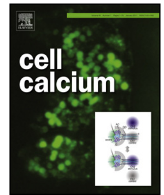
Cell Calcium 55 (2014) 104–118

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca



Effects of endogenous cannabinoid anandamide on excitation–contraction coupling in rat ventricular myocytes

Lina T. Al Kury [a], Oleg I. Voitychuk [d], Ramiz M. Ali [a], Sehamuddin Galadari [c], Keun-Hang Susan Yang [e], Frank Christopher Howarth [b], Yaroslav M. Shuba [d], Murat Oz [a,*]

[a] Laboratory of Functional Lipidomics, Department of Pharmacology, College of Medicine and Health Sciences, UAE University, Al Ain, Abu Dhabi, United Arab Emirates
[b] Department of Physiology, College of Medicine and Health Sciences, UAE University, Al Ain, Abu Dhabi, United Arab Emirates
[c] Department of Biochemistry, College of Medicine and Health Sciences, UAE University, Al Ain, Abu Dhabi, United Arab Emirates
[d] Bogomoletz Institute of Physiology and International Center of Molecular Physiology, National Academy of Sciences of Ukraine, Kyiv-24, Ukraine
[e] Department of Biological Sciences, Schmid College of Science and Engineering, Chapman University, One University Drive, Orange, CA 92866, USA

ARTICLE INFO

Article history:
Received 19 September 2013
Received in revised form
25 November 2013
Accepted 26 December 2013
Available online 5 January 2014

Keywords:
Endocannabinoid
Anandamide
Ventricular myocytes
Contraction
Intracellular calcium
Ventricular action potential

ABSTRACT

A role for anandamide (N-arachidonoyl ethanolamide; AEA), a major endocannabinoid, in the cardiovascular system in various pathological conditions has been reported in earlier reports. In the present study, the effects of AEA on contractility, Ca^{2+} signaling, and action potential (AP) characteristics were investigated in rat ventricular myocytes. Video edge detection was used to measure myocyte shortening. Intracellular Ca^{2+} was measured in cells loaded with the fluorescent indicator fura-2 AM. AEA (1 μM) caused a significant decrease in the amplitudes of electrically evoked myocyte shortening and Ca^{2+} transients. However, the amplitudes of caffeine-evoked Ca^{2+} transients and the rate of recovery of electrically evoked Ca^{2+} transients following caffeine application were not altered. Biochemical studies in sarcoplasmic reticulum (SR) vesicles from rat ventricles indicated that AEA affected Ca^{2+}-uptake and Ca^{2+}-ATPase activity in a biphasic manner. [^{3}H]-ryanodine binding and passive Ca^{2+} release from SR vesicles were not altered by 10 μM AEA. Whole-cell patch-clamp technique was employed to investigate the effect of AEA on the characteristics of APs. AEA (1 μM) significantly decreased the duration of AP. The effect of AEA on myocyte shortening and AP characteristics was not altered in the presence of pertussis toxin (PTX, 2 μg/ml for 4 h), AM251 and SR141716 (cannabinoid type 1 receptor antagonists; 0.3 μM) or AM630 and SR 144528 (cannabinoid type 2 receptor antagonists; 0.3 μM). The results suggest that AEA depresses ventricular myocyte contractility by decreasing the action potential duration (APD) in a manner independent of CB1 and CB2 receptors.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Endocannabinoids belong to a family of polyunsaturated fatty acid-based compounds that mimic most of the effects of tetrahydrocannabinol, the active ingredient of the marijuana plant *Cannabis sativa*. The most widely studied endogenous cannabinoids are N-arachidonoyl ethanolamide (AEA) or anandamide and 2-arachidonylglycerol [1,2]. In recent years extensive research focusing on the biological actions of these molecules has revealed the existence of an endocannabinoid system that regulates several physiological processes and pathological conditions [3]. It was suggested that the endocannabinoid system consists of at least the endocannabinoid receptors (such as CB1 and CB2 cannabinoid receptors), the enzymes regulating the synthesis (such as phospholipase-D, and monoacylglycerol), and the degradation (such as fatty-acid amide hydrolase and lipases) processes, and the proteins involved in their transport across the biological membranes [1,3]. The CB1 receptors are located in the brain and several peripheral tissues including the heart and the vasculature. The CB2 receptors, on the other hand, are expressed primarily in the immune system but recently their presence in the brain, myocardium, and smooth muscle cells have also been demonstrated [3].

Abbreviations: AP, action potential; APD, action potential duration; APD_{60}, action potential duration at 60% level of repolarization; AMP, amplitude; AA, arachidonic acid; APIII, antipyrylazo III; BSA, bovine serum albumin; DMSO, dimethylsulphoxide; DHP, dihydropyridine; NAEs, N-acylethanolamines; AEA, N-arachidonoyl ethanolamide, anandamide; NEM, N-ethylmaleimide; NO, nitric oxide; NT, normal tyrode; PTX, pertussis toxin; RCL, resting cell length; metAEA, R-methanandamide; SR, sarcoplasmic reticulum; T_{HALF}, time from peak to half; TPK, time to peak; TRP, transient-receptor potential.

* Corresponding author at: Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, UAE University, P.O. Box 17666, Al Ain, Abu Dhabi, United Arab Emirates. Tel.: +971 3 7137523; fax: +971 3 7672033.
E-mail address: murat_oz@uaeu.ac.ae (M. Oz).

0143-4160/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ceca.2013.12.005

Emerging evidence suggests a role for endocannabinoids in the cardiovascular system in various pathological conditions, such as hypertension, myocardial infarction and heart failure (for recent reviews [4,5]). AEA has profound and rather complex actions on blood pressure and cardiac function. For example, AEA has been shown to decrease arterial blood pressure in a triphasic manner by acting on the contractility of smooth muscle, the release of neurotransmitters from peripheral nerve endings, and the activation of autonomic reflex pathways [6]. Both cannabinoid receptor-dependent and independent mechanisms have been shown to play roles in AEA inhibition of smooth muscle contraction depending on the type of vascular structure, the presence of intact endothelium, the metabolic products of endocannabinoids and the activity of metabolizing enzymes [6–8].

Compared with a large body of information available on the vascular effects of endocannabinoids, surprisingly few studies have focused on the role of endocannabinoids in the regulation of contractility and Ca^{2+} signaling in cardiac muscle. Experiments with AEA performed in isolated Langendorff rat hearts and in isolated, electrically stimulated atrial appendages from human, rat, and rabbit [9–12] have revealed a negative inotropic effect of cannabinoids that may underlie the ability of AEA to decrease cardiac output as observed in studies performed in vivo (for reviews [4,7,8]). However, mechanisms underlying these cardiac actions of AEA remain largely unknown.

In the present study, we have hypothesized that the negative inotropic actions of anandamide are due to altered Ca^{2+} homeostasis and AP characteristics in ventricular myocytes. Thus, we have investigated the actions of AEA on contractile properties, Ca^{2+} signaling and AP waveforms in acutely dissociated rat ventricular myocytes.

2. Materials and methods

2.1. Ventricular myocyte isolation

Ventricular myocytes were isolated from adult male Wistar rats (264 ± 19 g) according to previously described techniques [13]. This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the UAE University. Briefly, the animals were euthanized using a guillotine and hearts were removed rapidly and mounted for retrograde perfusion according to the Langendorff method. Hearts were perfused at a constant flow of 8 ml g $heart^{-1}$ min^{-1} and at 36–37 °C with a solution containing (mM): 130 NaCl, 5.4 KCl, 1.4 $MgCl_2$, 0.75 $CaCl_2$, 0.4 NaH_2PO_4, 5 HEPES, 10 glucose, 20 taurine, and 10 creatine set to pH 7.3 with NaOH. When the heart had stabilized perfusion was continued for 4 min with Ca^{2+}-free isolation solution containing 0.1 mM EGTA, and then for 6 min with cell isolation solution containing 0.05 mM Ca^{2+}, 0.75 mg/ml collagenase (type 1; Worthington Biochemical Corp., USA) and 0.075 mg/ml protease (type X1V; Sigma, Germany). Ventricles were excised from the heart, minced and gently shaken in collagenase-containing isolation solution supplemented with 1% BSA. Cells were filtered from this solution at 4-min intervals and resuspended in isolation solution containing 0.75 mM Ca^{2+}.

2.2. Measurement of ventricular myocyte shortening

Ventricular myocytes were allowed to settle on the glass bottom of a Perspex chamber mounted on the stage of an inverted microscope (Axiovert 35, Zeiss, Germany). Myocytes were superfused (3–5 ml/min) with normal tyrode (NT) containing (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 10 glucose, 5 HEPES, and 1.8 $CaCl_2$ (pH 7.4). Shortening of myocytes was recorded using a video edge detection system (VED-114, Crystal Biotech, USA). Resting cell length (RCL) and amplitude of shortening (expressed as a % of resting cell length) were measured in electrically stimulated (1 Hz) myocytes maintained at 35–36 °C. Data were acquired and analyzed with Signal Averager software v 6.37 (Cambridge Electronic Design, UK). Experimental solutions were prepared from stock immediately prior to each experiment.

2.3. Measurement of intracellular Ca^{2+} concentration

Myocytes were loaded with the fluorescent indicator fura-2 AM (F-1221, Molecular Probes, USA) as described previously [13]. In brief, 6.25 μl of a 1 mM stock solution of fura-2 AM (dissolved in dimethylsulphoxide) was added to 2.5 ml of cells to give a final fura-2 concentration of 2.5 μM. Myocytes were shaken gently for 10 min at 24 °C (room temperature). After loading, myocytes were centrifuged, washed with NT to remove extracellular fura-2 and then left for 30 min to ensure complete hydrolysis of the intracellular ester. To measure intracellular Ca^{2+}concentration, myocytes were alternately illuminated by 340 and 380 nm light using a monochromator (Cairn Research, UK) which changed the excitation light every 2 ms. The resulting fluorescence emitted at 510 nm was recorded by a photomultiplier tube and the ratio of the emitted fluorescence at the two excitation wavelengths (340/380 ratio) was calculated to provide an index of intracellular Ca^{2+} concentration. Resting fura-2 ratio, TPK Ca^{2+} transient, T_{HALF} decay of the Ca^{2+} transient, and the amplitude of the Ca^{2+} transient were measured in electrically stimulated (1 Hz) myocytes.

2.4. Measurement of sarcoplasmic reticulum Ca^{2+} content

Sarcoplasmic reticulum (SR) Ca^{2+} release was assessed using previously described techniques [13,14]. After establishing steady state Ca^{2+} transients in electrically stimulated (1 Hz) myocytes maintained at 35–36 °C and loaded with fura-2, stimulation was paused for a period of 5 s. Caffeine (20 mM) was then applied for 10 s using a solution switching device customized for rapid solution exchange. Electrical stimulation was resumed and the Ca^{2+} transients were allowed to recover to steady state. SR-releasable Ca^{2+} was assessed by measuring the area under the curve of the caffeine-evoked Ca^{2+} transient. Fractional release of SR Ca^{2+} was assessed by comparing the amplitude of the electrically evoked steady state Ca^{2+} transients with that of the caffeine-evoked Ca^{2+} transient and refilling of SR was assessed by measuring the rate of recovery of electrically evoked Ca^{2+} transients following application of caffeine.

2.5. Assessment of myofilament sensitivity to Ca^{2+}

In some cells shortening and fura-2 ratio were recorded simultaneously. Myofilament sensitivity to Ca^{2+} was assessed from phase-plane diagrams of fura-2 ratio versus cell length by measuring the gradient of the fura-2-cell length trajectory during late relaxation of the twitch contraction. The position of the trajectory reflects the relative myofilament response to Ca^{2+} and hence, can be used as a measure of myofilament sensitivity to Ca^{2+} [15].

2.6. Preparation of cardiac sarcoplasmic reticulum vesicles

SR vesicles were obtained from rat ventricles by minor modifications of methods described earlier [16]. Adult male Wistar rats were anesthetized with intraperitoneal injection of sodium pentobarbital (100 mg/kg) and hearts were quickly removed and rinsed in Ca^{2+}-free Tyrode solution. The ventricles were blotted on filter paper to remove excess solution and homogenized in cold

Download English Version:

https://daneshyari.com/en/article/2166029

Download Persian Version:

https://daneshyari.com/article/2166029

Daneshyari.com