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Original article

Palmitate attenuates myocardial contractility through augmentation of repolarizing Kv currents

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ABSTRACT

There is considerable evidence to support a role for lipotoxicity in the development of diabetic cardiomyopathy, although the molecular links between enhanced saturated fatty acid uptake/metabolism and impaired cardiac function are poorly understood. In the present study, the effects of acute exposure to the saturated fatty acid, palmitate, on myocardial contractility and excitability were examined directly. Exposure of isolated (adult mouse) ventricular myocytes to palmitate, complexed to bovine serum albumin (palmitate:BSA) as in blood, rapidly reduced (by 54 ± 4%) mean (±SEM) unloaded fractional cell shortening. The amplitudes of intracellular Ca^{2+} transients decreased in parallel. Current–clamp recordings revealed that exposure to palmitate:BSA markedly shortened action potential durations at 20%, 50%, and 90% repolarization. These effects were reversible and were occluded when the K^+ in the recording pipettes was replaced with Cs^+, suggesting a direct effect on repolarizing K^+ currents. Indeed, voltage-clamp recordings revealed that palmitate:BSA reversibly *and selectively* increased peak outward voltage-gated K^+ (Kv) current amplitudes by 20 ± 2%, whereas inwardly rectifying K^+ (Kir) currents and voltage-gated Ca^{2+} currents were unaffected. Further analyses revealed that the individual Kv current components $I_{to,f}$, $I_{K,slow}$ and I_{ss}, were all increased (by 12 ± 2%, 37 ± 4%, and 34 ± 4%, respectively) in cells exposed to palmitate:BSA. Consistent with effects on both components of $I_{K,slow}$ ($I_{K,slow1}$ and $I_{K,slow2}$) the magnitude of the palmitate-induced increase was attenuated in ventricular myocytes isolated from animals in which the Kv1.5 ($I_{K,slow1}$) or the Kv2.1 ($I_{K,slow2}$) locus was disrupted and $I_{K,slow1}$ or $I_{K,slow2}$ is eliminated. Both the enhancement of $I_{K,slow}$ and the negative inotropic effect of palmitate:BSA were reduced in the presence of the Kv1.5 selective channel blocker, diphenyl phosphine oxide-1 (DPO-1).Taken together, these results suggest that elevations in circulating saturated free fatty acids, as occurs in diabetes, can directly augment repolarizing myocardial Kv currents and impair excitation–contraction coupling.

1. Introduction

Altered energy metabolism is a prominent feature of, and in some instances may cause, heart failure [1,2]. For example, cardiac dysfunction is a prominent feature of diabetes mellitus, and it is clear that impaired cardiac function can occur in individuals with diabetes without evidence of any other secondary risk factors for heart disease, including hypertension or atherosclerosis, suggesting that the metabolic consequences of diabetes alone are sufficient to impair cardiac function [3,4]. These observations also suggest that derangements of cardiac metabolism can have direct consequences on cardiac function. The potential molecular mechanisms that link altered metabolism with cardiac pathology are numerous [2], although poorly understood.

In the normal heart, lipid oxidation accounts for about 60% of the total ATP generated, while glycolysis supplies the remainder [5,6]. In the diabetic heart, in contrast, 80–90% of the ATP is generated from lipid oxidation as a direct result of increased circulating free fatty acids and reduced insulin sensitivity [7–9]. In previous studies, we demonstrated that transgenic mice (MHC-FATP) overexpressing fatty acid transport protein 1 (FATP-1) specifically in the myocardium exhibit increased myocardial lipid uptake, storage, and metabolism [10]. In addition, MHC-FATP mice have impaired diastolic function [10,11], one of the earliest signs of diabetic cardiomyopathy [12,13]. These findings support the hypothesis that altered cardiac metabolism alone is sufficient to impair cardiac function.

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Importantly, however, the later stages of diabetic cardiomyopathy in humans are also characterized by impaired systolic function [14–16]. In addition, marked systolic dysfunction has been observed in several animal models of diabetes, including streptozotocin-induced diabetes in rats and mice, Zucker diabetic fatty rats, and in *db*/*db* mice [9,17–19]. In contrast to the MHC-FATP transgenic mice in which the metabolic derangements are restricted to the myocardium [10], these animal models of diabetes exhibit systemic metabolic abnormalities, including increases in circulating saturated fatty acids [9,20]. The phenotypic differences between these models and the MHC-FATP mice also indicate that altered substrate usage in the heart alone is insufficient to explain the observed systolic dysfunction [10,11], further suggesting that the systemic increases in circulating free fatty acids that are associated with diabetes [19,21] might contribute directly to systolic dysfunction.

The experiments here were designed to explore directly the functional consequences of acute exposure to palmitate, complexed with bovine serum albumin (BSA) as in blood, on the mechanical and electrical properties of isolated (adult mouse) ventricular myocytes. Consistent with the notion that elevated circulating fatty acids may contribute to systolic dysfunction in cardiac disease, these experiments demonstrate reversible inhibition of unloaded cell shortening in response to elevated palmitate. In addition, the results of further electrophysiological experiments suggest that the negative inotropic effect of palmitate:BSA is due to shortening of action potential durations, resulting from palmitate-induced augmentation of repolarizing voltage-gated K^+ (Kv) currents.

2. Materials and methods

2.1. Animals

Adult wild-type (WT), SWAP [22], and Kv2.1$^{-/-}$ (Deltagen Corporation) C57BL6 mice were used in the experiments here. All animals were handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and all protocols were approval by the Washington University Animal Studies Committee.

2.2. Isolation of adult mouse ventricular myocytes

Myocytes were isolated from the left ventricles of adult (8–12 week) WT, SWAP, or Kv2.1$^{-/-}$ C57BL6 mice using enzymatic and mechanical methods that have been described previously in detail [23,24]. Following dispersion, isolated left ventricular myocytes were stored in isolation medium containing (in mM) NaCl, 116 mM; KCl, 5.3 mM; $CaCl_2$, 0.15 mM; NaH_2PO_4, 1.2 mM; glucose, 11.6 mM; $MgCl_2$, 3.7 mM; HEPES, 20 mM; L-glutamine, 2.0 mM; $NaHCO_3$, 4.4 mM; KH_2PO_4, 1.5 mM; and supplemented with 1× essential vitamins and amino acids (GIBCO) at room temperature. Cells were used in experiments within 12 h of isolation.

2.3. Solutions

For experiments, palmitate (Nu-Chek Prep #N-16-A) was complexed with fatty acid free bovine serum albumin (BSA, Sigma #A-6003). Stock solutions containing 20% BSA were prepared in sterile phosphate-buffered saline (PBS) and stored at 4 °C. Complexed palmitate:BSA solutions were then prepared by mixing aliquots of 20 mM palmitic acid (prepared fresh in sterile H_2O) and the 20% fatty acid free BSA stock solution at a 1:2 molar ratio in normal Tyrode solution. The resulting solutions were sterile filtered and stored at 4 °C (for up to 1 week) for use in experiments. The palmitate concentrations provided in the text represent the total (not the calculated free) palmitate concentrations in the solutions. The calculated free palmitate concentration, using the method of Richieri and colleagues [25], with a palmitate:BSA ratio of 1:2 was 42 nM. This final unbound palmitate concentration (of 42 nM) was selected based on previous reports that this concentration would not cause "lipotoxicity" [26]. This concentration is, however, expected to be in the low pathological range of unbound free fatty acid concentrations, reported to be as high as 100 nM [27].

2.4. Cell contractility and calcium transient measurements

Unloaded cell shortening and calcium transients were measured in isolated adult mouse left ventricular myocytes. For experiments, isolated left ventricular cells were transferred to a recording chamber mounted on the stage of a Nikon Diaphot inverted microscope and perfused with normal Tyrode solution containing (in mM) NaCl, 137; KCl, 5.4; NaH_2PO_4, 0.16; glucose, 10; $CaCl_2$, 1.8; $MgCl_2$, 0.5; HEPES, 5.0; $NaHCO_3$, 3.0; pH 7.3–7.4). All experiments were performed at room temperature. Video images of individual myocyte contractions were acquired and analyzed using a Myocam camera (IonOptix).

To determine the effect of acute palmitate applications on contractility, isolated myocytes were transferred to the recording chamber, field stimulated (20 V at 1 Hz) and superfused with normal Tyrode solution. After 30 s of recording in control solution, the superfusion solution was changed (using a rapid solution switcher; Warner Instruments) to Tyrode containing 75 μM palmitate:150 μM BSA (or the indicated concentrations of palmitate and BSA). At each solution change, contraction parameters were re-measured after 5 min of continuous superfusion or earlier when a new steady-state contraction was clearly reached prior to this (5 min) time point. This was often the case when the highest concentration of palmitate:BSA (75 μM) was applied (e.g., Fig. 1A). To assess the effects of the Kv channel blocker, diphosphine oxide 1 (DPO-1, Tocris Bioscience)[28], a similar experimental strategy, involving switching the superfusion solution during continuous field stimulation. Following 30 s of baseline recording, the solution was switched to Tyrode containing 1 μM DPO-1 and superfused for 5 min or until a new baseline was achieved. The superfusion solution was then switched to Tyrode containing 1 μM DPO-1 and 75 μM palmitate/150 μM BSA, followed by Tyrode lacking DPO-1, i.e., containing 75 μM palmitate/150 μM BSA alone; washout was obtained by superfusion of normal Tyrode solution.

In experiments focused on measuring intracellular free Ca^{2+} concentrations $[Ca^{2+}]i$, isolated ventricular myocytes were incubated in isolation medium (see above) containing (0.5–1 μM) fluo-4-AM (Molecular Probes, Inc.) and (0.02%) pluronic acid (Sigma) for 30–60 min at room temperature. Following washing with fresh isolation medium, fluo-loaded myocytes were transferred to the recording chamber and superfused with normal Tyrode solution supplemented with 500 μM probenecid (Sigma) to inhibit dye export [29]. Myocytes were then field stimulated and the superfusion solution was changed as described above. Emitted fluorescence was captured with a photomultiplier tube (IonOptix).

2.5. Electrophysiological recordings

Whole-cell current- and voltage-clamp recordings were obtained from myocytes isolated from the left ventricular apex (LVA) of adult mouse hearts. Current- and voltage-clamp recordings were obtained from cells within 12 h of isolation using a Dagan 3900A (Dagan Corporation) whole-cell patch clamp amplifier, interfaced to a Digidata 1332A A/D converter (Molecular Devices) with the pClamp (version 9.2, Molecular Devices) software package. For action potential recording, the bath solution contained (in mM) 136 NaCl, 4 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES and 10 glucose (pH 7.4; 300 mOsm). For recordings of voltage-gated K^+ (Kv) currents, clamp experiments, the bath solution also contained 20 μM Tetrodotoxin (TTX) and 0.5 mM $CdCl_2$ to block voltage-gated Na^+ and Ca^{2+} currents, respectively. Recording pipettes routinely contained (in mM) 135 KCl, 5 K_2ATP, 10