Effect of didrovaltrate on L-calcium current in rabbit ventricular myocytes

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Abstract

OBJECTIVE: To investigate the effect of didrovaltrate on L-type calcium current (I_{Ca}) in rabbit ventricular myocytes.

METHODS: We used the whole cell patch clamp recording technique.

RESULTS: Didrovaltrate at concentrations of 30 μg/L and 100 μg/L significantly decreased peak I_{Ca} (I_{Ca,max}) from (6.01±0.48) pA/pF to (3.45±0.27) pA/pF and (2.16±0.19) pA/pF (42.6% and 64.1%, n=8, P<0.01), respectively. Didrovaltrate shifted upwards the current-voltage curves of I_{Ca} without changing their active, peak and reverse potentials. Didrovaltrate affected the steady-state inactivation of I_{Ca}. The half activation potential (V_{1/2}) was significantly shifted from (-26±2) mV (n=6, P<0.05), with a significant change in the slope factor (k) (from 8.8±0.8 to 11.1±0.9, n=6, P<0.05). Didrovaltrate did not affect the activation curve.

CONCLUSION: Didrovaltrate blocks I_{Ca}, in a concentration-dependent manner and probably inhibits I_{Ca}, in its inactive state, which may contribute to its cardiovascular effect.

INTRODUCTION

The mildly sedating effects of valerian (Valeriana officinalis) and its many related Valerianaceae were noted by Greek, Roman, Chinese, and Indian scholars for more than 1000 years. The chemical composition of valerian includes sesquiterpenes of the volatile oil (including valeric acid), iridoids (valepotriates), alkaloids, furano-furan lignans, and free amino acids, such as γ-aminobutyric acid (GABA), tyrosine, arginine, and glutamine. Although the sesquiterpene components of the volatile oil are believed to be responsible for most of valerian’s biological effects, it is likely that all of the active constituents of valerian act in a synergistic manner to produce a clinical response. Research into the physiological activity of individual components of valerian has demonstrated direct sedative effects (valepotriates and valeric acid) and interaction with neurotransmitters, such as GABA (valeric acid and unknown fractions). In recent years, cardiovascular effects of valerian extract, such as hypotensive, negative inotropic, and antiarrhythmic actions, have been reported in many studies. However, the mechanisms underlying the beneficial effects of valerian extract on hearts have not been clearly established. It is
postulated that valerian extract has effects on the voltage-dependent I_{Ca-L}. Therefore, the purpose of the present study was to clarify the effects of valerian extract (didrovaltrate, one of the valepotriates) on the voltage-dependent I_{Ca-L} in rabbit cardiac myocytes.

MATERIALS AND METHODS

Cell isolation

Single rabbit ventricular myocytes were isolated by an enzymatic dissociation procedure modified from Tytgat. New Zealand White rabbits of either sex, weighing 1.0–2.0 kg (Experimental Animal Centre of Medical College, Xiamen University), were anesthetized with 1 mL/kg intramuscularly injected Hypnorm (10 mg/mL fluanisone and 0.315 mg/mL fentanyl citrate; Janssen Pharmaceutics, Tilburg, The Netherlands) and 0.1 mL heparin sodium (5000 U/mL) was injected intravenously. The heart was rapidly removed and mounted on a Langendorff perfusion apparatus. It was retrogradely perfused through the aorta with normal Tyrode’s solution for 10 min, followed by an additional 10 min of perfusion with Ca^{2+}-free Tyrode’s solution. Perfusion was then switched to Ca^{2+}-free Tyrode’s solution containing collagenase II (Sigma-Aldrich, USA) for 15 min. The enzymatic solution was recirculated. All solutions were saturated with 100% O_2 and the temperature was maintained at 37°C. Subsequently, the ventricles were minced and gently agitated in a beaker with low-Ca^{2+} Tyrode’s solution to obtain single myocytes. The calcium concentration was then increased stepwise by replacing approximately 75% of the low-Ca^{2+} Tyrode’s solution by normal Tyrode’s solution. This procedure was repeated four times at intervals of 10 min. The cells were placed in a high-K+ storage solution and gently triturated with a Pasteur pipette. Isolated myocytes were kept in the medium for at least 1 h at room temperature (20–22°C) before use.

Solutions and drugs

The normal Tyrode’s solution contained (mM): NaCl 140, KCl 5.4, MgCl_2 1.0, HEPES 5.0, and glucose 10. The pH was adjusted to 7.4 with NaOH. The pipette solution contained (mM): CsOH 110, TEACl 15, CsCl 20, MgCl_2 1.0, egtazic acid (EGTA, Sigma-Aldrich, USA) 5.0, pyruvic acid 5.0, MgATP 5.0, and HEPES 5.0 at pH 7.3 (adjusted with Tris). The external solution contained (mM): NMDG-Cl 140, MgCl_2 1.0, CaCl_2 1.8, HEPES 5.0, glucose 10, tetrodotoxin (TTX; Hebei Aquatic product Research Institute), and 0.002 and 4-aminopyridine (4-AP, Sigma-Aldrich, USA) 2.0. The pH was adjusted to 7.4 with NMDG. KB solution contained (mM): KOH 110, taurine 10, oxalic acid 10, glutamic acid 70, KCl 25, KH_2PO_4, 10, EGTA 5.0, HEPES 5.0, and glucose 10. The pH was adjusted to 7.4 with KOH. Other chemical reagents were purchased from China. Didrovaltrate was a kind gift from Professor Xue Cunkuan of the Geriatrics Institute of Huazhong University of Science & Technology.

Electrical recordings

Membrane currents and action potentials were recorded using an EPC-9 patch clamp amplifier (HEKA, Germany). Myocytes adhering to glass coverslips were placed in a small chamber mounted on the stage of an inverted microscope and superfused at 1.5 mL/min at room temperature (20–22°C). Action potential recordings were performed at 37°C. Other experiments were performed at room temperature. Only rod-shaped single cells that were quiescent and exhibiting well-defined cross-striations were studied. After establishment of whole-cell configuration and measurement of cell capacitance, series resistance (≤10 MΩ) was compensated by 50% to 70%. Junction potentials under these conditions were -3 mV and corrected. (I_{Ca-L}) was elicited by a series of depolarization steps of 200 ms duration applied in 10-mV increments from a holding potential of -40 mV. Action potentials were recorded in current-clamp mode. Borosilicate glass electrodes (1.0 mm OD) were pulled with a vertical puller (PB-7, Narishige, Tokyo, Japan) and had a resistance of 2-3 MΩ when filled with electrode internal solution. Data acquisition and analysis were carried out using pClamp software (Axon Instruments Inc., Foster City, CA, USA). Current signals were filtered at 3 kHz and sampled at a frequency of 10 kHz. Peak I_{Ca-L} amplitudes were estimated as the difference between the maximal inward current and zero-current.

Statistical analyses

Data are expressed as the mean±SD. Statistical analysis was performed using paired t-tests to determine statistical significance. A probability of P≤0.05 was considered significant.

RESULTS

Effects of didrovaltrate on voltage-dependent I_{Ca-L}

The effects of didrovaltrate on voltage-dependent I_{Ca-L} were examined in single ventricular myocytes (Figure 1). The membrane potential was maintained at -40 mV, and command voltage pulses (200 ms in duration) from -40 mV to +60 mV were applied at 0.2 Hz. Didrovaltrate at 30 μg/L and 100 μg/L significantly decreased peak I_{Ca-L} (I_{Ca-Lmax}) from (6.01±0.48) pA/pF to (3.45±0.27) pA/pF and (2.16±0.19) pA/pF (42.6% and 64.1%, n=8, P<0.01, respectively). After washout, I_{Ca-L} gradually returned to near control levels. The inhibitory effect was concentration dependent and was easily recovered after washout with Tyrode’s solution for
Effect of didrovaltrate on the current-voltage relationship of \( I_{\text{Ca-L}} \)

The current-voltage relationship of \( I_{\text{Ca-L}} \) and the didrovaltrate current-voltage (I-V) curve of L-type current were obtained by a number of depolarizing step pulses (200 ms) from the holding potential of -40 mV to test potentials between -40 mV and 60 mV. The test step pulses were delivered in 10 mV increments. \( I_{\text{Ca-L}} \) was activated at -30 mV and the peak amplitude occurred at the potential of 0 mV. Didrovaltrate at 30 μg/L and 100 μg/L shifted the I-V curve upwards without changes in their active, peak and reverse potentials (Figure 2).

Effect of didrovaltrate on the steady-state inactivation and activation of \( I_{\text{Ca-L}} \)

The effect of didrovaltrate on the steady-state inactivation of \( I_{\text{Ca-L}} \) is shown in Figure 3. The results were well fitted by the Boltzmann equation \( I/I_{\text{max}}=1/[1+\exp(Vm-V_{1/2}/k)] \), where \( I/I_{\text{max}} \) is the ratio of \( I_{\text{Ca-L}} \) amplitude to its maximal value, \( V_{1/2} \) is the voltage at which the normalized \( I_{\text{Ca-L}} \) amplitude is 0.5, and \( k \) is the slope factor. A concentration of 100 μg/L didrovaltrate caused a marked shift of the inactivation curve toward more negative potentials, significantly shifting \( V_{1/2} \) from (26±2) mV to (36±3) mV (n=6, \( P<0.05 \)), with a significant increase in \( k \) (from 8.8±0.8 to 11.1±0.9, \( n=6 \), \( P<0.05 \)). A concentration of 100 μg/L didrovaltrate did not affect the activation curve (data not shown).

DISCUSSION

Our findings demonstrated that didrovaltrate produced voltage-dependent blockade of \( I_{\text{Ca-L}} \) in a concentration-dependent manner. The steady-state inactivation kinetics of \( I_{\text{Ca-L}} \) were changed and the inactivation curve of \( I_{\text{Ca-L}} \) shifted towards more negative potentials. These findings indicated that didrovaltrate inhibited \( I_{\text{Ca-L}} \) by its effect on inactivation kinetics.
Two types of calcium channel have been distinguished on the basis of their electrophysiological and pharmacological characteristics in ventricular myocytes. The physiological significance of the L-type calcium channel is better understood than that of the T-type calcium channel. Calcium influx through the L-type calcium channel is responsible for the upstroke of the action potential in the sinoatrial node and atrioventricular node, and plays an important role in determining the plateau and eventual spike-dome appearance of the action potential in other cardiac cells. The L-type calcium channel is further responsible for the coupling between excitation and contraction, it induces release of Ca\(^{2+}\) from the sarcoplasmic reticulum, and regulates intracellular Ca\(^{2+}\) load. In this way, the L-type calcium channel determines activity of a number of mitochondrial and cytoplasmic Ca\(^{2+}\)-sensitive enzymes.

The underground organs of members of the genus Valeriana (Valerianaceae), as well as related genera such as Nardostachys, are used in the traditional medicine of many cultures as mild sedatives and tranquillizers and to aid the induction of sleep. The anticonvulsive, sedative, and hypnotic effects have been variously attributed to valeric acid, valeranone, and valepotriates. The chemical composition of valerian includes sesquiterpenes of the volatile oil (including valeric acid), iridoids (valepotriates), alkaloids, furanofuran lignans, and free amino acids, such as GABA, tyrosine, arginine, and glutamine. Although the sesquiterpene components of the volatile oil are believed to be responsible for most of valerian’s biological effects, it is likely that all of the active constituents of valerian act in a synergistic manner to produce a clinical response. Research into physiological activity of individual components has demonstrated direct sedative effects (valepotriates and valeric acid) and interaction with neurotransmitters, such as GABA (valeric acid and unknown fractions). In recent years, cardiovascular effects of valerian extract, such as hypotensive, negative inotropic, and antiarrhythmic effects, have been reported in some studies. Our findings demonstrate that didrovaltrate inhibits I\(_{\text{Ca-L}}\), which may contribute to its cardiovascular effects.

**REFERENCES**