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Research Paper

Antiarrhythmic effects of dehydroevodiamine in isolated human myocardium and cardiomyocytes

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ABSTRACT

Ethnopharmacological relevance: Dehydroevodiamine alkaloid (DeHE), a bioactive component of the Chinese herbal medicine Wu-Chu-Yu (*Evodiae frutus*), exerted antiarrhythmic effect in guinea-pig ventricular myocytes. We further characterize the electromechanical effects of DeHE in the human atrial and ventricular tissues obtained from hearts of patients undergoing corrective cardiac surgery or heart transplantation.

Materials and methods: The transmembrane potentials of human myocardia were recorded with a traditional microelectrode technique while sarcolemmal Na^+ and Ca^{2+} currents in single human cardiomyocytes were measured by a whole-cell patch-clamp technique. The intracellular pH (pH_i) and Na^+–H^+ exchanger (NHE) activity were determined using BCECF-fluorescence in human atria.

Results: In human atria, DeHE (0.1–0.3 μM) depressed upstroke velocity, amplitude of action potential, and contractile force, both in slow and fast response action potential. Moreover, the similar depressant effects of DeHE were found in human ventricular myocardium. Both in isolated human atrial and ventricular myocytes, DeHE (0.1–1 μM) reversibly, concentration-dependently decreased the Na^+ and Ca^{2+}currents. Moreover, DeHE (0.1 and 0.3 μM) suppressed delayed afterdepolarizations and after-contractions, induced by epinephrine and high $[Ca^{2+}]_o$ in atria. In human ventricular myocardium, the strophanthidin-induced triggered activities were attenuated by pretreating DeHE (0.3 μM). The resting pH_i and NHE activity were also significantly increased by DeHE (0.1–0.3 μM).

Conclusions: We concluded for the first time that, in the human hearts, DeHE could antagonize triggered arrhythmias induced by cardiotonic agents through a general reduction of the Na^+ and Ca^{2+} inward currents, while increase of resting pH_i and NHE activity.

1. Introduction

Arrhythmia, particularly rapid tachycardia, leads to inadequate ventricular filling, with irregular and disorganized contraction, resulting reduced cardiac output and, unless corrected, death (Taggart, 1989). Antiarrhythmic drugs suppress arrhythmias primarily by alteration of transmembrane ionic current flow (Campbell and Williams, 2001). However, unfortunately, attempts to prevent arrhythmias with drug treatment have not been rewarded with convincing satisfactions, because antiarrhythmic agents currently in clinical use may themselves dispose to proarrhythmic and lethal side effects. For example, flecainide, a type I antiarrhythmic drug, has been found useful to treat refractory ventricular arrhythmia, but, in contrast, it can aggravate pre-existing arrhythmia and congestive heart failure and/or induce life-threatening ventricular arrhythmia that is beyond treatment (Campbell and Williams, 2001). Therefore, development of a new drug with less or no adverse effects for treating life-threaten arrhythmias is still one of the important projects nowadays.

Abbreviations: DeHE, dehydroevodiamine; AP, action potential; DADs, delayed afterdepolarizations; EADs, early afterdepolarizations; ACs, aftercontractions; APD, action potential duration; MDP, maximum diastolic potential; dV/dtmax, maximum upstroke velocity of phase 0 depolarization; $I_{Ca,L}$, L-typed Ca^{2+} current; I_{Na}, Na^+ inward current; V_h, holding potential; pH_i, intracellular pH; NHE, Na^+–H^+ exchanger

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The popular traditional Chinese herb Wu-Chu-Yu (*Evodiae fructus*), the dry fruits of Evodia rutaecarpa (*Rutaceas Jussieu Bentham*), has been widely used in Chinese society to treat a variety of cardiovascular and systematic diseases, such as migraine, edema, emission, inflammation, and infection (Chen et al., 1981; Ueng et al., 2002; Liao et al., 2011). In cardiovascular experiments, Wu-Chu-Yu was demonstrated in vivo to produce a dose-dependent hypertension and an increased contractile force both in anesthetized cats and in conscious rats (Chen et al., 1981). Moreover, in vitro study, Wu-Chu-Yu caused dose-dependently positive inotropic and positive effects both in aortic strip preparations and auricle preparations (Chen et al., 1981). Dehydroevodiamine (DeHE) alkaloid is one of the active principles isolated from the Wu-Chu-Yu (Yang et al., 1990). In contrast to the effects of Wu-Chu-Yu, DeHE was shown to induce hypotensive and bradycardiac effects in rats (Yang et al., 1990). Furthermore, in rabbit and guinea-pig hearts, the similar observation was reported that DeHE (1–30 μM) decreased sinus rate and depressed slow response action potentials (Lin et al., 1990). Using the whole-cell patch-clamp technique, our group firstly demonstrated that a reduction of Ca^{2+} influx contributed mostly to DeHE induced negative chronotropic and ionotropic effects observed. In addition, in our previous study, DeHE (0.1–0.3 μM) was found to be remarkably effective at suppressing triggered arrhythmias in Ca^{2+}-overloaded guinea-pig myocytes induced by cardiotonic steroid in low-K^+ and high-Ca^{2+} superfusate in the guinea-pig cardiac cells (Loh et al., 1992). The ionic underlying mechanisms of these cardioprotective effects of DeHE were mainly due to the inhibitory action of the DeHE on I_{Na} and I_{Ca} (Loh et al., 1992). Therefore, this cardioprotective property of DeHE has attractive considerable interest recently. However, thus far, the electropharmacological and mechanical effects of this compound in human cardiac tissues have not been characterized yet.

Intracellular pH (pH_i) regulation is one of the major homeostatic systems within the cell. Many cellular mechanisms are sensitive to changes of pH_i. This pH homeostasis is especially important for cardiomyocytes, since changes in pH_i dramatically affect contractility and rhythm (Bountra and Vaughan-Jones, 1989; Orchard and Kentish, 1990; Orchard and Cingolani, 1994). The pH_i in cardiac cells is kept within a narrow range (7.0–7.2) through the combined operation of sarcolemmal transporters and intracellular buffering capacity (Leem et al., 1999; Loh et al., 2002a, 2002b, 1996). Sarcolemmal transporters can be divided into two main categories: acid extrusion carriers and acid loading carriers. Na^+–H^+ exchange (NHE), a ubiquitously expressed transmembrane protein, is one element of the acid extrusion system in the sarcolemma of the cardiac cell (Leem et al., 1999; Loh et al., 2002a, 2002b, 1996), which mediates electroneutral exchange of extracellular Na^+ for intracellular H^+ (Aronson, 1985). However, the effects of DeHE on resting pH_i and NHE activity in human myocardia await further investigation.

Therefore, the aims of the present experiments were to explore the electromechanical effects of DeHE in human atrial and ventricular tissues by means of a conventional microelectrode technique. Also, to explore the underlying ionic mechanisms, the effects of DeHE on transmembrane Ca^{2+} and Na^+ currents were also studied in single isolated human atrial and ventricular cells using the whole-cell patch-clamp technique. The microspectrofluorimetry technique was used to examine the effects of DeHE on pH_i and NHE activity in human atrium.

2. Methods

2.1. Human atrial and ventricular tissues

Human atrial tissues were excised from the right atrial appendage as part of the routine atriotomy procedure in 21 patients undergoing open-heart surgery for the treatment of a variety of congenital and acquired heart diseases. Ventricular tissues were obtained from 5 patients undergoing cardiac transplantation. Institutional rules for the protection of human subjects were observed. Prior to surgery, informed consent was acquired. Strands of atrial or ventricular trabeculae with a diameter around 1 mm and a length of 3–6 mm were removed from atrial or ventricular specimen as described previously (Loh et al., 2002c). The preparations were placed in a tissue bath perfused with oxygenated (95% O_2, 5% CO_2) Tyrode solution at 37 °C for further experiments. The composition of normal HEPES Tyrode solution was as follows (mM): NaCl 137, KCl 4, $NaHCO_3$ 21.9, NaH_2PO_4 0.5, $MgCl_2$ 0.5, $CaCl_2$ 2.7, and dextrose 5.5.

2.2. Measurement of transmembrane potentials and contractile force

The preparations were driven at 1 Hz with supra-threshold electrical stimuli of 2 ms duration. One end of the preparation was fixed and the other end was connected to Grass FT03C force-displacement transducer to record force change. Transmembrane potentials were recorded by means of conventional glass microelectrodes filled with 3 M KCl and connected to a Dagan 8500 preamplifier. Both electrical and mechanical events were displayed simultaneously on a Tektronix 5223 storage oscilloscope and a Gould ES 1000 recorder. Measurements were made of action potential amplitude (APA), action potential duration at 50% (APD_{50}) and 90% (APD_{90}) repolarization, maximum diastolic potential (MDP), and maximum upstroke velocity of phase 0 depolarization (dV/dtmax). Action potentials with a dV/dtmax greater than 50 V/s (fast response) were considered to be Na^+-dependent for excitation. To induce Ca^{2+}-dependent slow response action potential, $[K]_o$ was increased to 8 mM or 27 mM and 0.1–1 μM epinephrine was added (Sperelakis and Schneider, 1976; Lin et al., 1985) in the normal HEPES Tyrode solution. To evaluate the depressant effects of DeHE on triggered activity, the tissues were bathed in low-K (1 mM), high-Ca (9 mM) HEPES Tyrode solution plus 2 μM strophanthidin or 2.5 μM epinephrine (Loh et al., 1992).

2.3. Measurement and calibration of the intracellular pH

Measurement of the pH_i has been described in detail in our previous reports (Loh et al., 2002a, 2002b). In brief, the pH_i in the cultured HRASMC was measured using the pH-sensitive, dual excitation single-emission fluorescent dye, 2′,7′-bis(2-carboxethyl)-5(6)-carboxy-fluorescein-acetoxymethyl (BCECF-AM) (Molecular Probes). The preparations were loaded with BCECF-AM (5 μM) by incubating them for 30 min at room temperature and exciting them alternately with 490 and 440 nm wavelength light. The BCECF fluorescence emission ratio of the 510 nm emission at 440 nm and 490 nm excitation (490/440) was calibrated using the K^+-nigericin method (Loh et al., 2002a). Briefly, this method consisted of exposing a BCECF-loaded cell to the six nigericin calibration solutions (listed below in the Solution section) that clamps pH_i to the value of pH_o of the calibration solution. Fig. 1A shows the emission ratio changes seen on perfusing human artery smooth muscle cells with calibration solutions with different 6 pH values (5.5–9.5) in the presence of 10 μM nigericin. The emitted ratio 510 nm emission at 490 nm and 440 nm excitations (R; $R=F_{490}/F_{440}$) was increased as the pH value of superfusing solution was increased. R_{max} and R_{min} are, respectively, the maximum and minimum ratio values for the data curve. The fluorescence of BCECF at 490–440 nm is a function of pH_i and the overall sampling rate in the experiment was 0.5 Hz for the recorded fluorescent ratio (490 nm/440 nm). Using the linear regression fit of the data (shown in Fig. 7B) obtained from 6 calibration experiments similar to that shown in Fig. 7A, the mean apparent dissociation constant (pKa) at 37 °C was found to be 7.27, very close to the value determined by our previous study of the human heart, as well as the value determined by other investigators