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Conjugated Linoleic Acid (CLA) Consumption May Not Be Beneficial for Cardioprotection

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Abstract: The effects of Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) on cardiac hemodynamic and contractile parameters have been well established. On the other hand, the effects of Conjugated Linoleic Acid (CLA) on cardiac hemodynamic and contractile parameters and have never been investigated. The aims of this study were therefore to investigate the effects of CLA on these parameters in isolated rat heart and cardiac myocytes and to compare its effects to those of EPA and DHA. To investigate the effects on hemodynamic parameters, the aortic stump was cannulated and the heart was perfused with Tyrode's solution. A latex balloon was inserted into the Left Ventricle (LV) to measure LV Pressure (LVP) and Left Ventricular Developed Pressure (LVDP). The results showed that both 20 μM EPA and 20 μM DHA significantly reduced increases in LVP and LVDP induced by 0.1 mM ouabain, an inhibitor of Na^+-K^+ pump. Unlike EPA and DHA, 10 μM CLA further potentiated increases in LVP and LVDP induced by ouabain. To investigate the effects on contractile parameters (i.e., $[Ca^{2+}]_i$), propagated waves of calcium release in myocytes loaded with fluo 3 were imaged using laser scanning confocal microscopy. Imaging of the waves of calcium release showed that the amplitude and the rate of propagation of the wave did not increase in CLA but showed an increase in the rate constant for decay of calcium wave profile. Thus, the data clearly suggest that the effects of CLA are opposite to those of EPA and DHA suggesting different mechanisms actions. Instead of decreasing cardiac hemodynamic and contractile parameters, CLA increases them. The mechanisms whereby CLA increases cardiac hemodynamic and contractile parameters are probably due to the inhibition of sarcoplasmic reticulum calcium uptake.

Key words: Eicosapentaenoic acid, docosahexaenoic acid, conjugated linoleic acid, left ventricular pressure, isolated rat heart, calcium wave, cardiomyocyte

INTRODUCTION

Now-a-days, consumers are interested in nutritional values of products derived from animals and pay more attention to the relationship between food and health. Natural supplements which benefit for health (i.e., heart health) have been introduced in different ways to the production of animals. One of these is the use of polyunsaturated fatty acids both n-3 polyunsaturated fatty acids including Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) and n-6 polyunsaturated fatty acids found in milk and beef products such as Conjugated Linoleic Acid (CLA). These fatty acids were fed to animals by adding in feedstuffs, resulting in increased levels of such fatty acids in animal products including milk, eggs and meat. In addition to increase product value, fatty acid supplementation also provides health benefit to consumers.

It has been reported that consuming n-3 polyunsaturated fatty acids both EPA and DHA helps to reduce risk of atherosclerosis, lower cholesterol (Frenoux *et al*., 2001; Diniz *et al*., 2004), prevent cardiac arrhythmia (O'Neill *et al*., 2002; Kang and Leaf, 1994, 2000; Brouwer *et al*., 2006) whereas consuming n-6 polyunsaturated fatty acids, CLA, helps to prevent atherosclerosis and cancer, enhance immune system and lower cholesterol (Akahoshi *et al*., 2003; Kritchevsky *et al*., 2004; Park *et al*., 2005; Nestel *et al*., 2006; Simon *et al*., 2006; Lopes *et al*., 2008). Disorders mentioned above are accepted as major causes of early death in humans. Several studies have demonstrated the effects of EPA and DHA on cardiac hemodynamic (Shah *et al*., 2009a, b) and contractile parameters (Negretti *et al*., 2000). However, there is no or little information demonstrating the effects of CLA on both parameters. Thus, the aims of the present study were therefore to investigate the effects of CLA on hemodynamic parameters in isolated rat heart and to investigate the effects of CLA on contractile parameters (i.e., $[Ca^{2+}]_i$) in isolated cardiac myocytes. We particularly

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investigated the effects of CLA on Left Ventricular Pressure (LVP) and Left Ventricular Developed Pressure (LVDP) and compared its effects with those of EPA and DHA. The effects of CLA on propagated waves of calcium release in myocytes were also investigated using laser scanning confocal microscopy.

MATERIALS AND METHODS

Experimental animals: A total of 15 adult male Wistar rats (250-300 g) were used in the present study. Experiments were conducted in accordance with the advice of the Institutional Animal Care and Use Committee, Suranaree University of Technology, Thailand. The rats were maintained under environmentally controlled room at 24±1°C. They were fed with commercial food and allowed to access water *ad libitum*.

Langendorff perfused hearts: Rats were killed by stunning and cervical dislocation. The heart was then excised through a bilateral thoracotomy and pericardiotomy and immediately removed. The aortic root was cannulated with a 16 gauge cannula and heart was perfused in a retrograde fashion using a Langendorff apparatus. The perfusate consisted of Tyrode's solution which contained (mM): NaCl, 135; KCl, 4; Hepes, 10; glucose, 11; $MgCl_2$, 1; titrated to pH 7.4 with NaOH. The temperature of the perfusate solution was controlled at 37°C. In order to measure hemodynamic parameters, a latex balloon was carefully inserted into a left ventricle of the isolated heart. The balloon was inflated water to create a diastolic pressure (LV) of 7 mmHg. LVP were measured and monitored continuously by a physiological recoding system. Additionally, LVDP was defined as LVP-LV (mmHg).

Effects of polyunsaturated fatty acids: The measurements of polyunsaturated fatty acids on LVP were made whilst the heart was spontaneously beating and continually perfused with Tyrode's solution at constant perfusion pressure of 80 mmHg. The experiment consisted of four periods, each lasting 5 min (Fig. 1). After stabilization period (control phase), the perfusion with the tested substance started: 0.1 mM ouabain (an inhibitor of Na^+-K^+ pump; Kang and Leaf, 1994), 0.1 mM ouabain plus either 20 μM EPA, 20 μM DHA or 10 μM CLA and then 0.1 mM ouabain plus one of those fatty acids and free-fatty acid bovine serum albumin (BSA, 2 mg mL^{-1}).

Effects of CLA on propagated waves of calcium release: Effects of CLA on propagated waves of calcium release were performed on myocytes loaded with fluo 3 using laser scanning confocal microscopy as previously described (Negretti *et al*., 2000). Rats were killed by stunning and cervical dislocation. Rat myocytes were isolated using a collagenase and protease technique. Cells were loaded with the membrane permeant from fluo 3 for 5 and 20 min was allowed for esterification. Cells were placed in a superperfusion chamber on the stage of an inverted microscope. Fluo 3 fluorescence was excited at 488 nm and measured at 515 nm using the BioRad MRC 1024 confocal microscope. The bathing solution was Tyrode's solution. Initially, cells were bathed in the above solution at 1 mM $CaCl_2$; later this level was altered to 5 mM to induce spontaneous waves of calcium release and the effects of CLA on calcium wave profiles measured.

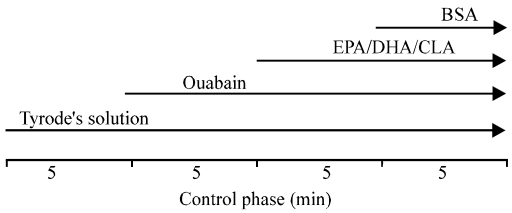

Fig. 1: Flowchart of the experiment

Statistical analysis: Data were analyzed by Student's t-test. $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Effects of polyunsaturated fatty acids on hemodynamic parameters: As shown in Table 1 and Fig. 2, an addition of 0.1 mM ouabain, an inhibitor of Na-K pump (Kang and Leaf, 1994) significantly increased LVP as well as LVDP compared with control. When 10 μM CLA was added in the continued presence of 0.1 mM ouabain both LVP and LVDP were further potentiated. The increment of these hemodynamic parameters was significantly higher than that of ouabain alone. It is interesting to note that the stimulatory effects of this fatty acid could not be washouted by 2 mg mL^{-1} BSA.

Unlike CLA, 20 μM EPA or 20 μM DHA significantly decreased LVP and LVDP when it was added after 0.1 mM ouabain. The decrement of these hemodynamic parameters was significantly lower than that of control. The inhibitory effects of these fatty acids could be slightly washouted by 2 mg mL^{-1} BSA.

Effects of polyunsaturated fatty acids on calcium wave profiles: Typical linescan measurements are shown in Fig. 3. The propagating wave of calcium release begins at the right of the cell and propagates to the left in both panels (Figs. 3a and b). In the presence of 10 μM CLA, it is clear that the peak calcium reached in the wave does

not differ from control (Fig. 3c). In CLA, the wave does not propagate faster as indicated by the same steep slope of the wavefront, i.e., the wave takes an equal amount of time to propagate along the cell (Fig. 3c). It is interesting to note that the rate constant for decay of calcium wave profiles with CLA was slower (Fig. 3c).

In the present study, CLA does not impair LVP but acts as a positive inotrope in both LVP in muscle preparations and isolated cardiac myocytes. This is opposite to those of EPA and DHA which have been shown to have a negative inotrope in both LVP in muscle preparations (Shah *et al*., 2009a, b) and isolated cardiac myocytes (Negretti *et al*., 2000). The mechanisms whereby EPA and DHA exerted their inotrope effects have been well documented in both isolated rat heart (Shah *et al*., 2009a, b) and isolated cardiac myocytes (Kang and Leaf, 1994; Negretti *et al*., 2000). However, the present study extends previous work by demonstrating that EPA and DHA not only effectively prevent and terminate lethal tachyarrhythmias (contracture/fibrillation) induced by high extracellular calcium concentrations or

Table 1: Hemodynamic data

Groups	Phase	Left ventricular pressure (mmHg)
CLA	Control	90.13±0.04
	Ouabain	91.16±0.06*
	Ouabain+CLA	99.52±0.36*
	Ouabain+CLA+BSA	99.65±0.28*
EPA	Control	88.28±0.12
	Ouabain	90.5 ±0.13*
	Ouabain+EPA	85.44±0.03*
	Ouabain+EPA+BSA	86.66±0.07*
DHA	Control	82.77±0.09
	Ouabain	85.19±0.19*
	Ouabain+DHA	79.43±0.16*
	Ouabain+DHA+BSA	80.20±0.35*

Data are expressed as mean±SEM; *p<0.05 compared with the control value in the group

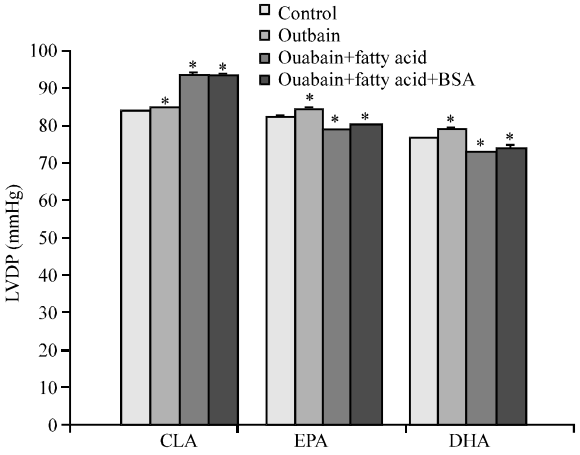

Fig. 2: Left ventricular developed pressure (LVDP); p<0.05 compared with the control value in the group

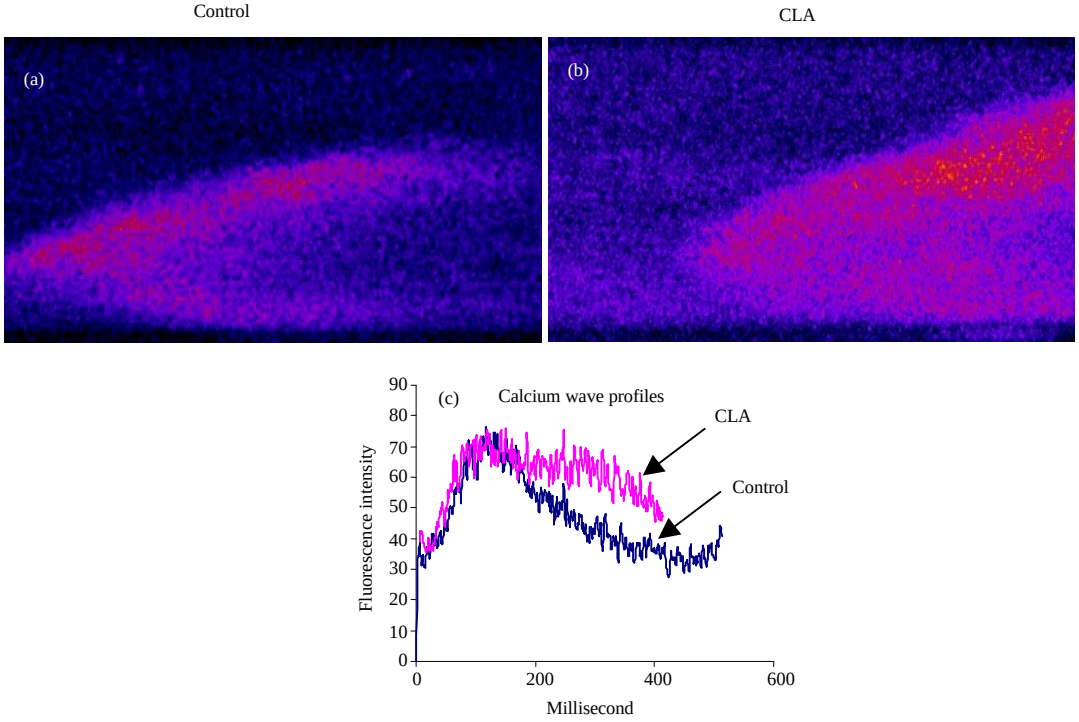

Fig. 3: Calcium wave propagation in a single ventricular myocyte loaded with fluo-3; a and b shows linescan images of calcium waves. Each scan shows a wave propagating along the cell in; a) control and b) 10 μM CLA. The cell was bathed in 5 mM external calcium to induce spontaneous waves. Wave propagation proceeds from right to left in both panels; c) Fluorescence intensity of calcium wave profiles plotted from a and b

ouabain in isolated cardiac myocytes (Kang and Leaf, 1994) but they also decrease cardiac contraction as indicated by the fall in LVDP (Fig. 2). Surprisingly, we have found that CLA did not decreased increases in cardiac contraction induced by ouabain but further potentiated the contraction as indicated by the rise in LVDP (Fig. 2). To date, there is no information demonstrating the mechanisms whereby CLA inserts its effects on hemodynamic parameters making difficulties in interpretation of the findings. We considered examining the effects of CLA on propagated waves of calcium release in myocytes loaded with fluo 3 using laser scanning confocal microscopy.

The results clearly showed that the effects of CLA on increasing cardiac contraction occurred via blocking of the sarcoplasmic reticulum calcium uptake. This conclusion comes from the imaging of the waves of calcium release and the fluorescence intensity of calcium wave profiles (Fig. 3). The results also confirmed that CLA do act beyond the surface membrane (i.e., the sarcoplasmic reticulum) as BSA could not prevent its effects.

CONCLUSION

The data clearly suggest that the effects of CLA are opposite to those of EPA and DHA suggesting different mechanisms of actions between n-6 and n-3 polyunsaturated fatty acids. Unlike EPA and DHA, consumption of CLA may not help to decrease risk for heart failure and attenuate pathologic cardiac remodeling in response to pressure overload.

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