European Journal of Pharmacology 668 (2011) 208–216

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular Pharmacology

Pharmacological evidence for Orai channel activation as a source of cardiac abnormal automaticity

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ARTICLE INFO

Article history:
Received 23 January 2011
Received in revised form 8 June 2011
Accepted 15 June 2011
Available online 5 July 2011

Keywords:
Orai
2-APB
SKF-96365
Abnormal automaticity
Arrhythmia

ABSTRACT

Calcium transport through plasma membrane voltage-independent calcium channels is vital for signaling events in non-excitable and excitable cells. Following up on our earlier work, we tested the hypothesis that this type of calcium transport can disrupt myocardial electromechanical stability. Our Western and immunofluorescence analyses show that left atrial and ventricular myocytes express the Orai1 and the Orai3 calcium channels. Adding the Orai activator 2-aminoethoxydiphenyl borate (2-APB) to the superfusate of rat left atria causes these non-automatic muscles to contract spontaneously and persistently at rates of up to 10 Hz, and to produce normal action potentials from normal resting potentials, all in the absence of external stimulation. 2-APB likewise induces such automatic activity in superfused rat left ventricular papillary muscles, and the EC_{50}s at which 2-APB induces this activity in both muscles are similar to the concentrations which activate Orais. Importantly, the voltage-independent calcium channel inhibitor 1-[2-(4-methoxyphenyl)-2-[3-(4-methoxyphenyl) propoxy] ethyl-1*H*-imidazole (SKF-96365) suppresses this automaticity with an IC_{50} of 11 ± 0.6 μM in left atria and 6 ± 1.6 μM in papillary muscles. 1-(5-Iodonaphthalene-1-sulfonyl)-hexahydro-1,4-diazepine (ML-7), a second voltage-independent calcium channel inhibitor, and two calmodulin inhibitors also prevent 2-APB automaticity while two calmodulin-dependent protein kinase II inhibitors do not. Thus an activator of the Orai calcium channels provokes a novel type of high frequency automaticity in non-automatic heart muscle.

1. Introduction

In the normal heart, the sinoatrial node spontaneously generates the electrical stimuli that transit through the conduction system to initiate myocyte depolarization and contraction (Adamson et al., 2005). By contrast to this typical setting, arrhythmogenic electrical activity can arise in ectopic sites like atrial and ventricular muscle, the cells of the conduction system, and the muscular sleeves of cardiac veins (Haissaguerre et al., 2002; Jais et al., 1997; Waldo and Wit, 1993). Three types of voltage-dependent phenomena are hypothesized to produce ectopic activity: (i) The abnormal propagation of electrical impulses through cardiac muscle which re-excites myocytes to produce impulse reentry (Panfilov and Pertsov, 2001), (ii) calcium leak from sarcoplasmic reticulum stores that activates transient inward currents which trigger abnormal impulses (Lehnart et al., 2006; Shannon et al., 2003), and (iii) myocyte partial depolarization in settings like ischemia which elicits the spontaneous action potentials that define abnormal automaticity (Vassalle, 1971). However, drugs that alter either the myocyte action potential or impulse conduction are poor anti-arrhythmics (Adamson et al., 2005; The CAST Investigators, 1989). In addition, arrhythmia occurs in normal heart under non-ischemic conditions that are unlikely to cause calcium leak or partial depolarization (Haissaguerre et al., 2002). Furthermore, myocytes isolated from failing hearts can spontaneously depolarize from normal resting potentials without major changes in calcium homeostasis (Nuss et al., 1999). Together these latter reports suggest that novel mechanisms may initiate arrhythmogenic ectopy.

Two families of plasma membrane voltage-independent calcium transporters sustain the calcium homeostasis essential for cell signaling; (i) the Orai channels that form the store-operated calcium channel (SOCC) and the arachidonate-regulated calcium channel (ARC), and (ii) the transient receptor potential protein (Trp) channels. In the case of the SOCC, depletion of endoplasmic reticulum (ER) calcium stores induces ER stromal interaction molecule-1 (Stim1) to translocate to ER-plasma membrane junctions. There Stim1 recruits plasma membrane proteins including Orai1 to form the SOCC which facilitates the calcium entry required to refill cell stores (Liou et al., 2005; Parekh and Putney, 2005). For ARC activity, receptor signaling generates free arachidonic acid which activates pentamers of Orai1 and Orai3 to provoke cell calcium

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doi:10.1016/j.ejphar.2011.06.025

entry independently of ER Stim1 (Shuttleworth et al., 2004). Finally, multiple effectors activate calcium entry into cells through the Trp channels, and the SOCC itself may contain Trps (Birnbaumer, 2009). Both families of voltage-independent calcium transporters contribute to muscle and sinoatrial node function (Hunton et al., 2004; Ju et al., 2007; Lyfenko and Dirksen, 2008; Stiber et al., 2008). However, no data suggest that calcium transported through either family of proteins influences the fundamental properties of non-automatic heart muscle including its electromechanical stability.

Several groups have reported that 2-aminoethoxydiphenyl borate (2-APB) activates Orai1 and Orai3 with EC_{50}s of 20 μM and 14 μM, respectively, to induce calcium entry into calcium-replete non-excitable cells (DeHaven et al., 2008; Lis et al., 2007; Peinelt et al., 2008; Zhang et al., 2008). Previously we showed that 2-APB causes sporadic or tachycardic ectopy in superfused rat left atria but had not identified a potential mechanism for this unusual activity (Wolkowicz et al., 2007a,b). Here we test whether this ectopy has characteristics of an Orai-dependent process and report data suggesting that the activation of these voltage-independent calcium channels may ignite high frequency automaticity in non-automatic cardiac muscle.

2. Materials and methods

2.1. Materials

2-APB, 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl) phenyl]-3-pyridinecarboxylic acid methyl ester (Bay K 8644), and 1-[2-(4-methoxyphenyl)-2-[3-(4-methoxyphenyl) propoxy]ethyl-1*H*-imidazole (SKF-96365) were from Tocris-Cookson (Ellisville MO, USA). 1-(2-Hydroxyethyl)-4-(3-(trifluoromethyl-10-phenothiazinyl) propyl)-piperazine (fluphenazine-N-2-choloroethane), *N*-[2-[[[3-(4-chlorophenyl)-2-propenyl]methylamino]methyl]phenyl]-*N*-(2-hydroxyethyl)-4-methoxybenzenesulphonamide (KN-93), 4-[(2*S*)-2-[(5-isoquinolinylsulfonyl)methylamino]-3-oxo-3-(4-phenyl-1-piperazinyl)propyl] phenyl isoquinolinesulfonic acid ester (KN-62), 1-(5-iodonaphthalene-1-sulfonyl)-hexahydro-1,4-diazepine (ML-7), and (*RS*)-*N*-benzyl-*N*-(2-chloroethyl)-1-phenoxy-propan-2-amine (phenoxybenzamine) were from Calbiochem (Gibbstown NJ, USA). Enhanced chemiluminescence reagent was from GE Healthsciences (Piscataway NJ, USA). All other reagents were standard laboratory grade.

Bay K 8644 and 2-APB were prepared as 100 and 150 mM DMSO stock solutions, respectively; SKF-96365 was suspended as a 100 mM aqueous stock solution.

2.2. Left atrial and left ventricular papillary muscle preparations

These investigations conform to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All rats were anesthetized with isoflurane prior to the harvesting of their hearts.

Rat left atrial appendages and intact right atria were isolated, superfused in Krebs-Henseliet (KH) buffer, and their mechanical function was assessed as described (Wolkowicz et al., 2007b). All left atria were pre-treated for 10 min with 300 nM (−)Bay K 8644 except those used in calmodulin inhibitor, calmodulin-dependent protein kinase II (CaMKII) inhibitor, and ML-7 studies. To acquire resting and action potentials, superfused left atria were impaled with glass microelectrodes containing 3 M KCl (Huang et al., 2004).

Anterior left ventricular papillary muscles were dissected and mounted in muscle baths that contained oxygenated KH (Topcuoğlu et al., 1999) maintained at 30 °C. Muscle resting tension was set to 0.8 g and all muscles were paced for 30 min at 1 Hz and 130% of capture voltage. Papillary muscle length then was adjusted to produce a maximum force of contraction and resting tension was set to 80% of this value. Typical muscles (n = 7) were 3.8 ± 0.25 mm long, 1.0 ± 0.10 mm wide, and weighed 4.3 ± 0.98 mg-wet-weight. They generated 14.6 ± 1.3 mN of force with a time-to-peak tension of 119 ± 2 ms, 50% relaxation times of 89 ± 1 ms, and 90% relaxation times of 157 ± 7 ms. All stabilized papillary muscles were pre-treated by adding 300 nM (−)Bay K 8644 to the superfusate 10 min before the start of any experiment. Bay K 8644 was maintained in the superfusate thereafter and it increased muscle force of contraction to 149 ± 7% of control.

2.3. The concentration-dependence of 2-APB-induced ectopy

Two sets of experiments assessed how 2-APB affects calcium-loaded cardiac muscle. First, left atria (n = 7) were superfused in KH at 30 °C, paced at 0.1 Hz, and eight increasing concentrations of 2-APB from 0 to 30 μM were added to the superfusate. After three to 10 min incubation at each concentration, pacing was stopped and we measured the rate at which these left atria spontaneously contracted. In separate experiments, 7.5 or 20 μM 2-APB was added to the superfusate of two groups of 0.1 Hz-paced left atria (n = 3 per) and their mechanical function was recorded for 15 min with or without pacing. Finally, we recorded the electrical activity of untreated, 1 Hz-paced (n = 3) and 2-APB-treated, spontaneously contracting left atria (n = 3).

The second set of experiments undertook similar analyses in papillary muscles. Seven concentrations of 2-APB from 0 to 28 μM were added to the superfusate of 1 Hz-paced papillary muscles (n = 7). After three to 10 min incubation at each concentration, pacing was stopped and we measured the rate at which papillary muscles spontaneously contracted. Two additional groups of muscles (n = 5 per) were superfused in KH at 30 °C, paced at 1 Hz, and 0 or 20 μM 2-APB was added to the superfusate. Five minutes later we stopped pacing and measured the rate at which these muscles spontaneously contracted.

2.4. The temperature dependence of 2-APB-induced left atrial ectopy

To estimate a physiological rate for 2-APB-induced ectopy, we superfused 0.1 Hz-paced left atria (n = 9) in KH at 30 °C, and added 20 μM 2-APB to the superfusate. Once these muscles spontaneously contracted, pacing was stopped and we measured the rate of this spontaneous activity. Additional 0.1 Hz-paced left atria (n = 9) then were superfused at 23 °C, 20 μM 2-APB was added to the superfusate, and the steady-state rate of spontaneous contraction was measured in the absence of pacing. Muscle bath temperature was rapidly increased to 37 °C and left atrial spontaneous contraction rate was measured again in the absence of pacing. For comparative purposes, the contraction rate of a group of untreated, unpaced intact rat right atria (n = 7) was measured at 30 °C as was the spontaneous contraction rate of a second group of untreated, unpaced right atria (n = 7) measured at 23 and 37 °C.

2.5. Assessment of 2-APB-induced ectopy as a triggered or an automatic event

Three groups of left atria (n = 5 per) and three groups of papillary muscles (n = 5 per) were superfused in KH at 30 °C, and paced at 1 Hz. Pacing was stopped and one group of each type of muscle remained untreated. 25 μM 2-APB was added to the superfusate of the second group of muscles 30 s after the last stimulus. The third group of left atria or papillary muscles were treated with 25 μM 2-APB 30 s after the last stimulus, and then with 40 μM SKF-96365 30 s later. We assessed whether any of these unpaced muscles spontaneously contracted during the next 10 min of superfusion.