF

Superfusion with $BaCl_2$ led to a depolarization of PF and development of rapid automatic rhythms (Figure 5, A and B, middle panels). Ivabradine but not ryanodine slowed the rate of barium-induced automaticity (Figure 5C). Neither compound affected the depolarized MDP (Figure 5D).

Discussion

Isolated superfused SN preparation

In this study we developed a new canine SN preparation – an isolated multicellular slice of the SN region. The rates and MDPs in these preparations are similar to those recorded previously in canine arterially-perfused right atrial preparations.[15, 16] In contrast to arterially-perfused preparations, the multicellular slices permit stable and long-lasting recordings of transmembrane potential. Control experiments demonstrated the stability of major parameters of pacemaker activity (the rate and MDP) during 3.5 hours. Therefore, we were able to test the effects of ivabradine and ryanodine on SN pacemaker activity.

Voltage clock in SN and PF

Our results allow us to estimate and compare the importance of voltage and calcium clocks for pacemaking in primary (SN) and secondary (PF) pacemakers. In the SN, ivabradine slowed basal pacemaking activity and partially decreased isoproterenol-induced rate acceleration. These results are in agreement with observations in disaggregated canine SN cells[24] and show that I_f is an important contributor to basal SN automaticity and to the rate acceleration induced by β-adrenergic receptor stimulation. However, because I_f blockade does not eliminate automaticity or completely prevent rate increases in response to isoproterenol, our data suggest that I_f-independent mechanisms also contribute to canine SN pacemaking.

In PF, in contrast to SN, ivabradine almost completely terminated basal automaticity and abolished the isoproterenol-induced rate acceleration. Similar results have been demonstrated in situ: in dogs with complete atrioventricular block, intravenous administration of ivabradine totally suppressed idioventricular rhythm.[25] These results point to I_f as a major determinant of automaticity in PF.

HCN2 and HCN4 are two major subunits of the HCN channels family that underlie I_f in pacemaker structures, with HCN4 being more expressed in the SN – along with HCN1 - and HCN2 in PF.[26-28] Importantly, HCN2 and HCN4 show nearly equal sensitivity to inhibition by ivabradine.[25, 29] Consequently, quantitatively different effects of ivabradine in the SN and PF likely do not result from a difference in sensitivity of I_f to the compound but reflect a different contribution of I_f to pacemaking in these structures.

Calcium clock in SN and PF

In the SN, ryanodine had no effects on basal pacemaker activity but significantly inhibited isoproterenol-accelerated SN automaticity. The effects of ryanodine on SN automaticity differ among various studies (from no effect to complete inhibition of automaticity) depending on species and experimental conditions.[30] Hence, it is interesting to compare our results with those obtained in canine preparations with a lesser and higher degree of SN tissue isolation: arterially- perfused right atrium[17] and single SN cells,[24] respectively. Studies of both preparations showed that ryanodine only marginally (and statistically insignificantly) reduced basal automaticity but considerably impaired the rate acceleration induced by isoproterenol. These data are similar to our results and suggest that in canine SN, a Ca^{2+}-dependent mechanism (Ca^{2+} clock) does not contribute substantially to basal automaticity but plays an important role in sinus acceleration during β-adrenergic stimulation. In PF, ryanodine at a low concentration had no effects on automaticity either in the absence or presence of isoproterenol. The latter effect is different from that in SN where a low ryanodine concentration maximally inhibited isoproterenol-induced rate acceleration. These data suggest that in PF, the Ca^{2+} clock does not contribute substantially to basal automaticity and to acceleration induced by β-adrenergic stimulation.

In PF, the high ryanodine concentration induced repetitive self-initiating and self-terminating episodes of automaticity at depolarized membrane potentials. The mechanism of this effect is unclear but the following explanation is plausible: block of SR Ca release induced by ryanodine inhibits calcium-dependent inactivation of calcium current.[31, 32] As a result, the current through L-type channels decays slowly and more Ca^{2+} ions enter the cell at each excitation. Intracellular calcium elevation activates the forward mode of the electrogenic Na^+/Ca^{2+} exchanger that generates an inward current.[33] Increased inward current leads to membrane depolarization and generation of automaticity. The Na^+/Ca^{2+} exchanger functions here to overload the cell with sodium, which activates the Na^+/K^+

pump.[34] Enhanced outward pump current hyperpolarizes the membrane and inhibits abnormal automaticity. Then this sequence of events is repeated.

Voltage and calcium clocks in abnormal automaticity in PF

The response to ivabradine and ryanodine is clearly different in the SN and PF preparations. One of the major differences between these pacemakers is the value of MDP: PFs are significantly more hyperpolarized than SN cells. The more positive MDP in the SN results from the difference in the background K^+ current (I_{K1}) in these pacemaker structures I_{K1} is almost absent in central SN cells.[35, 36] Therefore, we used Ba^{2+} to decrease I_{K1} in PF. Gradually increasing the Ba^{2+} concentration allowed us to depolarize MDP in PFs to values similar to those in the SN. In this MDP range, PFs exhibited automaticity comparable to that of SN. Thus, PFs depolarized with barium mimic the MDP and automaticity of the SN. Interestingly, the effects of ivabradine and ryanodine on this abnormal automaticity in PFs are comparable to those in SN: ivabradine moderately slows rate while ryanodine has no effect. The reduced ivabradine effects in depolarized PFs result, most likely, from a decreased availability of I_f at low membrane potentials. They also suggest that, as for SN, other (I_f-independent) mechanisms contribute to automaticity in PFs at depolarized level of membrane potentials. These results emphasize the role of the MDP in determining the mechanisms of automaticity in primary and secondary pacemakers.

Conclusions

Membrane and Ca clocks differentially contribute to canine SN pacemaking: I_f contributes to both basal automaticity and β-adrenergic-induced rate acceleration while the ryanodine-inhibited Ca clock appears more involved in β-adrenergic regulation of pacemaker rate. In PF, normal automaticity depends mainly on I_f. Inhibition of basal potassium conductance results in high automatic rate at depolarized membrane potentials with SN-like responses to inhibition of membrane and Ca clocks.

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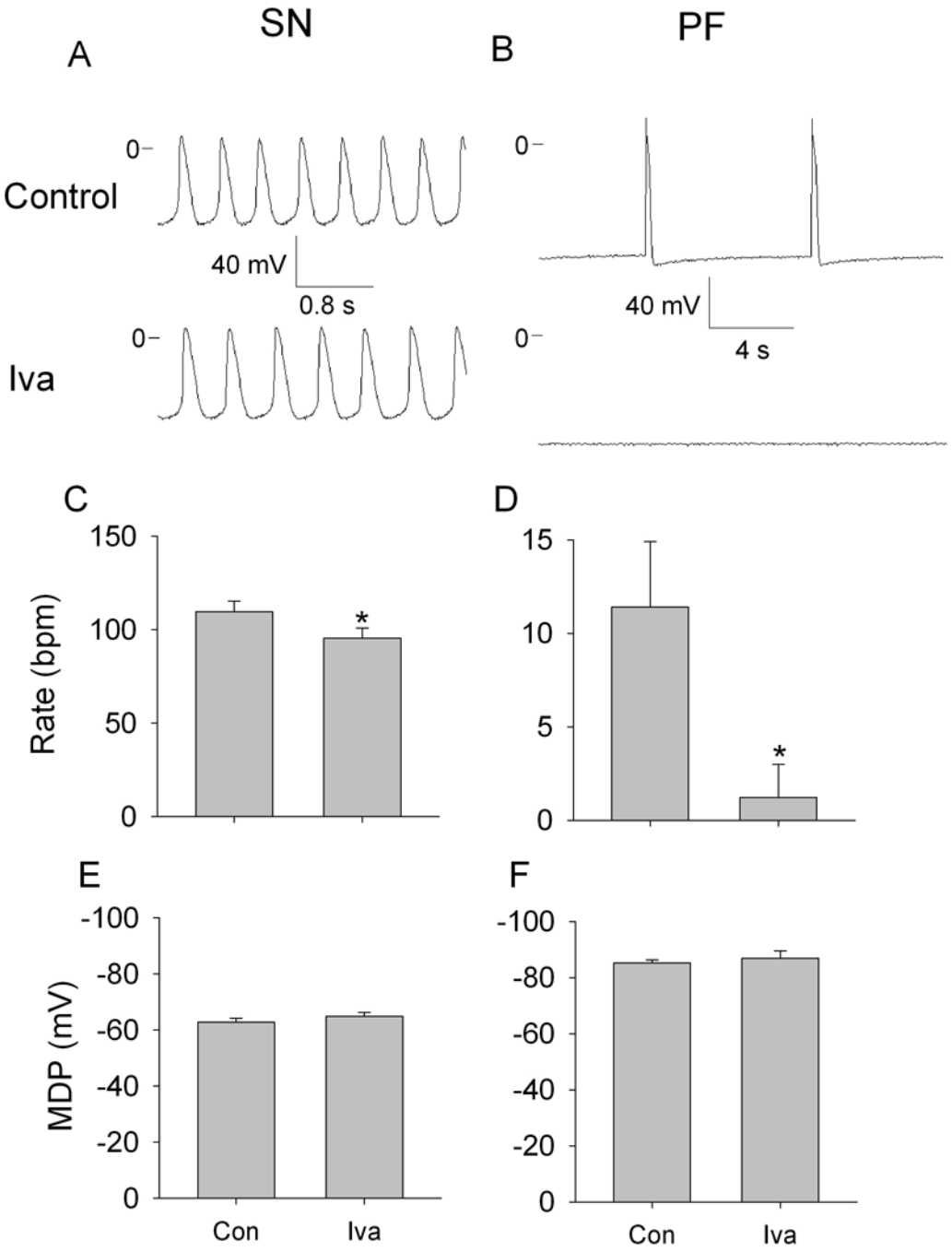

Figure 1.

Effects of ivabradine (Iva) on automaticity in SN and PF. A and B, representative transmembrane potentials recorded from spontaneously beating SN preparation and free running PF in control (upper traces) and in the presence of 3 μmol/L ivabradine (lower traces). C through F, respective summary data showing ivabradine effects on rate and maximum diastolic potential (MDP) in SN (C and E) and in PF (D and F). N=10 for SN, n=6 for PF. *P<0.05 versus respective control.

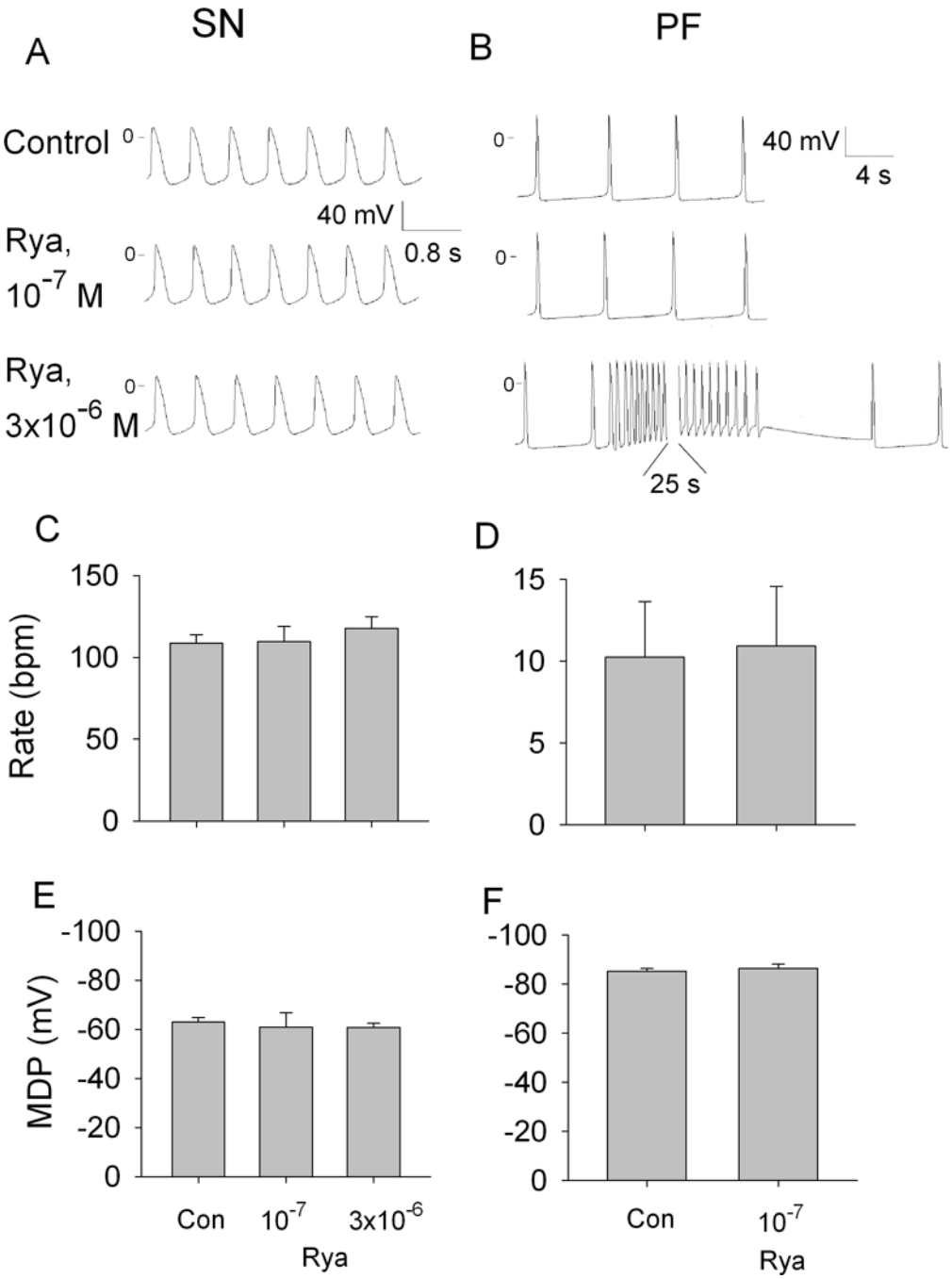

Figure 2.

Effects of ryanodine (Rya) on automaticity in SN and PF. Representative transmembrane potentials recorded from SN preparation (A) and PF (B) in control and in the presence of 0.1 and 3 μmol/L ryanodine. C through F, summary data showing the effects of 0.1 and 3 μmol/L ryanodine on the rate and maximum diastolic potential (MDP) in SN (C and E) and 0.1 μmol/L ryanodine in PF (D and F). N=6 for SN and PF.

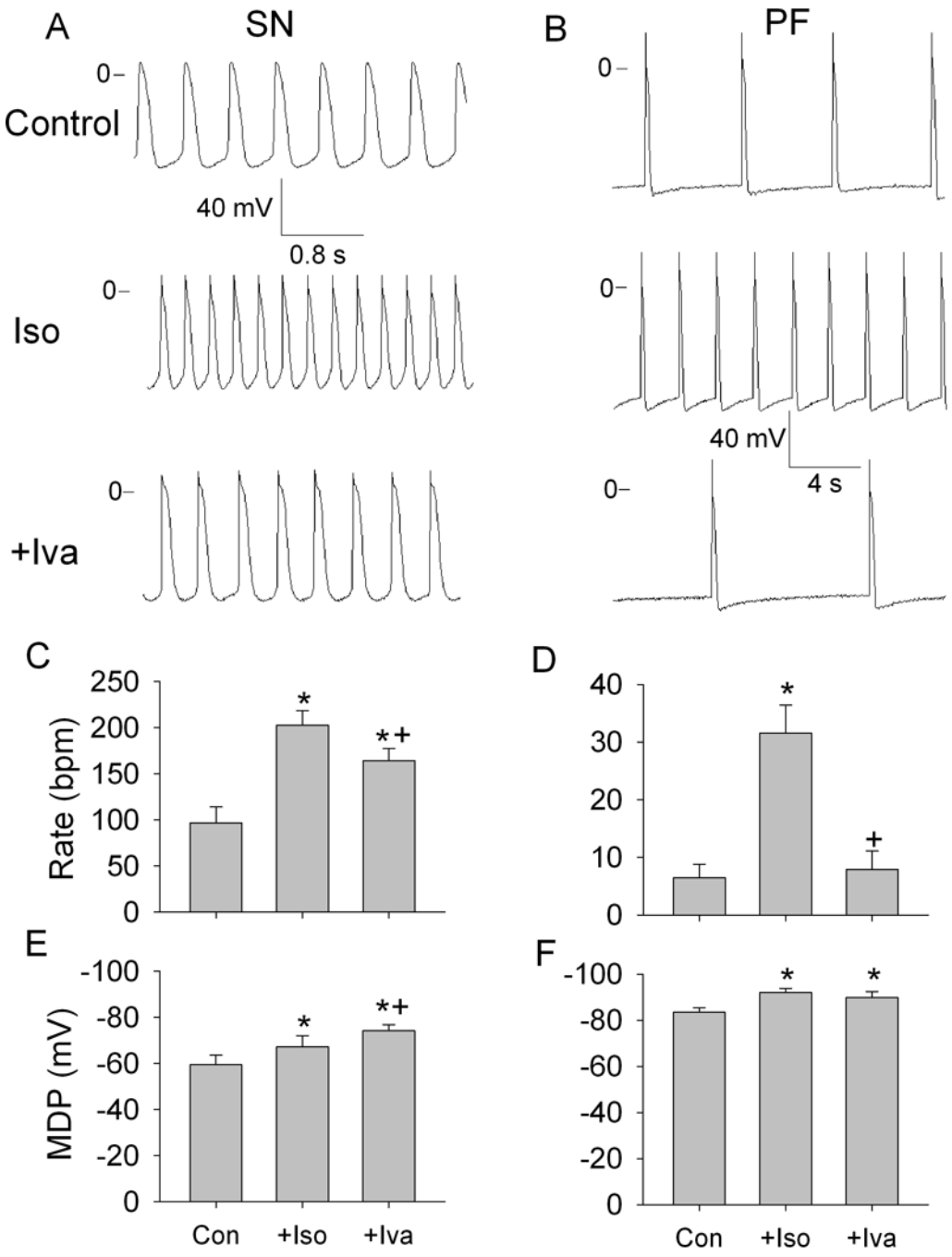

Figure 3.

Effects of ivabradine (Iva) on automaticity in the presence of isoproterenol (Iso) in SN and PF. A and B, representative transmembrane potentials recorded from spontaneously beating SN preparation and free running PF in control (upper traces), in the presence of 0.1 μmol/L isoproterenol (middle traces) and in the presence of isoproterenol and 3 μmol/L ivabradine (lower traces). C through F, respective summary data showing the rate and maximum diastolic potential (MDP) in SN (C and E) and in PF (D and F). N=6 for SN and for PF. *P<0.05 versus respective control. $^{+}$P<0.5 versus respective Iso.

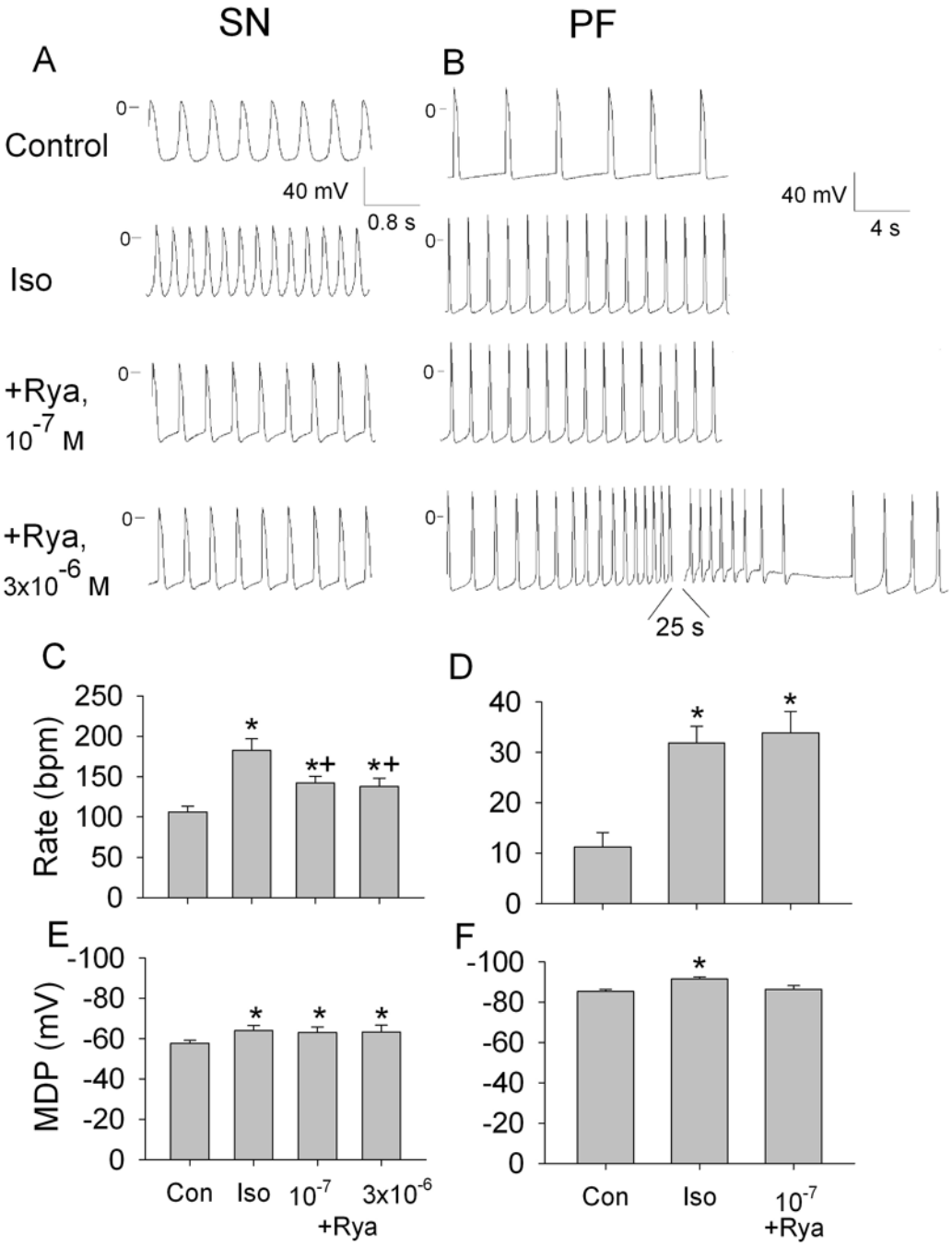

Figure 4.

Effects of ryanodine (Rya) on automaticity in the presence of isoproterenol (Iso) in SN and PF. A and B, representative transmembrane potentials recorded from spontaneously beating SN preparation and free running PF in control, in the presence of 0.1 μmol/L isoproterenol, in the presence of isoproterenol plus 0.1 μmol/L ryanodine and in the presence of isoproterenol plus 3 μmol/L ryanodine. C through F, respective summary data showing the rate and maximum diastolic potential (MDP) in SN (C and E) and in PF (D and F). N=6 for SN and n=8 for PF. *P<0.05 versus respective control. $^{+}$P<0.5 versus respective Iso.

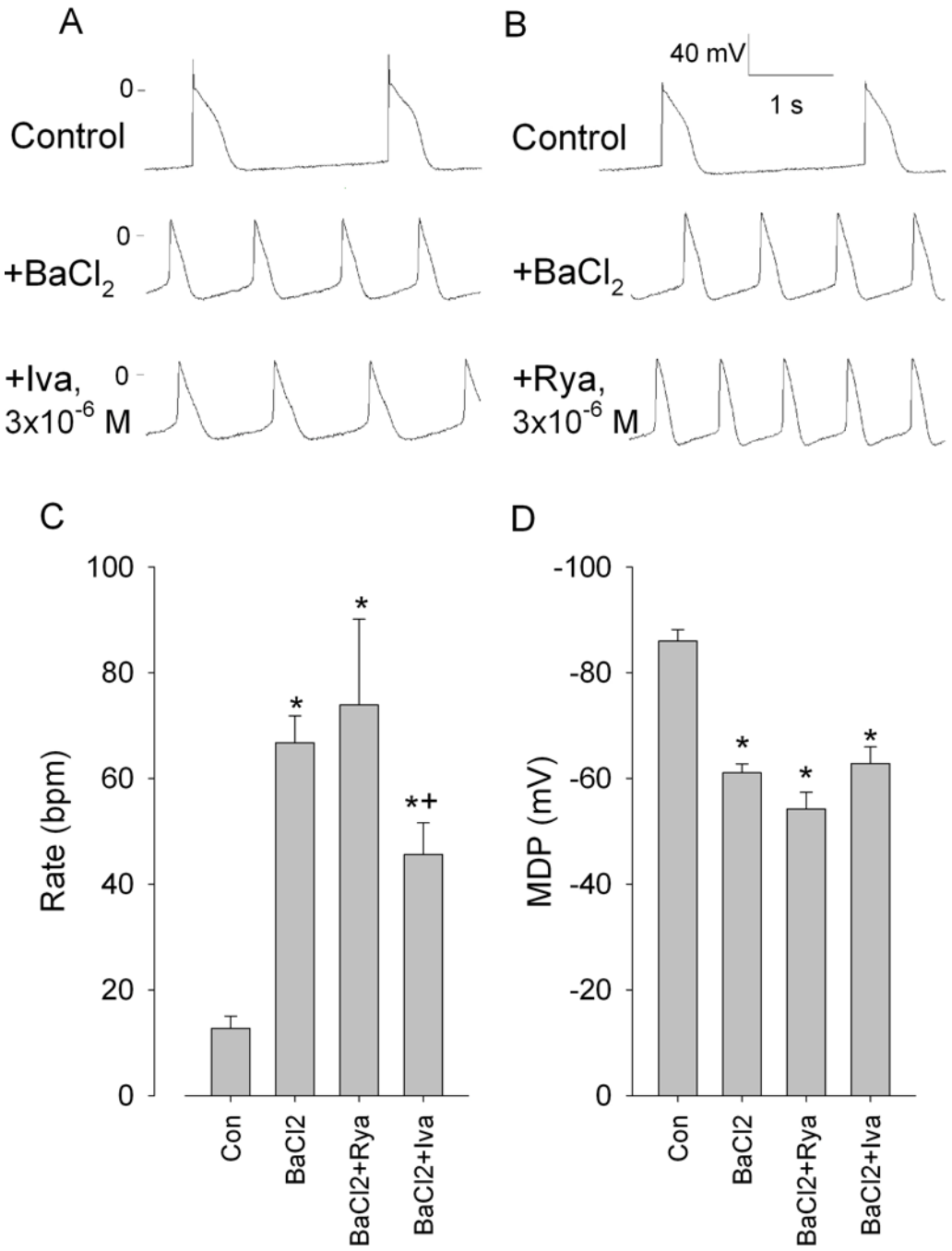

Figure 5.

Effects of ivabradine (Iva) and ryanodine (Rya) on barium-induced automaticity at low membrane potentials in Purkinje fibers. A and B, representative transmembrane potentials in control, in the presence of 0.4 mmol/L $BaCl_2$, and after adding Iva (3 μmol/L), or Rya (3 μmol/L). C and D, summary data showing the rate and maximum diastolic potential (MDP). N=5 for Iva and Rya. *P<0.05 versus respective control. $^{+}$P<0.05 versus respective $BaCl_2$.