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European Journal of Pharmacology 297 (1996) 97–106

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Protective effects of free polyunsaturated fatty acids on arrhythmias induced by lysophosphatidylcholine or palmitoylcarnitine in neonatal rat cardiac myocytes

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Received 29 June 1995; revised 15 September 1995; accepted 20 October 1995

Abstract

Cultured, spontaneously beating, neonatal rat cardiac myocytes were used to examine the effects of various free fatty acids added to the medium perfusing the cells on lysophosphatidylcholine (LPC)- or acylcarnitine-induced arrhythmias. Perfusion of the cells with LPC or palmitoylcarnitine (2–10 μM) induced sustained tachyrhythmia with episodes of spasmodic contractures and fibrillation. Free PUFA (10–15 μM) including eicosapentaenoic acid (EPA, 20:5n–3), docosahexaenoic acid (DHA, 22:6n–3), α-linolenic acid (18:3n–3), arachidonic acid (AA, 20:4n–6) and linoleic acid (18:2n–6) were able to effectively prevent as well as terminate the LPC or acylcarnitine-induced arrhythmias. In contrast, monounsaturated oleic acid (18:1n–9) and saturated stearic acid (18:0) did not have such effects. The protective effects of the polyunsaturated fatty acids (PUFA) could be reversed by cell perfusion with delipidated bovine serum albumin. To determine the potential primary action by which the PUFA exert the antiarrhythmic effects, measurements of intracellular Ca^{2+} levels and the response of the cells to electrical pacing in the absence or presence of the PUFA were performed and the effects of verapamil (a L-type Ca^{2+} channel blocker), tetrodotoxin (a Na^{+} channel blocker) and Ca^{2+} ionophore A23187 on the cell contraction and the cytosolic Ca^{2+} levels were compared with that of the PUFA. Results suggest that an inhibitory effect on the electrical automaticity/excitability of the cardiac myocyte rather than a reduction in cytosolic Ca^{2+} underlie the protective effects of PUFA. In conclusion, free PUFAs are able to effectively protect the cardiac myocytes against the arrhythmias induced by low concentrations of lysophosphatidylcholine or palmitoylcarnitine.

Keywords: Lysophosphatidylcholine; Acylcarnitine; Fibrillation; Fatty acid; Excitability; Antiarrhythmic

1. Introduction

Ventricular arrhythmias remain the leading cause of death from acute myocardial infarctions. Numerous substances accumulating in ischemic myocardium have been implicated as putative arrhythmogenic factors (Curtis et al., 1993). Lysophosphatidylcholine (LPC) which accumulates in the ischemic heart (Sobel et al., 1978; Corr et al., 1982, 1987b; Shaikh and Downar, 1981; Man et al., 1983) has been incriminated as one of the biochemical factors for the production of cardiac arrhythmias (for reviews see refs. Curtis et al., 1993; Corr et al., 1984, 1987a; Corr, 1990; Katz and Messineo, 1981). Perfusion of cardiac myocytes with LPC produces a number of arrhythmogenic electrophysiological derangements including a depolarization of the resting membrane potential (Corr et al., 1979, 1981, 1982; Arnsdorf and Sawicki, 1981), increases in automaticity (Corr et al., 1979, 1981; Arnsdorf and Sawicki, 1981; Clarkson and Ten Eick, 1983; Duan and Moffat, 1991) and occurrence of delayed afterdepolarizations and triggered activity (Pogwizd et al., 1986). These electrophysiological alterations produced by exogenous LPC appear to mimic those observed in ischemic tissue. Perfusion of the isolated heart or myocytes with LPC has also been shown to produce arrhythmias/cell contractures associated with cytosolic Ca^{2+} overload

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SSDI 0014-2999(95)00701-6

(Sedlis et al., 1983; Woodley et al., 1991; Liu et al., 1991). Furthermore, a correlation between the elevation of LPC levels and the production of arrhythmias in the ischemic heart has been established (Corr et al., 1987b; Kinnaird et al., 1988). Such studies provide support for the notion that LPC is involved in the generation of arrhythmias during cardiac ischemia (Corr, 1990; Corr et al., 1984, 1987a; Katz and Messineo, 1981). Palmitylcarnitine, another amphiphile known to accumulate in ischemic tissue (DaTorre et al., 1991), produces similar arrhythmogenic effects on the myocardium including alterations of both electrophysiologic and mechanical properties (Corr et al., 1981; DaTorre et al., 1991), and has also been proposed as an endogenous chemical mediator of ventricular arrhythmias in ischemic heart disease (Curtis et al., 1993; Corr, 1990; Corr et al., 1987a; Katz and Messineo, 1981).

Recent studies have revealed a role for ω-3 fatty acids in the prevention of fatal ventricular arrhythmias. Clinical trials in humans have shown a significant reduction in the incidence of sudden death from coronary heart disease in subjects whose diets contained long-chain ω-3 fatty acids (Burr et al., 1989; De Logeril et al., 1994). McLennan et al. have found that a diet high in fish oil, in contrast to saturated fat and monounsaturated fat (olive oil), prevented ventricular fibrillation induced by coronary artery ligation in rats (McLennan et al., 1985, 1988) and increased the electrical ventricular fibrillation thresholds in marmosets (McLennan et al., 1992). Billman et al. (1994) have shown that an intravenous infusion of an emulsion comprised largely of eicosapentaenoic acid (EPA, 20:5n–3) and docosahexaenoic acid (DHA, 22:6n–3) can prevent ischemia-induced ventricular fibrillation in conscious, prepared dogs. Our previous studies also indicate that free long-chain polyunsaturated fatty acids, but not saturated and monounsaturated fatty acids, at 5–10 μM can markedly reduce the contraction rate of isolated, spontaneously beating cardiomyocytes (Kang and Leaf, 1994), inhibit membrane electrical excitability of the myocytes (Kang et al., 1995), and prevent or terminate the tachyarrhythmias and fibrillation induced by ouabain (Kang and Leaf, 1994), high extracellular Ca^{2+} (Kang and Leaf, 1994) or a β-adrenoceptor agonist (Kang and Leaf, 1995). In the present study, we used a defined system to test the efficacy of various fatty acids to prevent LPC or acylcarnitine-induced arrhythmias in neonatal rat cardiac myocytes and examined the possible mechanisms responsible for their protective effects. We report here that free polyunsaturated fatty acids but not monounsaturated or saturated fatty acids at 10–15 μM can effectively prevent and terminate the arrhythmias induced by low concentrations (2–10 μM) of LPC or acylcarnitine, and that the protective effects of PUFA may be related to their inhibitory effect on the automaticity/excitability of the cardiac myocytes with resulting stabilization of their electrical excitability.

2. Materials and methods

2.1. Cell culture

Cardiac myocytes were isolated from 1 day old rats using the Neonatal Cardiomyocyte Isolation System (Worthington Biochemical Corp., NJ). The method is based on that described by Torasson et al. (1989) in which the minced tissue is incubated overnight with trypsin at 4°C. The isolated cells were placed on 15 mm glass coverslips in petri dishes and cultured at 37°C in air with 5% CO_2 added and 98% relative humidity in a tissue culture incubator (Model 3123, Forma Scientific, OH). The culture medium was changed every other day. After 48 h in culture, cells exhibited regular spontaneous contractions. Cells were used for experiments after 3–5 days of culture.

2.2. Measurement of cell contraction

Changes in amplitude of contraction and beating rate of cultured cardiomyocytes were determined using a phase contrast microscope and video-monitor edge-detector as described previously (Barry and Smith, 1984). A glass coverslip with attached cultured myocytes was placed in a chamber which was continuously superfused during contractility measurements with a Hepes-buffered saline solution (in mM: 140 NaCl, 5 KCl, 1.0 $MgCl_2$, 1.2 $CaCl_2$, 1.0 Na_2HPO_4, 5.0 Hepes, 10 glucose, pH was adjusted to 7.4 with NaOH). The chamber was placed on the stage of a Zeiss Axiovert 10 inverted microscope which was enclosed by a temperature-controlled, lucite box heated to 37°C. The inlet to the perfusion system was connected by a 4-way manifold with polyethylene tubing connecting to two syringe pumps (Harvard Apparatus, MA) so that the coverslip could be sequentially superfused with different solutions. The flow rate was 20 ml/h. The cells were magnified with a 32 × objective. Plastic 2–3 μm diameter microspheres, which were added to the culture on day 2 provided an improved image for measurement of motion. The image was monitored with a CCD video camera (Javelin Electronics, Los Angeles, CA) attached to the microscope observation port and displayed on a television screen. Motion along a selected rastor line segment was quantitated by a video motion detector system (Barry and Smith, 1984). After a 5–10 min stable baseline during superfusion with normal Hepes-buffered solution, motion signals were recorded. Perfusion with various test solutions was then carried out, and motion signals recorded with a Panasonic

AG-1960 tape recorder and a Grass 79D strip chart recorder (Grass Instrument Co., Quincy, MA).

2.3. Measurement of intracellular Ca^{2+}

Changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were measured with the fluorescent dye fura-2 (Grynkiewicz et al., 1985) using a IonOptix Whole-cell Photometry System (Milton, MA). Cultured myocytes attached to coverslips were loaded with fura-2 by incubating the cells in the Hepes-buffered solution containing 3 μM fura-2-AM and 0.05% Pluronic F-127 at 37°C for 60 min. After washing 3 times with fresh buffer solution, cells loaded with fura-2 were placed on the microscope stage and illuminated with a dual-wavelength excitation light source. A collimated light beam from a 75-W xenon arc lamp was passed through a computer-controlled electronic shutter, and then through a narrow bandwidth interference filter driven by a precision stepper motor, and was conveyed to the microscope by a flexible light guide/coupling system to provide alternating 360- and 380-nm excitation light to the cells. Light (fluorescence emission wavelength) returning through the objective and dichroic mirror passed through a fluorescence emission barrier filter and was collected by a photomultiplier tube. The photon generated current spikes in the photomultiplier tube were converted to digital voltage levels by an amplifier/discriminator so they could be counted by the digital counter on a photon counting circuit board connected inside an IBM PC (486) computer. The cells were also illuminated with light from the standard microscope light source passed through a deep red (700 nm) filter. This wavelength did not interfere with fluorescence detection and was matched to the spectral sensitivity of the video camera used to detect cell motion, so that cell motion and fluorescence intensity could be simultaneously recorded. The fluorescence information (raw data, ratios or $[Ca^{2+}]_i$) and cell contraction were stored in the computer and could be displayed in real-time on the computer monitor. In the present study we used the 360/380 nm fluorescence ratio to estimate changes in $[Ca^{2+}]_i$.

2.4. Electrical pacing of myocytes

Cells were paced with a programmed series of square-wave impulses (10–30 V with a duration of 100 ms). The electrical impulses were generated by a S4E Stimulator (Grass Instrument Co., Quincy, MA) and were delivered to the cells through a pair of platinum electrodes that were immersed in the perfusion solution at the two ends of the perfusion chamber.

2.5. Materials

Neonatal Cardiomyocyte Isolation System Kit was purchased from Worthington Biochemical Corp., NJ. Fura-2, pluronic F-127 and 4-bromo A23187 were obtained from Molecular Probes, Eugene, OR. Lysophosphatidylcholine, palmitoylcarnitine, fatty acids, ETYA (eicosatetraynoic acid), verapamil and fatty acid-free bovine serum albumin were obtained from Sigma Chemical Co., St Louis, MO. Tetrodotoxin was pur-

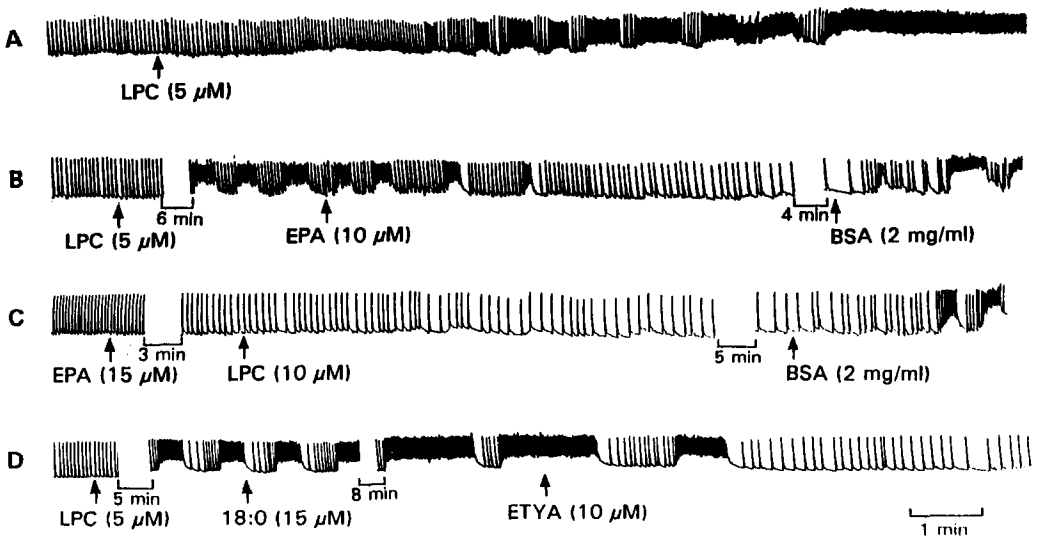

Fig. 1. Protective effects of PUFA on LPC-induced arrhythmias in isolated neonatal rat cardiomyocytes. (A) Typical arrhythmia induced by 5 μM LPC. (B) After induction of arrhythmia by LPC (5 μM), addition of EPA (10 μM) to the perfusate terminated the tachyarrhythmia and contractures followed by a slow beating rate. Subsequent addition of BSA (2 mg/ml) still in the presence of LPC reinstated the tachyrhythmia and contractures ($n = 13$). (C) After perfusion with 15 μM EPA for 5 min, addition of 10 μM LPC failed to induce tachyarrhythmias. But when BSA (2 mg/ml) was subsequently added to the medium, the cell contractures occured ($n = 5$). (D) Addition of stearic acid (15 μM) could not stop the arrhythmias induced by LPC (5 μM). Subsequent addition of 10 μM ETYA quickly terminated the arrhythmias ($n = 5$).

chased from Biomol, Plymouth Meeting, PA. Fatty acids were dissolved weekly in ethanol at a concentration of 10 mM and stored under a nitrogen atmosphere at −20°C. The final concentration of ethanol was negligible and had no effect on myocyte contraction.

3. Results

Perfusion of the cultured myocytes with medium containing LPC induced an increased basal beating rate with intermittent severe tachyarrhythmias, contractures, fibrillations. The effect of LPC on the contraction of the cells is concentration-related and time-dependent. The minimal concentration required to induce arrhythmias in our experimental conditions was as low as 2 μM. The onset times of the effect of LPC at 2, 5 and 10 μM were 6.5 ± 1.8, 3.5 ± 1.5 and 2.0 ± 1.0 min (n = 15), respectively. Fig. 1A shows an example of the arrhythmias induced by 5 μM LPC. To test the effects of various fatty acids on LPC-induced arrhythmias, individual fatty acids were added to the perfusion solution either prior to or after induction of arrhythmias by LPC. As shown in Fig. 1B, addition of 10–15 μM eicosapentaenoic acid (EPA, C20:5n–3) stopped the tachyarrhythmias induced by 5–10 μM LPC within 2–3 min followed by a slowing of the beating rate. Subsequent addition to the perfusate of delipidated bovine serum albumin (BSA, 2 mg/ml), which has a high affinity for free fatty acids and can extract the fatty acid out of the myocytes, reversed the effect and the arrhythmia recurred (n = 13). (Perfusion of the cells with BSA alone at this concentration did not affect the cell contractions). Alternatively, pre-perfusion of the cells with 10–15 μM EPA for 3–5 min or simultaneous addition of EPA and LPC completely prevented the occurrence of the arrhythmias induced by 5–10 μM LPC (n = 5), but tachyarrhythmias would occur if delipidated BSA (2 mg/ml) was subsequently added to the medium (Fig. 1C). With 5–10 μM LPC less than 5 μM EPA generally failed to stop the arrhythmia, whereas EPA concentrations of 10–15 μM stopped the arrhythmia, indicating a concentration dependence of the PUFA effect. However, if the LPC concentration was higher than 15 μM, application of 10–15 μM EPA, in most cases, failed to protect the cells from arrhythmias.

The effects of other fatty acids on LPC-induced arrhythmias have also been tested. Results showed that the polyunsaturated fatty acids including docosahexaenoic acid (DHA, C22:6n–3), α-linolenic (C18:3n–3), linoleic acid (C18:2n–6) and arachidonic acid (AA, C20:4n–6) had similar effects (n = 5 for each fatty acid, data not shown). In addition, eicosatetraynoic acid (ETYA), a nonmetabolizable analog of AA and a potent inhibitor of the cyclooxygenase, lypoxygenase, and expoxygenase enzymatic pathways, also exhibited the protective effects as did EPA (Fig. 1D). In contrast, saturated palmitic (C16:0) or stearic (C18:0) acid and monounsaturated oleic acid did not show any protective effect against LPC-induced arrhythmias (Fig. 1D) (n = 5 for each fatty acid). To rule out the possibility

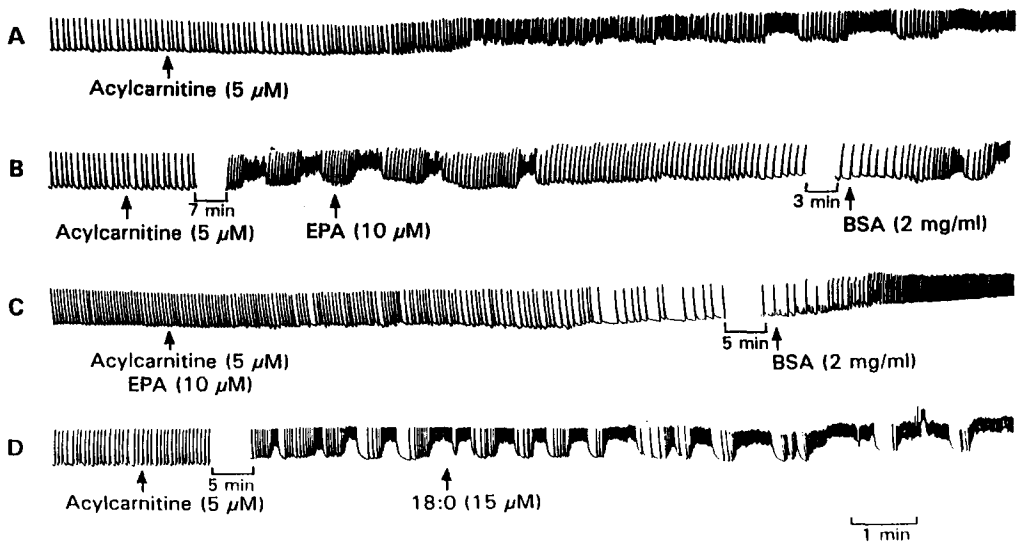

Fig. 2. Protective effects of PUFA on palmitoylcarnitine-induced arrhythmias in isolated neonatal rat cardiomyocytes. (A) Perfusion of the cells with 5 μM palmitoylcarnitine induced tachyarrhythmias and contractures. (B) After induction of arrhythmia by palmitoylcarnitine (5 μM), addition of EPA (10 μM) to the perfusate terminated the tachyarrhythmia and contractures followed by a slow beating rate. Subsequent addition of BSA (2 mg/ml) still in the presence of the acylcarnitine reinstated the contractures (n = 5). (C) Simultaneous addition of 5 μM palmitoylcarnitine and 10 μM EPA exhibited no significant tachyarrhythmias. But when BSA (2 mg/ml) was subsequently added to the medium, the cell contractures occurred (n = 3). (D) Addition of stearic acid (15 μM) failed to terminate the arrhythmias induced by 5 μM palmitoylcarnitine (n = 3).

that failure of saturated and monounsaturated fatty acids to show protective effects was due to their poor solubility in the medium, these fatty acids were added together with BSA in a molar ratio of 7–10:1 (FA/BSA). Results showed that the saturated and monounsaturated fatty acids so transported still failed to show any protective effect, whereas the EPA added with BSA in the same ratios was still protective (data not shown).

Perfusion with palmitoylcarnitine (2–10 μM) induced arrhythmias similar to that observed with LPC (Fig. 2). Similarly, the polyunsaturated fatty acids, but not the saturated and monounsaturated fatty acids, at concentrations of 10–15 μM could prevent and terminate the tachyarrhythmias induced by 2–10 μM palmitoylcarnitine ($n = 5$ for EPA, $n = 3$ for each other fatty acid).

Since previous studies have suggested that the LPC-induced contracture of cardiac myocytes is due to an increase in the intracellular Ca^{2+} ion concentration ($[Ca^{2+}]_i$)(Woodley et al., 1991; Liu et al., 1991), we tested whether PUFAs have an effect on $[Ca^{2+}]_i$ in the cells. Both contractile motion and changes in $[Ca^{2+}]_i$ were simultaneously measured before and after addition of fatty acid in cells with or without stimulation of LPC. Fig. 3A shows a simultaneous recording of cell contraction and the 360/380 nm fluorescence intensity ratio, which indicates changes in $[Ca^{2+}]_i$ within the cells, prior to and 10 min after addition of EPA (10 μM) in the absence of LPC. Although a marked reduction in the beating rate occurred, there was no significant change in systolic and diastolic $[Ca^{2+}]_i$ as indicated by 360/380 fluorescence intensity signal ($n = 6$). Perfusion with 5 μM LPC elevated both diastolic and systolic $[Ca^{2+}]_i$ by 30–100% with intermittent further increases as cells developed episodic contractures (Fig. 3B). Addition of EPA (10 μM) abolished the intermittent fluctuations of $[Ca^{2+}]_i$ and cell contractures followed by slow rates of Ca^{2+} transients and cell contractions without a significant reduction in the amplitudes of the Ca^{2+} transients or contractions. However, the basal levels of $[Ca^{2+}]_i$ after addition of EPA remained higher than the original levels ($n = 6$). To see whether blocking L-type Ca^{2+} ion channels mimics the effects of EPA, either verapamil or nitrendepine, L-type Ca^{2+} channel blockers, was added to the cells after induction of tachyarrhythmias by LPC. As shown in Fig. 4A, verapamil (1 μM) could quickly stop LPC-induced cell contractures and suppressed the elevated $[Ca^{2+}]_i$. But unlike EPA, verapamil did not slow the rate of contractions or Ca^{2+} transients, but induced a progressive reduction in the amplitudes of both and finally stopped them. Furthermore, the basal Ca^{2+} after exposure to verapamil was close to or even lower than the original level ($n = 5$). In contrast, tetrodotoxin, a specific Na^+ channel blocker, exerted effects similar

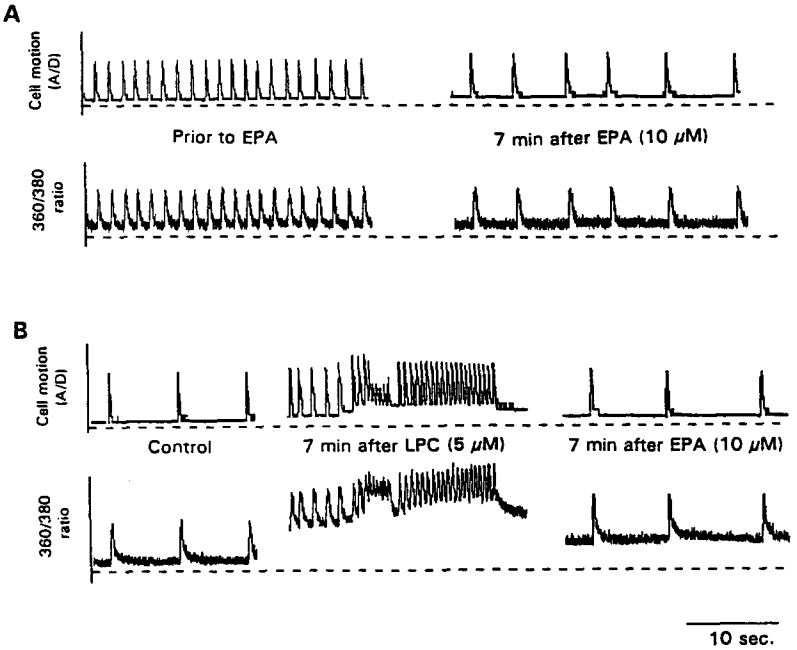

Fig. 3. Simultaneous measurements of $[Ca^{2+}]_i$ (as indicated by 360/380 ratio) and cell motion (as shown by A/D signal) showing the effects of EPA and LPC in cultured myocytes. (A) A representative recording illustrate the $[Ca^{2+}]_i$ transients (lower trace) and cell contractions (upper trace) before and after perfusion with EPA (10 μM) in the absence of LPC ($n = 6$). (B) Tracings show that 5 μM LPC induces an elevation of basal $[Ca^{2+}]_i$ levels with chaotic transients as cell contratures or tachyarrhythmias occurs, and addition of EPA (10 μM) results in a slow beating and $[Ca^{2+}]_i$ transient with a basal $[Ca^{2+}]_i$ level between the control and the LPC alone ($n = 6$).

to EPA on LPC-induced arrhythmias and $[Ca^{2+}]_i$ (Fig. 4B, $n = 4$) (although their effects on the beating rate were not identical). Furthermore, EPA was also able to protect the cells against the tachyarrhythmias induced by Ca^{2+} ionophore A23187 despite a persistent high level of intracellular Ca^{2+} (Fig. 4C, $n = 5$).

Taken together, the above results suggest that the primary action by which PUFAs exert their protective effects against LPC-induced arrhythmias is not directly on the L-type Ca^{2+} channels but likely on the automaticity/excitability of the cardiac myocyte which may secondarily modulate Ca^{2+} movement.

Our previous studies showed that the PUFA reduced the electrical excitability of cardiac myocytes (Kang and Leaf, 1994; Kang et al., 1995). This can be demonstrated directly, as shown in Fig. 5, by their response to electrical pacing. Prior to addition of EPA to the cells, application of a series of stimulating impulses elicited a rapid beating synchronized with the impulse rate. 3–5 min after perfusion of the cells with 15 μM EPA, when a slowing of the beating rate had occurred, application of the same (15 V) or even stronger electrical stimuli failed to boost the beating rate. When the cells were washed with medium containing delipidated BSA (2 mg/ml) for 2–3 min, stimulation of the cells with 15 V field strength induced a response similar to that observed prior to addition of EPA (Fig. 5A). (BSA alone at 2 mg/ml did not affect the response). Alternatively, when the cells were paced with continuous impulses, addition of EPA to the cells was still able to slow the beating rate and subsequent addition of BSA (2 mg/ml) to the medium reversed the beating rate (Fig. 5B). The minimal stimulation strength required to pace the cells increased from 15 V

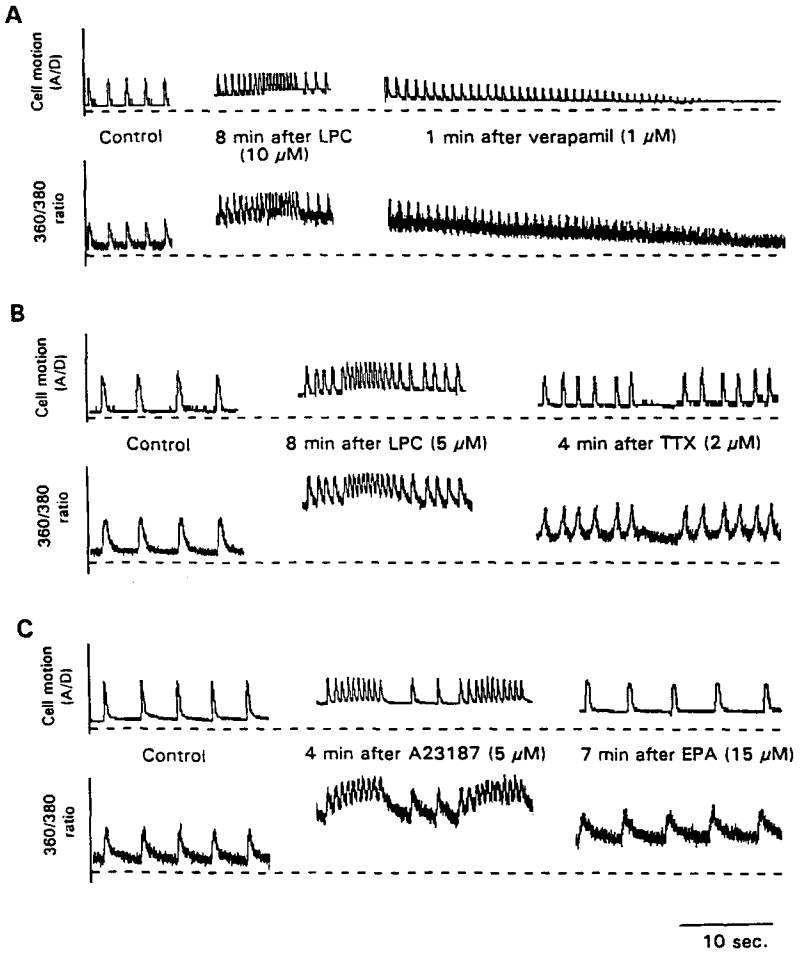

Fig. 4. Panel A: effects of verapamil on the cell contraction (upper trace) and the $[Ca^{2+}]_i$ (lower trace) during perfusion with LPC. Addition of 1 μM verapamil not only abolished the tachyarrhythmias induced by 10 μM LPC but also progressively reduced the amplitude of cell motion and $[Ca^{2+}]_i$ transient and quickly reversed the basal $[Ca^{2+}]_i$ ($n = 5$). Panel B: effects of tetrodotoxin (TTX) on LPC-induced arrhythmia (upper trace) and the $[Ca^{2+}]_i$ (lower trace). Perfusion with TTX (2 μM) could stop the tachyarrhythmias and reduced the elevated $[Ca^{2+}]_i$ level caused by LPC ($n = 4$). Panel C: effects of EPA on the cell contraction (upper trace) and the $[Ca^{2+}]_i$ (lower trace) during perfusion with Ca^{2+} ionophore A23187. Perfusion with 4-bromo-A23187 (5 μM) quickly increased the $[Ca^{2+}]_i$ and caused tachyarrhythmia and contractures. Addition of EPA (15 μM) resulted in a slow beating and $[Ca^{2+}]_i$ transients, but the basal $[Ca^{2+}]_i$ level remained high.

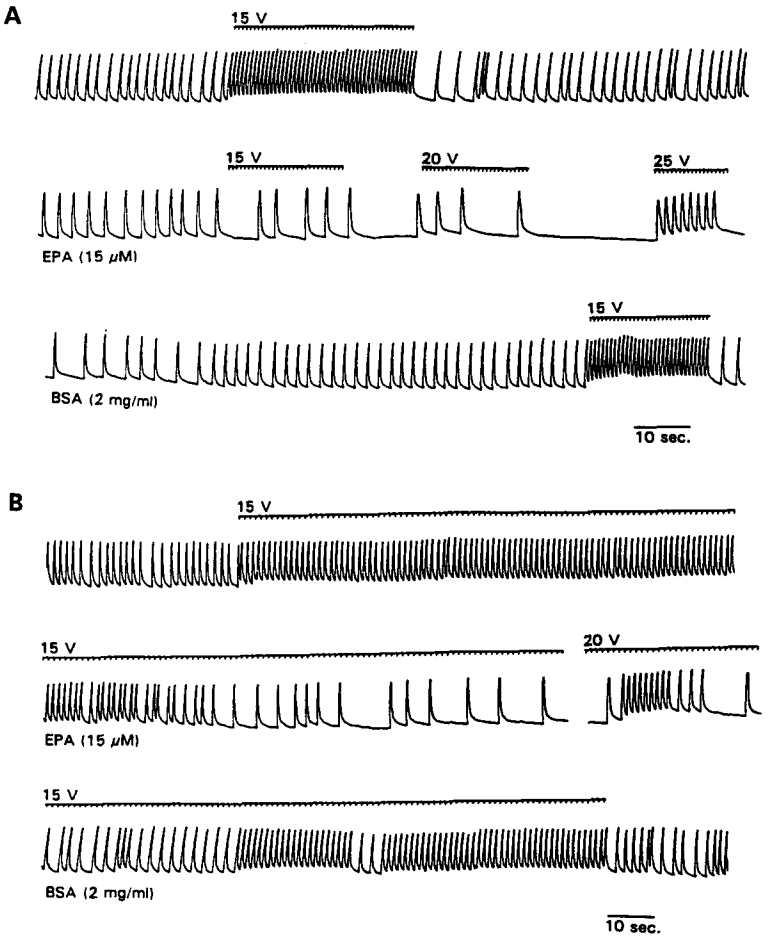

Fig. 5. Effect of EPA on the response of cultured myocytes to electrical pacing. Cells were paced as described in Methods. (A) Tracings show the response of cell contraction to the electrical stimuli (as indicated by the bars above the contraction trace) before perfusion with EPA (top trace), during perfusion with 15 μM EPA (middle trace) and after washout with BSA. (B) Tracings show the effect of EPA on the contraction rate during the continuous electrical pacing.

to 30 V ($\Delta = 15 \pm 5$, $n = 7$, $P < 0.01$) after 5 min of perfusion with 15 μM EPA. These results confirm that EPA has an inhibitory effect on the electrical automaticity/excitability of the cardiac myocytes. These findings are consistent with our earlier observation as to the effect of PUFA on the electrophysiologic properties of these myocytes (Kang et al., 1995).

4. Discussion

The isolated, cultured, neonatal rat cardiac myocytes provide an appropriate preparation for assessing the arrhythmogenic or antiarrhythmic effects of an agent (Kang and Leaf, 1994, 1995), since cultured cells can be placed in direct, uniform contact with a defined concentration of specific agents without confounding effects of possible neurogenic or circulating humoral factors. The myocytes grow as syncytia of ten or more cells which spontaneously beat synchronously and rhythmically. The edge monitor follows the motion of a single cell within a syncytium. Moreover, cultured myocytes have been shown to develop arrhythmias similar to that in the in vivo heart (Thandroyen et al., 1991), and to have cellular Ca^{2+} kinetics similar to those operating in adult hearts from several species (Sedlis et al., 1983; Langer et al., 1975). Using this preparation, we have examined the effects of various fatty acids on LPC or acylcarnitine-induced arrhythmias. The results presented here demonstrate that free polyunsaturated fatty acids, but not monounsaturated or saturated fatty acids, at concentrations of 10–20 μM, were able to prevent or terminate the tachyarrhythmias (or fibrillations) induced by 2–10 μM LPC or acylcarnitine.

Consistent with previous findings showing that LPC and palmitoylcarnitine are arrhythmogenic (Corr, 1990; Corr et al., 1984, 1987a; Katz and Messineo, 1981), we found that LPC or palmitoylcarnitine at concentrations of 2–10 μM caused sustained tachyarrhythmias with intermittent contractures within 2–10 min in the neonatal rat cardiac myocytes. The concentrations of LPC or acylcarnitine which are sufficient to induce

arrhythmias in the cultured cells are much lower than those reported by previous studies (Woodley et al., 1991; Liu et al., 1991). This disparity in sensitivity of cells to LPC may be due to a species difference.

LPC and acylcarnitine are amphiphilic molecules. By inserting into the membrane as free monomers and altering the lipid environment, LPC and acylcarnitine may have specific effects on integral membrane proteins such as ion channels or transporters. At higher concentration, these amphiphilic lipids are more likely to form micelles, causing nonselective membranes leakiness and eventually disrupting biological membranes via a nonspecific detergent action (Woodley et al., 1991; Liu et al., 1991). Since the LPC concentration used in this study (2–10 μM) was lower than the reported critical micellar concentration, which may range from 10 to 80 μM (Bergmann et al., 1981; Stafford et al., 1989), and the LPC-induced arrhythmias could be prevented by verapamil, tetrodotoxin and PUFAs, the observed effects of LPC appeared to be nondetergent.

Our previous studies (Kang and Leaf, 1994, 1995) have shown the protective effects of free PUFAs against arrhythmias induced by high extracellular Ca^{2+}, ouabain or isoproterenol. The present findings that PUFAs are able to prevent and stop the arrhythmias induced by LPC or acylcarnitine indicates that the PUFA may prevent that contribution by LPC and acylcarnitine to ischemia-induced sudden cardiac death. Consistent with our earlier findings (Kang and Leaf, 1994, 1995; Kang et al., 1995), only polyunsaturated fatty acids, but not monosaturated or saturated fatty acids, are effective. The free fatty acid is the form responsible for the antiarrhythmic effect independent of any covalent incorporation into membrane components since the effects of PUFA can be reversed quickly by addition of delipidated BSA, which binds fatty acid with high affinity extracting them from the myocytes. Furthermore, ETYA, a nonmetabolizable AA analog, exhibits the same antiarrhythmic effects as EPA, indicating that metabolites of the fatty acids are not required for their antiarrhythmic effects, as we have previously demonstrated (Kang and Leaf, 1994, 1995; Kang et al., 1995).

The arrhythmogenic action of LPC is thought to be mediated via its association with the sarcolemmal membrane (Man et al., 1990). Since free fatty acids also quickly partition into the lipid bilayer of cell membrane, it is possible, if this accumulation involves specific binding to some components of the membrane, that the antiarrhythmic effect of PUFA may result from competition between the PUFA and LPC for the binding site.

Since LPC can be converted to phosphatidylcholine (PC) by acyl:LPC acyltransferase in the presence of free fatty acids, another possible explanation for the antiarrhythmic effect of PUFA against LPC is that PUFA, as substrate, facilitate the acylations of LPC thereby lowering the LPC level in the cell membrane. However, this possibility is refuted by the observations that the antiarrhythmic effect of PUFA could be reversed promptly by addition of BSA which would extract only the free fatty acid, but not any covalently bound fatty acid, out of the membrane. Nevertheless, the possibility that increased levels of exogenous free fatty acids inhibit LPC production by suppression of PLA_2 activity in ischemic hearts in vivo can not be excluded.

Several previous studies have shown an association of increases in the intracellular Ca^{2+} concentrations with LPC-induced arrhythmias (Sedlis et al., 1983; Woodley et al., 1991; Liu et al., 1991). The factors that may contribute to LPC-induced increase in $[Ca^{2+}]_i$ include a direct effect on sarcolemmal permeability to Ca^{2+} (Woodley et al., 1991; Liu et al., 1991) or an increase in Ca^{2+} influx via L-type Ca^{2+} channels (Sedlis et al., 1983) or Na^+-Ca^{2+} exchangers (Karli et al., 1979). In our experiments, myocyte contracture and increased $[Ca^{2+}]_i$ induced by LPC at concentrations of 2–10 μM were prevented by additions of 1.0 μM verapamil. This finding is consistent with those previous observations showing that verapamil does effectively block or delay some of the LPC-induced electrophysiologic abnormalities (Pogwizd et al., 1986; Nakaya et al., 1984a, b) and Ca^{2+} influx (Sedlis et al., 1983). The effect of EPA on $[Ca^{2+}]_i$ may be either the primary action of EPA responsible for its antiarrhythmic effect or an effect secondary to other electrophysiologic changes, such as reduction in automaticity/excitability. In fact, the beating rate can modulate $[Ca^{2+}]_i$. Our results indicate that an effect on automaticity/excitability is more likely, because (i) a slowing of the beating rate was always observed when the protective effect occurs (Figs. 1–5); (ii) the characteristics of the effects of EPA on the $[Ca^{2+}]_i$ and contractions are different from that of Ca^{2+} channel blockers (i.e. verapamil) which induce a progressive reduction in the amplitude of contractions and $[Ca^{2+}]$ transients and could totally reverse the increased basal (diastolic) $[Ca^{2+}]_i$ caused by LPC (Fig. 4A); (iii) the Na^+ channel blocker (TTX) which does not have a direct effect on Ca^{2+} channels, can mimic most of the effects of EPA on LPC-induced arrhythmia and $[Ca^{2+}]_i$ (Fig. 4B); (iv) EPA is also able to block Ca^{2+} ionophore-induced tachyarrhythmia despite the presence of high $[Ca^{2+}]_i$ (Fig. 4C). Thus, it is feasible to propose that the primary action responsible for the antiarrhythmic effect against LPC is an inhibition (or stabilization) of electric excitability/automaticity. This suggestion is validated directly as shown in Fig. 5 in which EPA significantly increases the electric stimulation strength required to pace the myocytes. This observation is

consistent with our earlier electrophysiologic data showing that PUFA increased the threshold for gating the fast Na^+ channel which initiates the action potential; the resting or diastolic membrane potential; and the refractory period duration in the cardiac myocyte. Our preliminary data also show an inhibiting effect of PUFA on Na^+ channels (Xiao et al., 1995). The observed electrophysiologic effects of PUFA are just opposite to those induced by LPC, which include reduction in the resting membrane potential (Corr et al., 1979, 1981, 1982; Arnsdorf and Sawicki, 1981), increase in Na^+ channel activity (Undrovinas et al., 1992; Burnashev et al., 1991) and increase in automaticity (Corr et al., 1979, 1981; Arnsdorf and Sawicki, 1981; Clarkson and Ten Eick, 1983; Duan and Moffat, 1991).

Finally, it seems that LPC and PUFA, by inserting into cell membrane, can modulate the lipid bilayer properties and/or interact with specific membrane proteins (i.e. ion channels) in *different (or opposite) manners* resulting in arrhythmogenic and antiarrhythmic effects, respectively. However, the ion channels underlying the antiarrhythmic effect of PUFA and how the fatty acids affect the ion channels remain to be determined.

Acknowledgements

This study was supported by National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK 38165. We are grateful to Caren Kachoris for her assistance with the cell cultures.

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