Environmental Medicine, Vol. 46 No.1, 2, 2002 (page 84 ~ 85)

Inhibitory Action of Mibefradil on T-Type Calcium Channels in Early Embryonic Mouse Ventricular Myocytes

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Abstract: Mibefradil has been reported to preferentially block T-type Ca^{2+} channel currents in many different tissues including heart with variable potencies. We recorded T-type Ca^{2+} currents (I_{Ca-T}) in early embryonic (9.5 day post coitum (dpc)) mouse ventricular myocytes using a whole-cell patch clamp method and studied the effects of mibefradil. I_{Ca-T} were inhibited by mibefradil in a concentration-dependent manner with half inhibitory concentration (IC_{50}) at 3.4 ± 0.2 μM. This value is higher than those reported for native adult and neonatal atrial myocytes (0.1–1.5 μM). Factors affecting the mibefradil sensitivity of I_{Ca-T}, in particular different subtypes of α1 subunits, are discussed.

Key words: mibefradil, T-type Ca^{2+} channels

Mibefradil is a tetralol derivative chemically distinct from other Ca^{2+} channel antagonists and characterized by preferential block of T-type Ca^{2+} currents (I_{Ca-T}).[1,2] Mibefradil has been shown to block of I_{Ca-T} in many different tissues including cardiac myocytes,[3,4] vascular smooth muscle cells[2,5] and spermatogenic cells[6] with considerable variation of sensitivity. Mouse cardiac ventricles at embryonic and neonatal period were shown to possess functional T-type Ca^{2+} channels.[7,8] In our recent experiments I_{Ca-T} were recognized in mouse ventricular myocytes at early embryonic period (9.5 day post coitum (dpc)), immediately after initiation of spontaneous beats (unpublished data).

In the present study I_{Ca-T} in mouse ventricular myocytes at 9.5 dpc were investigated the effects of mibefradil.

Material and Methods

Single ventricular myocytes were prepared from early embryonic, 9.5-dpc mouse hearts following the previously described method.[9] In short, pregnant mice were killed and embryos were removed. Cardiac ventricles were dissected from embryos and incubated in enzyme containing solution; 0.3 mg/ml collagenase (Worthington, type II) to isolate single myocytes. Isolated myocytes were cultured overnight in Dulbecco's modified Eagle's Medium (Gibco) with 10 % fetal bovin serum (GIBCO) and 10 μM gentamycin before currents recording.

Current recordings were performed by a whole-cell patch clamp method with an Axopatch-200D (Axon Instruments, Inc.). pClamp 7 software (Axon Instruments, Inc.) was used for data acquisition. The pipette solution contained (mM) CsOH 60, CsCl 80, L-Aspartic acid 40, HEPES 5, EGTA 10, MgATP 5, Na_2-creatinephosphate 5 and $CaCl_2$ 0.65 (pH 7.2 with CsOH). The bath solution contained (mM) TEA-Cl 140, $MgCl_2$ 1, HEPES 5, Glucose 10, $CaCl_2$ 5, 4-aminopyridine 5 and tetrodotoxin (TTX) 0.005 (pH 7.4 with CsOH). Mibefradil was diluted in the bath solution to the desired concentration (0.1 μM to 100 μM) from a stock solution (1 mM in distilled water). Experiments were conducted at 32–35°C. Data are indicated as mean ± SEM.

Results

Figure 1A shows representative records of I_{Ca-T} from mouse ventricular myocytes at 9.5 dpc. The membrane potential was depolarized from holding potential (HP) at −100 mV to −40 mV for 200 ms (a subthreshold limit for activation of L-type Ca^{2+} currents, I_{Ca-L}). Cumulative increase of mibefradil concentration resulted in progressive reduction of I_{Ca-T} peak amplitude. Figure 1B shows the dose-response curve. The data were obtained from 7 ventricular myocytes. The concentration of half inhibition (IC_{50}) of mibefradil was calculated to be 3.4 ± 0.2 μM.

Discussion

The present study has revealed I_{Ca-T} in mouse ventricular myocytes at an early embryonic stage (9.5 dpc). The value of

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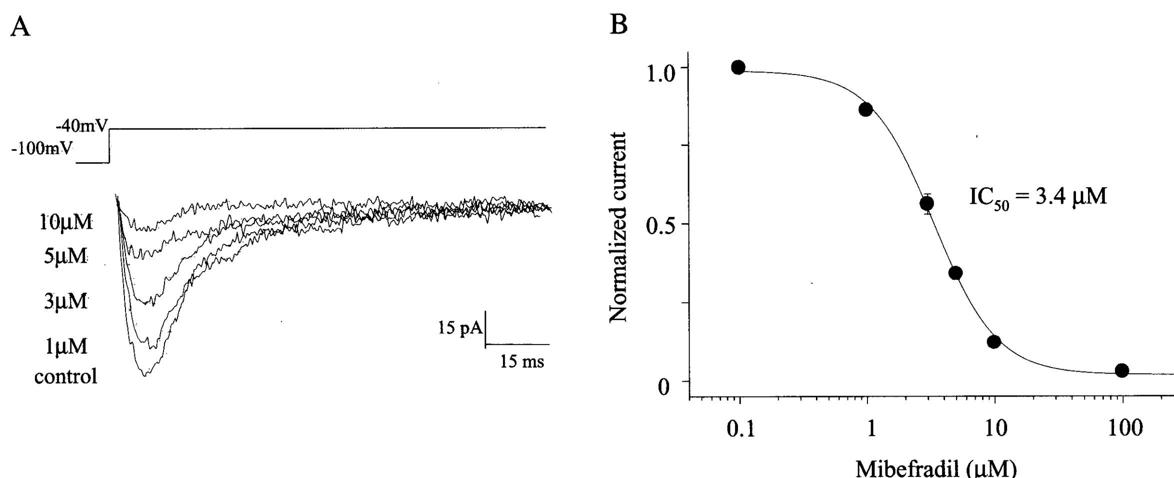

Fig. 1 Effects of mibefradil on T-type Ca^{2+} currents of 9.5-dpc ventricular myocytes.
A, Representative T-type currents elicited during test pulses to −40mV in control condition and in the presence of increasing concentration of mibefradil.
B, Dose-response analysis of mibefradil block. Inhibition of T-type Ca^{2+} currents were normalized to control and then plotted as a function of drug concentration. The smooth curve was generated from a fit to the data (n = 7) with a sigmoidal dose-response equition.

IC_{50} (3.4 μM) is higher than those reported for neonatal rat atrial myocytes (0.1 μM)[3] or adult guinea pig atrial myocytes (1.5 μM).[4]

Recently cloning of T-type Ca^{2+} channels have identified three subtypes of gene, α1G, α1H and α1I.[10,11,12] Molecular analyses have indicated that α1G and α1H subunit gene were expressed in the heart.[11,13] Low sensitivity to mibefradil might attribute to the difference of channel isoform.

The available data were obtained under various recording conditions, i.e., different charge carrier and different temperature. As the charge carrier, both Ca^{2+} and Ba^{2+} have been used and the concentration has ranged from 5 to 30 mM. Cloned T-type Ca^{2+} channels study demonstrated mibefradil had a higher affinity in 2mM Ca^{2+} than in 2mM Ba^{2+} (2.5-fold) and increasing charge carrier concentration reduced the affinity.[14] In addition, most of the previous experiments have been performed at room temperature (20–25°C), while the present study have done under physiological condition at 32–35°C. Martin RL et al. pointed the decrease in the efficacy of the mibefradil at a temperature close to physiological (35°C).[14]

Other possible explanation is that the variation of pulse protocol might reflect diversity of results. Difference of HP and pulse interval could alter the channel status to modify the channel affinity to mibefradil.

In conclusion, the mibefradil blocks $I_{Ca\text{-}T}$ in cardiomyocytes of 9.5-dpc mouse. The sensitivity difference might be due to isoform variation and/or the experimental condition. More studies are required to resolve the low sensitivity for mibefradil in cardiomyocytes of early embryonic mouse.

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Received June 3, 2002; accepted June 14, 2002