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Effects of cannabidiol on contractions and calcium signaling in rat ventricular myocytes

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ABSTRACT

Cannabidiol (CBD), a major nonpsychotropic cannabinoid found in *Cannabis* plant, has been shown to influence cardiovascular functions under various physiological and pathological conditions. In the present study, the effects of CBD on contractility and electrophysiological properties of rat ventricular myocytes were investigated. Video edge detection was used to measure myocyte shortening. Intracellular Ca^{2+} was measured in cells loaded with the Ca^{2+} sensitive fluorescent indicator fura-2 AM. Whole-cell patch clamp was used to measure action potential and Ca^{2+} currents. Radioligand binding was employed to study pharmacological characteristics of CBD binding. CBD (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. CBD (1 μM) significantly decreased the duration of APs. Further studies on L-type Ca^{2+} channels indicated that CBD inhibits these channels with IC_{50} of 0.1 μM in a voltage-independent manner. Radioligand studies indicated that the specific binding of [^{3}H]Isradipine, was not altered significantly by CBD. The results suggest that CBD depresses myocyte contractility by suppressing L-type Ca^{2+} channels at a site different than dihydropyridine binding site and inhibits excitation–contraction coupling in cardiomyocytes.

1. Introduction

Cannabidiol (CBD) is a major nonpsychoactive phytocannabinoid found in *Cannabis sativa*. Although it is devoid of psychoactive properties, in earlier studies CBD has been shown to possess anti-apoptotic, anti-oxidant, and anti-inflammatory effects [for reviews, 1, 2]. Interestingly, CBD displays low affinity for the cannabinoid CB_1 and CB_2 receptors [for reviews, 3, 4]. Thus, pharmacological actions of CBD have been suggested to be mediated mainly by its direct actions on various enzymes and ion channels or through a novel cannabinoid (non-CB_1 and non-CB_2) receptor [for reviews, 3, 4].

Several earlier studies indicate that CBD has beneficial effects in various cardiovascular pathologies such as myocardial infarction, ischemia-induced arrhythmias, and diabetic cardiomyopathy [5–8]. Although anti-oxidant and anti-inflammatory actions have been suggested to be involved in these beneficial effects [9], the exact mechanisms of CBD actions are currently unknown.

Abbreviations: AP, action potential; APD, action potential duration; APD_{60}, action potential duration at 60% level of repolarization; AMP, amplitude; BSA, bovine serum albumin; CBD, cannabidiol; THC, Δ^9-tetrahydrocannabinoid; DMSO, dimethylsulphoxide; NT, normal tyrode; RCL, resting cell length; SR, sarcoplasmic reticulum; T_{HALF}, time from peak to half; TPK, time to peak; TRP, transient receptor potential.

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Haeomodynamic effects of CBD have been investigated in earlier in vivo and in vitro studies [10]. In pentobarbitone anaesthetized rats, CBD has been shown to cause a significant but transient fall in mean arterial blood pressure [6]. Similarly, in arterial segments taken from rat mesenteric vascular bed that have been pre-constricted with phenylephrine, CBD has been shown to cause a concentration-dependent vasorelaxation [11]. Likewise, in rat isolated aortae, application of CBD (1–30 μM) caused vasorelaxation. In agreement with these findings, in human mesenteric arteries, it has been shown that CBD causes vasorelaxation of endothelin-1 pre-constricted arterial segments [12]. In these studies, both cannabinoid receptor-dependent and independent mechanisms have been shown to play roles in CBD inhibition of smooth muscle contraction. Further contributions to the complexity of CBD actions include the type of vascular structure, the presence of intact endothelium, the metabolic products of endocannabinoids [13] and the activity of metabolizing enzymes have also been shown to modulate the actions of CBD on cardiovascular system [10].

Compared with the information available on the vascular effects of CBD, to our knowledge, there have been few studies focusing on the role of CBD in the regulation of contractility and Ca^{2+} signaling in cardiac muscle. In earlier in vitro studies, negative inotropic and bradycardic effects of CBD have been reported [14]. In addition, in perfused rat heart, CBD has been shown to antagonize the Δ^9-tetrahydrocannabinoid-induced positive inotropic effect and tachycardia, one of the most consistent cardiovascular effects of *Cannabis* intoxication [15]. In the present study, we have hypothesized that the negative inotropic actions of CBD observed in earlier studies are due to the inhibition of excitation–contraction coupling in ventricular myocytes. Thus, we have investigated the effects of CBD on contractility and electrical properties of acutely dissociated rat ventricular myocytes.

2. Materials and methods

2.1. Ventricular myocyte isolation

Ventricular myocytes were isolated from adult male Wistar rats (248 ± 17 g) according to previously described techniques [16]. 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 X1 V; 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, 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 stocks 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 [17]. 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. Measurements of sarcoplasmic reticulum Ca^{2+} content

Sarcoplasmic reticulum (SR) Ca^{2+} release was assessed using previously described techniques [18]. 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 vs. 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, was used as a measure of myofilament sensitivity to Ca^{2+} [19].

2.6. Electrophysiological measurements of action potentials

Action potentials (APs) were measured using whole-cell patch clamp technique. Recordings were made with an Axopatch 200B amplifier (Molecular Devices, Downington, PA, USA) coupled to an A/D interface (Digidata 1322; Molecular Devices, Downington, PA, USA). Patch pipettes were fabricated from filamented GC150TF borosilicate glass (Harvard Apparatus, Holliston, MA, USA) on a

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