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Short communication

Mibefradil, an $I_{Ca,T}$ blocker, effectively blocks $I_{Ca,L}$ in rabbit sinus node cells

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Abstract

To test the hypothesis that the Ca^{2+} channel blocker mibefradil slows heart rate due to inhibition of T-type Ca^{2+} current in pacemaker cells, we studied effects of mibefradil on action potentials and ionic currents of isolated rabbit sinus node cells using the patch clamp technique. Mibefradil (100 nM and 1 μM) reduced spontaneous rate, decreased action potential amplitude and finally stopped impulse initiation. This action was not due to the drug effect on hyperpolarization-activated pacemaker current, but can be explained by attenuation of both T- and L-type Ca^{2+} currents, which were inhibited by mibefradil almost equally (55% and 64% inhibition with 1 μM for T- and L-types, respectively).

Keywords: Mibefradil; Sinus node cell; Ca^{2+} current

1. Introduction

Mibefradil, a Ca^{2+} channel blocker, was found to be effective in treatment of chronic heart failure in experimental models as well as in clinics. Although mibefradil has been withdrawn from the market because of serious interactions with amiodarone, digoxin, β-blockers and some other drugs used in cardiotherapy (Krayenbühl et al., 1999), it is important to study its effects on ionic channels because mibefradil is considered to be the first representative of a new group of Ca^{2+} channel blockers, those that are selective for T-type Ca^{2+} current ($I_{Ca,T}$) (Clozel et al., 1997). Mibefradil selectively blocks $I_{Ca,T}$ in vascular smooth (Mishra and Hermsmeyer, 1994) and atrial (see Clozel et al., 1997) cells. In sinus node cells, $I_{Ca,T}$ has been suggested to contribute to impulse generation (Hagiwara et al., 1988). Mibefradil has a modifying effect on sinus rhythm (Rosenquist et al., 1997) leading to the suggestion that selective inhibition of $I_{Ca,T}$ accounts for the sinus slowing (Clozel et al., 1997). However, relative selectivity of mibefradil for $I_{Ca,T}$ over L-type Ca^{2+} current ($I_{Ca,L}$) appears to be tissue specific (Clozel et al., 1997; Pinto et al., 1999), and to date there are no data on effects of mibefradil on ionic currents in isolated sinus node cells. We present results of mibefradil on $I_{Ca,T}$, $I_{Ca,L}$, hyperpolarization activated pacemaker current (I_f), and on the action potentials of spontaneously beating sinus node cells. The results demonstrate that mibefradil inhibits both $I_{Ca,L}$ and $I_{Ca,T}$ with no obvious preference for $I_{Ca,T}$. It arrests action potentials in spontaneously beating sinus node cells after only a modest slowing of rate, probably due to its effect on both Ca^{2+} currents.

2. Materials and methods

The sinus node region was isolated from 40–47-day-old female rabbits and cells were prepared using a previously described method (DiFrancesco et al., 1986). Cells were placed in the experimental chamber (35°C), and superfused with Tyrode solution of the following composition (mM): 140 NaCl, 5.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, 10 glucose (pH 7.4). Membrane currents or potentials were recorded using the patch technique. Borosilicate glass pipettes were filled with a solution of the following composition (mM): 90 Aspartic acid, 10 NaCl, 100 CsOH, 30 CsCl, 2 $MgCl_2$, 5 EGTA, 2 $CaCl_2$, 10 HEPES, 2 ATP Na_2, 0.1 GTP Na_2, pH 7.2.

The whole cell configuration of the patch clamp was used to study $I_{Ca,T}$. Cells were superfused with a modified

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Tyrode solution (in mM: 50 NaCl, 5 CsCl, 90 tetraethylammonium chloride, 0.5 $MgCl_2$, 10 HEPES, 2 $CaCl_2$, 5.5 glucose, 0.01 tetrodotoxin, pH 7.4). The current–voltage (I–V) relationship for $I_{Ca,T}$ was determined from a holding potential of −80 mV. The protocol included 200 ms step to potentials ranging from −70 to 50 mV (increment 10 mV) without or with a 50 ms prepulse to −50 mV to inactivate $I_{Ca,T}$. $I_{Ca,T}$ was obtained from the difference current.

To study $I_{Ca,L}$, the perforated patch technique was employed; it allowed us to avoid rundown of the current. The composition of the pipette solution was as described earlier except for the addition of amphotericin B (Sigma), 100–200 μg/ml. Two to ten minutes after forming a seal, and after series resistance was reduced to 15–20 MΩ, the superfusate was exchanged for one containing 10 mM CsCl, 3 μM tetrodotoxin and no KCl. To construct I–V curves, 200 ms depolarizing voltage steps ranging from −40 to 50 mV were used from a holding potential of −50 mV.

The perforated patch approach also was used to study I_f and spontaneous action potentials. The pipette solution contained (in mM) 130 aspartic acid, 146 KOH, 10 NaCl, 2 $CaCl_2$, 5 EGTA, 10 HEPES, 2 ATP Mg, 100–200 μg/ml amphotericin (pH 7.2). Action potentials were recorded from cells superfused with Tyrode solution; for I_f, 1 mM $BaCl_2$ and 2 mM $MnCl_2$ were added to the Tyrode solution. I_f was induced by 3-s long hyperpolarizing pulses ranging from −45 to −105 mV followed by a 400-ms long deactivating pulse to −5 mV. Holding potential was −35 mV.

Mibefradil was applied at final concentrations of 100 nM and 1 μM; each cell was exposed to a single concentration. Effects of mibefradil on ionic currents were evaluated 2–3 min after beginning of drug administration; effects on action potential were constantly monitored throughout the experiment. Data are presented as mean ± S.E. Statistical significance was determined by Student's *t*-test or Analysis of Variance (ANOVA) as appropriate. A value of $P < 0.05$ was regarded as significant.

3. Results

3.1. Action potential

In three cells, 100 nM mibefradil reduced maximal diastolic potential and decreased total action potential amplitude such that in two cells, impulse initiation halted in 60 and 90 s (Fig. 1). Heart rate was moderately slowed (cycle length increased to 124 ± 5% of control as measured in two cells before they were arrested and in the third cell at the steady state level of the chronotropic effect; $P = 0.048$). 1-μM mibefradil (two cells) decreased heart rate and action potential amplitude and arrested action potentials within 80 s of application. When washout

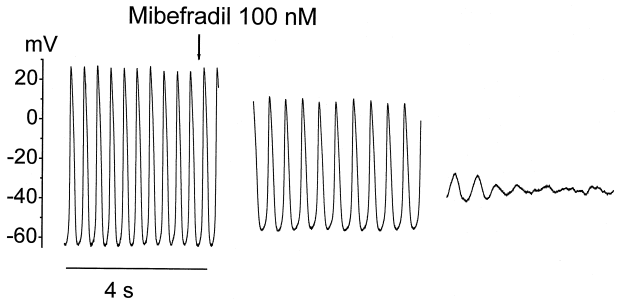

Fig. 1. Effect of mibefradil (100 nM) on action potentials of a spontaneously beating sinus node cell. Perforated patch, current clamp mode, 35°C. Interval between the first and the third recordings is 90 s.

was monitored for an extended period, full recovery of rate was observed ($n = 2$).

3.2. $I_{Ca,T}$

After rupture, 200 ms pulses to 20 mV preceded by short steps to −50 mV from a holding potential of −80 mV were repeated every 5 s to estimate the dynamics of $I_{Ca,L}$ rundown. Within 1.5–2 min, the currents had stabilized with at least two thirds of original size. $I_{Ca,T}$ was then determined as described in Materials and methods. The I–V curve constructed from the difference currents had a threshold between −60 and −50 mV and a peak of −4.8 ± 0.6 pA/pF ($n = 11$) at −20 mV (Fig. 2A). This rapidly decaying inward current was not a fast sodium current since adult sinus node cells have little or no fast Na current, and any Na current present would be suppressed by 10 μM TTX (Baruscotti et al., 1996). In two cells, nickel 100 μM, which is reported to preferentially inhibit $I_{Ca,T}$ in sinus node cells (Hagiwara et al., 1988), decreased the current to 30% of control (data not shown). Thus, the difference current is $I_{Ca,T}$. Both 100 nM and 1 μM mibefradil decreased the current amplitude (Fig. 2A), I–V curves being significantly different from corresponding control I–V curves (ANOVA, $P = 0.002$ and $P < 0.001$, respectively). 1-μM mibefradil reduced peak current by 55%.

3.3. $I_{Ca,L}$

Using the perforated patch method, there was no rundown of $I_{Ca,L}$ during repetitive 0.2-Hz depolarizations from −50 to 20 mV. The control I–V curve had a peak current density of −14.7 ± 2.0 pA/pF at −10 mV (n = 9). As is seen in the Fig. 2B, 100 nM and 1 μM of mibefradil demonstrated inhibition of the current, and the effect was significant for both concentrations used (I–V curves significantly different from corresponding control I–V curves by ANOVA; $P < 0.001$ for both). Similar to the result with $I_{Ca,T}$, mibefradil 1 μM produced 64% inhibition of the peak current density. Although recovery was not routinely monitored, recovery (up to 20%) was seen in some cells in 3–5 min after the drug was washed

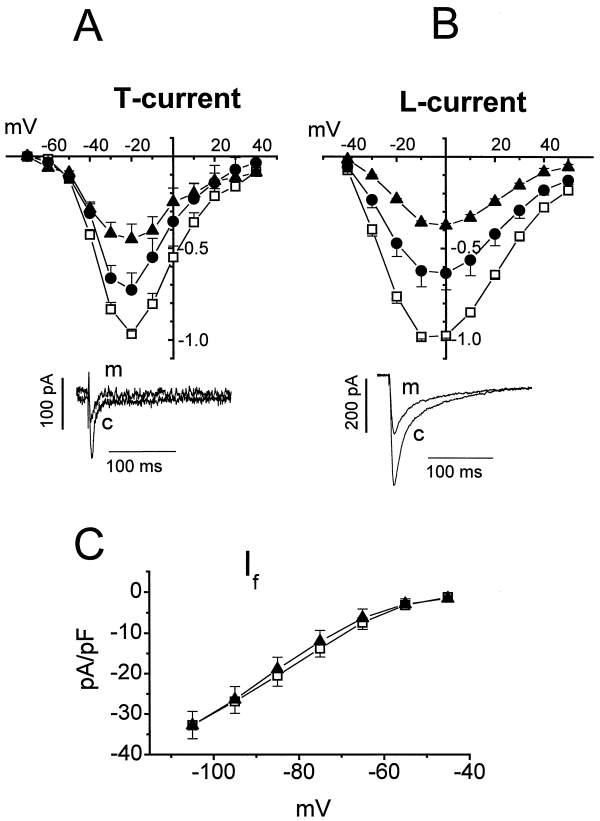

Fig. 2. Effects of mibefradil on currents responsible for development of action potentials in sinus node cells. (A and B) Top: $I-V$ curves obtained in control conditions and in the presence of mibefradil. Data are means and S.E. Ordinates in A and B are current densities normalized in individual experiments to control peak current densities; absolute values for peak current densities and number of experiments are given in the text. (A and B) Bottom: original traces of peak currents in control (c) and with mibefradil 1 μM (m). (A) T-type calcium current found as a difference between currents obtained at holding potential of −80 mV and those at −50 mV (see Materials and methods); whole cell patch. (B) L-type calcium current, holding potential −50 mV; perforated patch. (C) $I-V$ curves for hyperpolarization activated pacemaker current (I_f), holding potential −35 mV; perforated patch. In A, B, and C (□) is control, (●) is mibefradil 100 nM, and (▲) is mibefradil 1 μM.

out. In two experiments, nifedipine (10 μM), a dihydropyridine Ca^{2+} channel blocker, was used instead of mibefradil; it decreased the current at least by 95% (data not shown), confirming that the current was $I_{Ca,L}$.

3.4. I_f

Mibefradil (1 μM) did not change the magnitude or kinetics of I_f in three cells. Current density–voltage curves in the presence of mibefradil did not significantly differ from those in control (Fig. 2C).

4. Discussion

Mibefradil increases cyclic length and reduces the amplitude of sinus node cell action potentials, and eventually stops spontaneous activity of individual sinus node cells. This is consistent with the chronotropic effect of mibefradil in human heart (Rosenquist et al., 1997). It should be noted, however, that in our experiments using isolated sinus node cells the chronotropic effect of mibefradil was moderate. Spontaneous activity of sinus node cells ceased as a result of a progressive decrease in action potential amplitude and maximal diastolic potential rather than due to prolongation of cycle length. This is consistent with the strong effect of mibefradil on $I_{Ca,L}$ in sinus node cells, measured at a holding potential of −50 mV, which approximates the maximal diastolic potential. In this respect, it is relevant that in Purkinje fibers, where mibefradil also inhibits both T- and L-currents, spontaneous firing rate was not affected (Pinto et al., 1999). This probably reflects the differing role of $I_{Ca,L}$ in action potential upstroke and plateau in sinus node cells versus that in Purkinje fibers, as well as perhaps the more negative maximal diastolic potential, where mibefradil is less potent (Pinto et al., 1999).

Unexpectedly, we did not find any selectivity for $I_{Ca,T}$. It should be noted that Liang-min and Osterrieder (1991) have demonstrated strong potential-dependence to the effect of mibefradil on $I_{Ca,L}$ in rat ventricular myocytes. In this case, a $-80/-50$ difference current used to define $I_{Ca,T}$ could result in an underestimate of the effect of mibefradil on $I_{Ca,T}$. However, the activity of mibefradil shown in our experiments, where 1 μM mibefradil produced 55% inhibition of $I_{Ca,T}$, agrees with that demonstrated for other cardiac T-channels (Cribbs et al., 1998; Clozel et al., 1997), although greater sensitivity was reported in Purkinje fibers. Thus, the non-selectivity of mibefradil in sinus node cells does not arise from reduced $I_{Ca,T}$ sensitivity, but rather from enhanced $I_{Ca,L}$ sensitivity. While we cannot exclude the possibility that at more negative holding potentials the effect of mibefradil on $I_{Ca,L}$ would be reduced relative to that on $I_{Ca,T}$, at physiologically relevant voltages for the sinus node (−50 mV) mibefradil does not demonstrate selectivity for $I_{Ca,T}$ over $I_{Ca,L}$. It should also be noted that this study only investigated mibefradil's action on Ca^{2+} currents and I_f. Additional effects on other currents, such as $I_{K,ATP}$ (Gomora et al., 1999) cannot be excluded.

In conclusion, in rabbit sinus node cells mibefradil can have a significant inhibitory effect on $I_{Ca,L}$. Inhibition of $I_{Ca,L}$ likely contributes to the effect of mibefradil on action potential amplitude and rate. Thus, mibefradil's efficacy in slowing heart rate cannot be taken as evidence for the contribution of $I_{Ca,T}$ to sinus automaticity. This question awaits the development of more selective sinus node T-channel blockers for its resolution.

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