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Inhibition of Cardiomyocyte Contractile/Relaxation by MN9202 and Mechanisms Involved

Xiaoxing Zhu,[a,b,*] Xiaoling Zhu,[d,*] Xiaolin Niu,[a] Jianming Pei,[c] Lize Xiong,[d] Shaoyang Chen,[d] Shun-Yan Lu,[c] Jin Wei,[a] Bin Xing,[b] Li Liu,[b] Qibing Mei,[b] and Miaozhang Zhu[c,*]

[a]*Department of Cardiology, Xi'an Jiaotong University, Xi'an, Shaanxi, PR China,*
[b]*Department of Pharmacology,* [c]*Department of Physiology,* [d]*Department of Anesthesiology, Fourth Military Medical University, Xi'an, Shaanxi, PR China*

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Background. The cardiac contractile function of hypertensive patients is higher than non-hypertensive patients so that it is beneficial for lowering cardiac contractile function of hypertensive patients. It remains unclear if MN9202, a dihydropyridine calcium channel blocker, has effects on positive inotropic responses induced by tetraethylammonium chloride (TEA), an antagonist of calcium-activated potassium channels, forskolin (FSK), an activator of adenylyl cyclase, isoproterenol (Iso), an activator of β-adrenergic receptors, and methylene blue (MB), an inhibitor of guanylyl cyclase, in electrically stimulated rat cardiomyocytes. Myocyte shortening and intracellular calcium transients were assessed and the underlying mechanisms were investigated.

Methods. Twitch amplitude was measured by a video edge tracker method. Cell shortening/relengthening indexes including peak height (ph), peak height/baseline percent (ph/bl%), maximal velocity of shortening ($+dL/dt$), and maximal velocity of relengthening ($-dL/dt$) were recorded and analyzed by computer. Calcium transient amplitude (ΔFFI) indicates intracellular calcium transients.

Results. Iso, FSK, TEA, and MB enhanced electrical stimulation induced contraction as evidenced by increased ph, ph/bl%, $\pm dL/dt$, and calcium transient amplitude (ΔFFI) compared with those in the control group. Under basal conditions, MN9202 decreased electrically induced contraction (ph, ph/bl%,$+dL/dt$,$-dL/dt$) in a concentration-dependent manner from 3×10^{-10} to 3×10^{-6} mol/L. MN9202 significantly decreased calcium transient amplitude. Moreover, MN9202 (3×10^{-6} mol/L) partially but significantly blocked the positive inotropic effect induced by Iso, FSK, MB, and TEA through blocking ΔFFI.

Conclusions. Iso, FSK, TEA, and MB increased the shortening and relengthening function of cardiomyocytes, which were partially blocked by MN9202. These results suggest that MN9202 may not only block the dihydropyridine receptor but may also inhibit other calcium influx. The exact mechanism of the action of MN9202 requires further study.

Key Words: MN9202, Cell shortening, Cell relengthening, Forskolin, Methylene blue, Tetraethylammonium chloride, Isoproterenol.

Introduction

Intracellular calcium plays an important role in regulating basic physiological functions of cardiac muscles. Calcium channel blockers have many pharmacological actions: they possess negative inotropic effects, inhibit cardiac hypertrophy, improve hemorheology, vasodilate, protect endothelium, and retard atherosclerosis (1–3). Calcium

*These authors contributed equally to this study.
Address reprint requests to: Xiaolin Niu, Department of Cardiology, Xi'an Jiatong University, No. 5 West Road, Xi'an, Shaanxi, 710032, PR China; E-mail: xx_zhu2003@yahoo.com.cn

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channel blockers are widely used in the treatment of hypertension. Methylpenty1,4-dihydro-2,6-dimethyl-4-(3-nitro-phenyl)-3,5-pyridinediboxylate (MN9202) is a calcium channel blocker and demonstrates antithrombotic, antishock effects in rabbit intestinal ischemia/reperfusion (4–6). In addition, it can inhibit cardiac hypertrophy induced by L-thyroxine and decrease blood viscosity in rats. However, the effects of MN9202 on the performance of cardiac myocyte contraction and relaxation have not been fully investigated.

The application of isolated myocytes provides unique information on cardiac physiology. The presence of heterogeneous cell types and nerve terminals often make the results obtained from multicellular preparations such as papillary muscles difficult to interpret. Mechanical function of the myocardium may be affected by non-myocyte factors such as the coronary vasculature and/or interstitial connective tissue. For example, alterations in contractile performance exposed to ethanol may simply be due to enhanced interstitial fibrosis, not reduced function of individual myocytes. Therefore, it is imperative that there be a tool for physiologists to study the myocyte specifically, regardless of the function of its surrounding tissues. This can be accomplished by video-based edge-detection of the isolated ventricular myocyte. Therefore, the present study was performed to investigate the effects of MN9202 on cardiomyocyte contractile/relaxation function and to identify the mechanisms involved by a video-based edge-detection model of the isolated ventricular myocyte.

Materials and Methods

Reagents

Forskolin (FSK), methylene blue (MB), tetraethylammonium chloride (TEA), and isoproterenol (Iso) were purchased from Sigma Chemical Co. (St. Louis, MO). Collagenase (Worthington Biochemical Corp., Freehold, NJ) and fura-2/AM (Alexis Biochemicals, San Diego, CA) were gifts from Dr. Z. Gao. Methylpenty1,4-dihydro-2,6-dimethyl-4-(3-nitro-phenyl)-3,5-pyridinediboxylate (MN9202) and larcidipine were synthesized by our department.

Animal Preparation

Animals were injected with heparin (1000 U/kg, IP) 20 min prior to the experimental protocol. Animals were anesthetized with ketamine/xylazine (0.1 mL/100 g, IP). Once anesthetized, each animal underwent thoracotomy and its heart was removed. The aorta was suspended using two forceps, and the heart was placed on a perfusion cannula through which buffer perfuse at a slow rate. The aorta was clamped to a perfusion needle and tied inferiorly with surgical thread. The buffer flow rate was increased until the drops fell between 5 and 10 times/min. The carotid arteries filled with buffer and the heart then began beating rhythmically, and the effluent solution became clear.

Isolation Procedure of Rat Ventricular Myocytes

Once successfully suspended, the heart was perfused (at 37°C) with Krebs-Henseleit bicarbonate (KHB) buffer containing (in mM): 118 NaCl, 4.7 KCl, 1.25 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH2PO_4, 25 $NaHCO_3$, 10 N-[2-hydro-ethyl]-piperazine-N′-[2-ethanesulfonic acid] (HEPES) 11.1 mmol/L glucose, equilibrated with 5% CO_2/95% O_2. Next, a nominally Ca^{2+}-free KHB buffer was injected for 2–3 min until spontaneous contractions ceased, followed by a 15–20 min perfusion (depending on the animal's physiological condition) with Ca^{2+}-free KHB containing 223 U/mL collagenase (Worthington Biochemical Corp.) and 0.1 mg/mL hyaluronidase (Sigma). After perfusion, ventricles were removed and minced under sterile conditions and incubated with the Ca^{2+}-free KHB with collagenase solution for 3–5 min. Cells were further digested with 0.02 mg/mL trypsin (Sigma) before being filtered through a nylon mesh (300 μm) and subsequently separated from the collagenase/trypsin solution by centrifugation (60 g for 30 sec). Myocytes were then resuspended in a new aliquot of buffer to remove remnant enzyme and to increase extracellular Ca^{2+} incrementally. This washing process was repeated five times, resulting in a final concentration of 1 mmol/L Ca^{2+}. Myocytes were resuspended in Tyrode's buffer containing (in mM): 131 NaCl, 4 KCl, 1 $MgCl_2$, 10 HEPES, and 10 mmol/L glucose 1 $CaCl_2$. Myocytes with obvious sarcolemmal blebs or spontaneous contractions were not used. Only rod-shaped myocytes with clear edges were selected for recording of mechanical properties as previously described (7).

Cell Shortening/Relengthening

Mechanical properties of ventricular myocytes were assessed using a video-based edge-detection system (IonOptix Corporation, Milton, MA). Cells were placed in a Warner chamber mounted on the stage of an inverted microscope (IX-70; Olympus, Center Valley, PA) and superfused (1 mL/min at 25 °C) with a Tyrode buffer containing (in mM): 131 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, pH 7.4. The cells were field stimulated with suprathreshold voltage at a frequency of 0.5 Hz, with 3 msec duration, using a pair of platinum wires placed on opposite sides of the chamber connected to an FHC stimulator (Bowdoin, ME). The polarity of the stimulatory electrodes was frequently reversed to avoid possible build-up of electrolyte by-products. The studied myocyte was displayed using an IonOptix MyoCam camera, achieving sharp amplitude and velocity shortening/relengthening fidelity with rapidly image scans q8.3 msec (using soft-edge software IonOptix).

Intracellular Ca^{2+} Fluorescence Measurement

Myocytes were loaded with fura-2/AM (0.5 μM; Alexis Biochemicals) for 30 min at 25°C, and fluorescence