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A monounsaturated fatty acid (oleic acid) modulates electrical activity in atrial myocytes with calcium and sodium dysregulation

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ABSTRACT

Background: Obesity and metabolic syndrome are important risk factors for atrial fibrillation. High plasma concentrations of monounsaturated fatty acids, including oleic acid (OLA), are frequently noted in obese individuals and patients with metabolic syndrome. However, it is not clear whether monounsaturated fatty acids (MUFAs) can directly modulate the electrophysiological characteristics of atrial myocytes.

Methods: Whole-cell patch clamp, indo-1 fluorescence, and Western blot analyses were used to record the action potentials (APs), ionic currents, and protein expressions of HL-1 myocytes incubated with and without (control) OLA (0.5 mM) for 24 h.

Results: Compared to control myocytes ($n = 14$), OLA-treated myocytes ($n = 16$) had shorter APD_{90} (65 ± 6 vs. 85 ± 6 ms, $p < 0.05$) and APD_{50} (24 ± 6 vs. 38 ± 4 ms, $p < 0.05$) with a higher incidence of delayed afterdepolarizations (35.7% vs. 7%, $p < 0.05$), which were suppressed by 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS, a blocker of the calcium-activated chloride current). In addition, OLA-treated myocytes ($n = 19$) exhibited larger calcium transients (0.54 ± 0.06 vs. 0.38 ± 0.05 R410/485, $p < 0.05$), and sarcoplasmic reticular calcium contents (0.91 ± 0.05 vs. 0.64 ± 0.08 R410/485, $p < 0.05$) than control myocytes ($n = 15$). OLA-treated myocytes had larger late sodium currents, smaller sodium–calcium exchanger currents, and smaller sodium–potassium pump currents. Moreover OLA-treated myocytes had higher expressions of sarcoplasmic reticular Ca^{2+}-ATPase and calmodulin kinase II, but lower expression of the sodium–potassium ATPase protein than control myocytes.

Conclusions: MUFAs can regulate atrial electrophysiological characteristics with calcium and sodium dysregulation, which may contribute to atrial arrhythmogenesis.

1. Introduction

Atrial fibrillation (AF) is the most common persistent cardiac arrhythmia in clinical practice, and often induces cardiac dysfunction and stroke [1–3]. Obesity and metabolic syndrome (MS) are important risk factors for the genesis of AF [4–6]. Pericardial adipose tissues release free fatty acids (FFAs), which may have direct cardiac impacts or systemic effects [7,8]. High plasma concentrations of FFAs, including oleic acid (OLA, a monounsaturated omega−9 fatty acid), are frequently found in atherosclerotic patients with type 2 diabetes and in obese hypertensive patients [8]. Hyperinsulinemia is associated with larger FFA release and leads to elevated postprandial FFAs in obesity [8]. Our previous study showed that adipocyte-incubated left atrium (LA) myocytes had a higher incidence of delayed afterdepolarizations (DADs) [9]. Moreover, epicardial fat in heart failure (HF) patients can induce atrial triggered activity and tachyarrhythmias [10]. Therefore, adipocytes

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contain arrhythmogenic potential. However, the mechanisms underling the effects of adipocytes have not been fully elucidated.

ω3 polyunsaturated fatty acids (ω3-PUFAs) from fish oil may prevent AF [11,12]. Acute administration of the ω3-PUFAs, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), inhibits the formation of noradrenalin-induced triggered activity and reduces DADs and calcium transients [13,14]. On the contrary, knowledge of the electrophysiological effects of monounsaturated fatty acids (MUFAs) is limited. Abnormal Ca^{2+} regulation may play an important role in the genesis of AF [15]. However, the effects of MUFAs on Ca^{2+} regulation remain unclear. An excess of FFAs (linolate and palmitate) was shown to facilitate the genesis of ventricular tachyarrhythmias [16]. In addition, OLA can inhibit the sodium–potassium (Na^+/K^+)-ATPase of the heart, which may induce calcium or sodium dysregulation [17]. The purpose of this study was to investigate whether OLA can enhance atrial arrhythmogenesis through modulating electrical activity in atrial myocytes and to evaluate its underlying mechanisms.

2. Methods

2.1. Cell culture

HL-1 cells derived from mouse atrial cardiac muscle cells (kindly provided by Dr. Claycomb, Louisiana State University Health Sciences Center, New Orleans, LA) were cultured in a humidified atmosphere of 5% CO_2 at 37 °C in Claycomb medium (JRH Biosciences, Lenexa, KS) as described previously [18,19]. HL-1 cells were cultured with or without 0.5 mM OLA (Sigma–Aldrich, St Louis, MO) in 1% bovine serum albumin for 24 h. Both control and OLA-treated cells were seeded in fibronectin/gelatin pre-coated dishes as described previously [18,19]. After 24 h of cell seeding, we added OLA or vehicle solution into cells for OLA-treated or control cells respectively, and further incubation for 24 h. Cells were plated at a density of 5×10^5 cells/well in 6-well culture plates.

2.2. Whole-cell patch-clamp study

A whole-cell patch clamp was performed in control and OLA-treated HL-1 cells using an Axopatch 1D amplifier (Axon Instruments, Union City, CA) at 35 ± 1 °C. Borosilicate glass electrodes (o.d., 1.8 mm) with tip resistances of 3–5 MΩ were used. Before formation of the membrane-pipette seal, tip potentials were zeroed in Tyrode solution. Junction potentials (9 mV) were corrected for action potential (AP) recording. APs were recorded in the current-clamp mode, and ionic currents were measured in the voltage-clamp mode as described previously [9,18,20]. A small hyperpolarizing step from a holding potential of −50 mV to a test potential of −55 mV for 80 ms was delivered at the beginning of each experiment. The area under the capacitive current was divided by the applied voltage step to obtain the total cell capacitance. Normally, 60%–80% series resistance (R_s) was electronically compensated for. The resting membrane potential (RMP) was measured during the period between the last repolarization and the onset of the subsequent AP. The AP amplitude (APA) was obtained from the measurement of RMP to the peak of AP depolarization. AP durations were measured at 20% (APD_{20}), 50% (APD_{50}), and 90% (APD_{90}) repolarization of the amplitude at a driven rate of 1 Hz. The DAD was defined as the presence of spontaneous depolarization of an impulse after full repolarization had occurred. 4,4′-Diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS, 100 μM, a Ca^{2+}-activated chloride current blocker, Sigma, St. Louis, MO) was perfused in cells with DAD.

Micropipettes were filled with a solution containing the following (in mM): NaCl 10, CsCl 130, EGTA 5, HEPES 5, glucose 5, and ATP-Mg 5 for the late sodium current ($I_{Na\text{-}Late}$); containing (in mM) NaCl 20, CsCl 110, $MgCl_2$ 0.4, $CaCl_2$ 1.75, tetraethylammonium chloride (TEACl) 20, BAPTA 5, glucose 5, MgATP 5, and HEPES 10, titrated to a pH of 7.25 with CsOH for experiments on the sodium–calcium exchanger (NCX) current; containing (in mM) NaCl 15, $MgCl_2$ 1, CsCl 8, HEPES 10, EGTA 5, MgATP 5, creatine phosphate 5, $CsCH_3O_3S$ 90, $NaCH_3O_3S$ 35, titrated to a pH of 7.16 for the experiments on Na/K ATPase pump current (I_p); and containing (in mM) KCl 20, K aspartate 110, $MgCl_2$ 1, Mg_2ATP 5, HEPES 10, EGTA 0.5, LiGTP 0.1, and Na_2 phosphocreatine 5, titrated to a pH of 7.2 with KOH for experiments on the AP.

The $I_{Na\text{-}Late}$ included a step/ramp protocol (−100 mV stepping to +20 mV for 100 ms, then ramping back to −100 mV over 100 ms) at room temperature with an external solution containing (in mM): NaCl 130, CsCl 5, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 10, and glucose 10 at pH 7.4 adjusted with NaOH. Micropipettes were filled with a solution containing (in mM): CsCl 130, Na_2ATP 4, $MgCl_2$ 1, EGTA 10, and HEPES 5 at a pH 7.3 adjusted with NaOH. An equilibration period (5–10 min) for dialysis was allowed to adequately clamp the cell currents. $I_{Na\text{-}Late}$ was measured as the tetrodotoxin (TTX, 30 μM)-sensitive portion of the current traces obtained during voltage ramped back to −100 mV.

The NCX current was elicited by test potentials between −100 and +100 mV from a holding potential of −40 mV for 300 ms at a frequency of 0.1 Hz. Amplitudes of the NCX current were measured as 10-mM nickel-sensitive currents. The external solution consisted of (in mM) NaCl 140, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 5, and glucose 10 at a pH of 7.4 and contained strophanthidin (10 μM), nitrendipine (10 μM), and niflumic acid (100 μM).

The Na/K pump current (I_p) was measured at 0 mV using the ruptured-patch technique. The standard extracellular solution contained (in mM): NaCl 140, KCl 5, $MgCl_2$ 1, $NiCl_2$ 2, $BaCl_2$ 1, glucose 10, and HEPES 10 at pH 7.4. K-free extracellular solutions were made by removing KCl from the solution, with no correction for the osmolarity. Under these conditions, the I_p can be defined as that inhibited by the removal of extracellular K. Currents were continuously recorded and were quantified every 5 min for 20 min.

2.3. Measurement of calcium transients and intracellular calcium concentration ($[Ca^{2+}]_i$)

The $[Ca^{2+}]_i$ was recorded by a fluorometric ratio technique by a previously described procedure [18,20]. The fluorescent indicator, indo-1, was loaded by incubating cardiomyocytes at room temperature for 20–30 min with indo-1/AM (10 μM, Sigma). HL-1 cells were then perfused with normal Tyrode's solution at 35 ± 1 °C for at least 30 min to wash out the extracellular indicator and allow for intracellular deesterification of the indo-1. Background and cell autofluorescence levels were canceled out by zeroing the output of the photomultiplier tubes using cells without indo-1 loading. Ultraviolet light of 360 nm from a monochromator was used to excite the indo-1 from a xenon arc lamp which was controlled by a microfluorometric system (OSP100-CA, Olympus, Tokyo, Japan). The excitation light beam was directed into an inverted microscope (IX-70, Olympus). The emitted fluorescence signals from the indo-1/AM-loaded cardiomyocytes were digitized at 200 Hz. We recorded the ratio of fluorescence emissions at 410 and 485 nm. The $R_{410/485}$ value was used as an index of the $[Ca^{2+}]_i$. Ratios of the $[Ca^{2+}]_i$ transient, peak systolic $[Ca^{2+}]_i$, and diastolic $[Ca^{2+}]_i$ values were measured during 2-Hz field stimulation with 10-ms square-wave pulses at twice the diastolic threshold strength. The sarcoplasmic reticular (SR) Ca^{2+} content was estimated by adding 20 mM caffeine after electric stimulation at 2 Hz for at least 30 s. The total SR Ca^{2+} content was measured from the peak amplitude of caffeine-induced $[Ca^{2+}]_i$ transients or measured from integrating the NCX current during rest with the membrane potential clamped to −40 mV. The time integral of the NCX current was converted to amoles (10^{-18} mol) of Ca^{2+} released from the SR.

2.4. Western blot analysis of ion channel proteins

Control and OLA-treated HL-1 cells were washed with cold phosphate-buffered saline (PBS) and lysed on ice for 30 min in radioimmunoprecipitation assay buffer containing 50 mmol/L Tris at pH 7.4, 150 mmol/L NaCl, 1% Nonidet P-40 (NP40), 0.5% sodium deoxycholate, 0.1% sodium dodecylsulfate (SDS), and a protease inhibitor cocktail (Sigma-Aldrich). The protein concentration was determined with a Bio-Rad protein assay reagent (Bio-Rad, Munich, Germany). Equal amounts of proteins were subjected to SDS-polyacrylamide gel electrophoresis (PAGE). Blots were probed with primary antibodies against Na^+/K^+ ATPase α1 subunit (Cell signaling Technology, Danvers, MA), sarcoplasmic reticular Ca^{2+}-ATPase (SERCA2a; Santa Cruz Biotechnology, Santa Cruz, CA), calmodulin kinase II (CaMKII; Abcam, Cambridge, UK), NCX (Swant, Bellinzona, Switzerland), and secondary antibodies conjugated with horseradish peroxidase. Bound antibodies were detected with an ECL detection system (Millipore, Billerica, MA) and analyzed with ALPHAEASEFC software (Alpha Innotech, San Leandro, CA). Targeted bands were normalized to GAPDH to confirm equal protein loading.

2.5. Statistical analysis

All continuous variables are expressed as the mean ± standard error of the mean (SEM). Differences in AP durations and ionic currents between control and OLA-treated HL-1 cells were compared by a Mann–Whitney rank-sum test or an unpaired *t*-test depending on the outcome of the normality test. Pearson's Chi-squared test was used to compare the incidence of DADs. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Effects of OLA on the electrical activity of HL-1 cells

Fig. 1 shows the AP morphology from control and OLA-treated HL-1 cells. OLA-treated HL-1 cells had shorter APD_{90} and APD_{50}, but had a similar APD_{20} value compared to control myocytes. Moreover, OLA-treated HL-1 cells exhibited a higher incidence of DADs than control myocytes, which could be suppressed by DIDS (100 μM, Fig. 1). The APD_{90}/APD_{50} (2.7 ± 1.0 versus 2.2 ± 1.3, $p > 0.05$) was not significantly different in OLA-treated and control HL-1 cells.

3.2. Effects of OLA on calcium handling of HL-1 cells

As shown in Fig. 2A, OLA-treated HL-1 cells exhibited an increased SR Ca^{2+} content measured from integrating the caffeine-induced NCX current in OLA-treated HL-1 cells. In addition, OLA-treated HL-1 cells exhibited a larger amplitude of $Ca^{2+}{}_i$ transients, with larger systolic and