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Electrophysiological and mechanical effects of caffeic acid phenethyl ester, a novel cardioprotective agent with antiarrhythmic activity, in guinea-pig heart

Gwo-Jyh Chang [a,*], Chi-Jen Chang [b], Wei-Jan Chen [b], Yung-Hsin Yeh [b], Hsiao-Yu Lee [a]

[a] Graduate Institute of Clinical Medicinal Sciences, College of Medicine, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan
[b] First Cardiovascular Division of Medicine, Chang Gung Memorial Hospital, 5 Fu-Shing St, Kwei-Shan, Tao-Yuan 333, Taiwan

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ABSTRACT

Caffeic acid phenethyl ester (CAPE) is an active component of propolis that exhibits cardioprotective and antiarrhythmic effects. The detailed mechanisms underlying these effects, however, are not entirely understood. The aim of this study was to elucidate the electromechanical effects of CAPE in guinea-pig cardiac preparations. Intracardiac electrograms, left ventricular (LV) pressure, and the anti-arrhythmic efficacy were determined using isolated hearts. Action potentials of papillary muscles were assessed with microelectrodes, Ca^{2+} transients were measured by fluorescence, and ion fluxes were measured by patch-clamp techniques. In a perfused heart model, CAPE prolonged the atrio-ventricular conduction interval, the Wenckebach cycle length, and the refractory periods of the AV node and His–Purkinje system, while shortening the QT interval. CAPE reduced the occurrence of reperfusion-induced ventricular fibrillation and decreased LV pressure in isolated hearts. In papillary muscles, CAPE shortened the action potential duration and reduced both the maximum upstroke velocity and contractile force. In fura-2-loaded single ventricular myocytes, CAPE decreased cell shortening and the Ca^{2+} transient amplitude. Patch-clamp experiments revealed that CAPE produced a use-dependent decrease in L-type Ca^{2+} current ($I_{Ca,L}$) (IC_{50}=1.1 μM) and Na^{+} current (I_{Na}) (IC_{50}=0.43 μM), caused a negative-shift of the voltage-dependent inactivation and a delay of recovery from inactivation. CAPE decreased the delayed outward K^{+} current (I_K) slightly, without affecting the inward rectifier K^{+} current (I_{K1}). These results suggest that the preferential inhibition of Ca^{2+} inward and Na^{+} inward currents by CAPE may induce major electromechanical alterations in guinea-pig cardiac preparations, which may underlie its antiarrhythmic action.

1. Introduction

Caffeic acid phenethyl ester (CAPE) is an active flavonoid-like component of honeybee propolis extracts and has been widely used in folk medicine for many years. CAPE exhibits antioxidant (Sud'ina et al., 1993), anti-inflammatory (Michaluart et al., 1999), and immunomodulatory (Park et al., 2004) activities characteristic of flavonoids, as well as inhibiting the growth of different types of transformed cells (Chen et al., 2001; Chung et al., 2004). In accordance with the above effects, CAPE has been shown to be a potent and specific inhibitor of NF-κB activation (Natarajan et al., 1996).

Throughout the last decade a number of reports have demonstrated many diverse beneficial effects of CAPE on the cardiovascular system. CAPE has been shown to prevent ischemia/reperfusion-induced myocardial injury, possibly as a result of its antioxidant, anti-inflammatory, or anti-apoptotic activity (Cagli et al., 2005; Ince et al., 2006; Ozer et al., 2004; Parlakpinar et al., 2005; Tan et al., 2005). CAPE has also been reported to exhibit antiplatelet activity (Hsiao et al., 2007) and to protect the heart against oxidative injury induced by various cardiotoxic agents (Fadillioglu et al., 2004; Rezzani et al., 2005; Motawi et al., 2011). In addition, CAPE was reported to promote vasodilatation in isolated rat thoracic aorta and this effect was likely due to the inhibition of calcium influx through the smooth muscle cell membrane (Cicala et al., 2003). A recent study further revealed that the vasodilatory activity might also involve the activation of NO/cGMP and β-adrenoceptor/cAMP pathways (Long et al., 2009).

Although several studies have reported on CAPE-induced protection on ischemia/reperfusion-induced myocardial injury, studies investigating its antiarrhythmic actions are relatively rare. We are aware of only one such study, showing that CAPE prevents ischemia- and reperfusion-induced arrhythmias in anaesthetized rats (Huang et al., 2005). The authors speculated that this effect

* Corresponding author. Tel.: +886 3 211 8800x3481; fax: +886 3 328 0170.
E-mail address: gjchang@mail.cgu.edu.tw (G.-J. Chang).

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of CAPE might be related to its antioxidant or vasodilatory or to upregulation of NO activity of CAPE, however, a definite underlying mechanism was not determined. Most antiarrhythmic agents are ionic channel blockers, classified on the basis of their effects on the cardiomyocyte action potential (Vaughan Williams, 1989). Since electrophysiological changes are proposed to be the ultimate mechanism of arrhythmogenesis during both myocardial ischemia and reperfusion (Manning and Hearse, 1984; Pogwizd and Corr, 1987), it is necessary to clarify whether CAPE can modify ion fluxes in cardiomyocytes. Several reports have shown stimulatory effects of CAPE on large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) (Lin et al., 2004) and TREK-1 (a member of the twin-pore background K^+ channels) (Danthi et al., 2004) in neuroendocrine cells and inhibitory effects on Ca^{2+}-release-activated Ca^{2+} channels (CRAC) and K^+ channels in T lymphocytes (Nam et al., 2009). However, to our knowledge, the effects of CAPE on cardiovascular ion channels have never been investigated. Therefore, the aim of this study was to get insight into the electrophysiological and mechanical basis for the antiarrhythmic action of CAPE in guinea-pig heart. Specifically, this study examines the effects of CAPE in isolated hearts, myocardial tissues and single cardiomyocytes.

2. Materials and methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996) and was approved by our institutional review board. Adult male Hartley guinea-pigs weighing 300–350 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and then sacrificed by cervical dislocation.

2.1. Intracardiac electrocardiogram recording

The guinea-pig hearts were quickly excised, mounted in a Langendorff perfusion apparatus, and retrogradely perfused at a rate of 6 ml/min/(g cardiac tissue) with oxygenated (95% O_2 and 5% CO_2) normal Tyrode solution (137 mM NaCl, 5.4 mM KCl, 1.1 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.33 mM NaH_2PO_4, 1.8 mM $CaCl_2$, and 11 mM dextrose) at 37 °C as described previously (Chang et al., 2008). A tungsten spring-soldered platinum electrode was used to record the His bundle electrogram, while the other platinum electrode was used to record ventricular signals. To pace the atrium and ventricle, one pacing electrode was placed on the right atrium and the second one was placed on the right ventricular apex, respectively. Pacing stimuli (1-ms duration, twice-threshold voltage) were delivered by a programmable stimulator (DTU 215, Fischer imaging Co., Denver, CO, USA). The signals were recorded on a chart recorder (WindowGraf, Gould Inc., Cleveland, OH, USA) and digitized simultaneously with a computer-based data acquisition system (IWX/214, iWorx, Dover, NH, USA). Electrophysiological studies were performed according to standard methods described previously (Chang et al., 2008). The QT interval (interval between ventricular depolarization and T wave) was used as the parameter for monitoring ventricular repolarization. The right atrium was then paced at a constant rate which was slightly faster than the spontaneous heart rate. At this constant rate (250 ms cycle length) pacing, the QT interval and the intra-atrial, AV nodal and His–Purkinje conduction times were measured. Incremental right atrial pacing was used to determine the Wenckebach cycle length (WCL), at which the 1:1 AV conduction was lost. Atrial premature extrastimulation (S_2) was then performed to obtain the refractory periods of the atria, AV node, and His–Purkinje system. The ventricular effective refractory period (VERP) was determined using a ventricular extrastimulation study protocol.

2.2. Global ischemia/reperfusion-induced arrhythmias

The guinea-pig hearts were retrogradely perfused through the aorta with normal Tyrode solution at a constant pressure as described previously (Chang et al., 2008). Following a 10-min equilibration period, the hearts were administered vehicle or drug for 10 min. After this pre-ischemic period, the aortic cannula was clamped to institute global no-flow ischemia. The electrograms were recorded from a low atrial and a ventricular electrode. After 30-min ischemia, the aortic cannula was unclamped to permit reperfusion and the incidence and duration of ventricular arrhythmias were recorded and subsequently analyzed. Vehicle or drug was included in the perfusion solution throughout the ischemia and reperfusion period.

2.3. Intraventricular pressure recording experiment

The guinea-pig hearts were retrogradely perfused at a constant pressure of 55 mmHg with normal Tyrode solution. The solution was equilibrated with a gas mixture consisting of 95% O_2 and 5% CO_2 at 37 °C. A latex balloon (size No. 5; Radnoti) connected to a pressure transducer (P23XL-1, Becton Dickinson, USA) using a short stainless steel catheter was then inserted into the LV cavity via left atrium and inflated with approximately 0.04 ml of distilled water, sufficient to produce an LV end-diastolic pressure (LVEDP) of 8–12 mmHg. The ventricles were paced electrically at 250 beats/min. In addition to a polygraph (WindowGraf, Gould) recording, all data were digitized with a computer-based data acquisition system (PowerLab/4sp with Chart 5 software, ADInstruments, NSW, Australia). Each preparation was allowed to equilibrate for at least 2 h before the application of drug. The LV developed pressure (LVDP) was calculated by subtracting the LVEDP from the LV peak systolic pressure. The maximal rates of LV pressure (LVP) development ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) were determined by differentiation of the LV pressure signal. Heart contractility under varying treatment conditions was evaluated following the analysis of $+dP/dt_{max}$.

2.4. Electromechanical recording of papillary muscles

Transmembrane potentials were recorded by using a conventional microelectrode technique (Chang et al., 2008). In brief, the right ventricular papillary muscles (0.5–1 mm in diameter and 3–5 mm in length) were dissected and mounted in a tissue chamber and superfused at a rate of 20 ml/min with an oxygenated normal Tyrode solution at 37 °C. The preparations were stimulated with 1.5 times-threshold strength pulses (pulse duration: 1 ms) at 1 Hz. Each preparation was stretched to a length at which maximum-developed force was evoked and allowed to equilibrate for at least 2.5 to 3 h before the commencement of the experiments. Transmembrane potentials were recorded with a microelectrode filled with 3 M KCl (tip resistance: 15–25 MΩ), which was connected to an Axoclamp 2B amplifier (Molecular Devices, Sunnyvale, CA, USA). The contractile response was recorded by a bridge amplifier (ADInstruments). Action potentials and contractions were digitized at 10 kHz by a PowerLab/4sp digitizer via Chart 5.0 software (ADInstruments) for off-line analysis.

2.5. Measurements of fura-2 fluorescence ratio and cell shortening

Ventricular myocytes were isolated by enzymatic dissociation as described previously (Chang et al., 2006). After digestion, cells were stored in a 0.5 mM Ca^{2+} containing HEPES-buffered Tyrode solution until use. The nominally Ca^{2+}-free HEPES-buffered Tyrode solution contained (mM): NaCl 137, KCl 5.4, KH_2PO_4 1.2, $MgSO_4$ 1.22, dextrose 22, HEPES 6; pH adjusted to 7.4 with NaOH. Cytosolic loading of fura-2 was achieved by incubating cardiomyocytes with