Journal of Molecular and Cellular Cardiology 65 (2013) 120–126

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Original article

Muscarinic stimulation and pinacidil produce similar facilitation of tachyarrhythmia induction in rat isolated atria

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ARTICLE INFO

Article history:
Received 13 September 2013
Accepted 9 October 2013
Available online 17 October 2013

Keywords:
Atrial tachyarrhythmia
Electrical propagation
Inward rectifier potassium currents
K_{ATP} channel
Action potential

ABSTRACT

Atrial tachyarrhythmias, the most common type of cardiac arrhythmias, are associated with greater stroke risk. Muscarinic cholinergic agonists have been shown to facilitate atrial tachyarrhythmia maintenance in the absence of cardiac disease. This has been attributed to action potential shortening, which enhances myocardial electrical anisotropy, and thus creates a substrate for reentrant excitation. In this study, we describe a similar effect of the ATP-sensitive K^+ channel (K_{ATP}) opener pinacidil on tachyarrhythmia induction in isolated rat atria. Pinacidil, which activates a weakly inwardly-rectifying current in isolated atrial myocytes, enhanced arrhythmia induction in the right and left atria. This effect was abolished by the K_{ATP} blocker glibenclamide, but not by atropine, which rules out a possible indirect effect due to stimulation of acetylcholine release. However, pinacidil attenuated carbachol-induced tachyarrhythmia facilitation, which may indicate that the action of these agonists converges to a common cellular mechanism. Both agonists caused marked action potential shortening in isolated atrial myocytes. Moreover, during arrhythmia in the presence of pinacidil and carbachol, the atrial vectorelectrographic patterns were similar and consistent with reentrant propagation of the electrical activity. From these results, we conclude that the K_{ATP} channel opening is pro-arrhythmic in atrial tissue, which may pose as an additional risk in the scenario of myocardial hypoxia. Moreover, the similarity of the electrophysiological effects of pinacidil and carbachol is suggestive that the sole increase in background K^+ conductance is sufficient for atrial tachyarrhythmia facilitation.

1. Introduction

Atrial tachyarrhythmias (AT), the most frequent type of sustained arrhythmias, are associated with significant morbidity and mortality, and considered as a risk factor for stroke, cardiomyopathy and peripheral embolism (e.g., [1–4]). Because AT prevalence increases with age [1,5], it is expected that a considerable part of the population worldwide should be afflicted by it in the next decades, in face of the progressive rise in life expectancy in most countries. Thus, it is not surprising that much effort has been expended for better understanding of the mechanisms underlying this type of arrhythmia, as well as for improvement of the therapeutic and preventive approaches [3].

It is well known that stimulation of muscarinic cholinergic receptors can facilitate AT occurrence and maintenance [6–9], a mechanism likely to underlie the vagally-mediated form of atrial fibrillation [10–12]. This effect has been largely attributed to the establishment of a functional substrate for reentrant propagation of the electrical activity by abbreviation of the action potential (AP) and thus of the refractory period of atrial myocytes [6,7,9,13,14]. In the adult heart, the predominant muscarinic receptors are of the m2 subtype, which are coupled to a pertussis toxin-sensitive G protein (G_i/G_o). Classically, muscarinic stimulation produces functional antagonism of the β-adrenergic pathway. Most of this antagonism is exerted on the cAMP synthesis, as the α subunit of the G protein coupled to m2 receptors inhibits adenylate cyclase activity [15]. In the atrial tissue, m2 receptor stimulation recruits an additional mechanism: the activation of inward rectifier K^+ channels by the βγ subunits of the G protein, resulting in the activation of a ligand-regulated, background hyperpolarizing current ($I_{K(ACh)}$), to which is largely attributed the negative chronotropic effect, as well as the AP shortening effect of muscarinic agonists [15–19].

Using an in vitro rat model of muscarinic AT [20], Zafalon et al. [8] observed that arrhythmia induction and maintenance could be suppressed by agents that activate the cAMP–protein kinase A signaling

Abbreviations: ACh, acetylcholine; AP, action potential; APD, action potential duration; AR, right atrial spontaneous rate; AT, atrial tachyarrhythmia; cAMP, 3′-5′ cyclic adenosine monophosphate; CCh, carbachol; *High*, high-inducibility stimulation protocol; $I_{K(ACh)}$, acetylcholine-regulated K^+ current; $I_{K(ATP)}$, ATP-sensitive K^+ current; K_{ATP} channel, ATP-sensitive K^+ channel; *Low*, low-inducibility stimulation protocol; SUR, sulfonylurea receptor; TI, tachyarrhythmia induction index; V_m, membrane potential.

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http://dx.doi.org/10.1016/j.yjmcc.2013.10.004

pathway, such as β-adrenoceptor agonists, as well as phosphodiesterase and protein phosphatase inhibitors. However, amiodarone, which blocks $I_{K(ACh)}$ and prevents AP shortening by muscarinic agonists [21,22], was also able to antagonize the proarrhythmic effects of muscarinic stimulation [8]. Thus, although both mechanisms seem to be involved in the muscarinic tachyarrhythmia facilitation, it was not clear if this effect could be produced by recruitment of only one of them. It has been shown that $I_{K(ACh)}$ activation is necessary for the manifestation of muscarinic proarrhythmogenesis [23]. However, it still remained to be demonstrated whether the increase in background K^+ conductance alone is sufficient for AT facilitation.

The goal of the present study was to investigate the ability of the induction of the ATP-sensitive K^+ current ($I_{K(ATP)}$), a muscarinic-independent, background K^+ current, to reproduce the proarrhythmic effect of muscarinic stimulation. The pore-forming subunits of the channels that conduct $I_{K(ACh)}$ and $I_{K(ATP)}$ belong to the family of inward rectifier K^+ channels (Kir family: Kir3.x and Kir6.x, respectively), and are well expressed in atrial cells [19,24]. The ATP-sensitive K^+ (K_{ATP}) channel has constitutive activity and is negatively regulated by intracellular adenine nucleotides: $I_{K(ATP)}$ may be induced by ATP dissociation from its binding sites at the channel or by pharmacological agents that cause the channel to open by binding to the sulfonylurea receptor (SUR), a regulatory subunit [19]. In this study, we compared the effects of the K_{ATP} channel opener pinacidil [25] and of the muscarinic cholinergic agonist carbachol (CCh) on the electrical behavior of both unicellular and multicellular atrial preparations, to explore the hypothesis that the increase in sarcolemmal background conductance to K^+ is sufficient to promote AT facilitation.

2. Materials and methods

2.1. Isolated atrial preparations

The right and left atria (weighing 42.4 ± 5.2 mg, N = 22; and 34.4 ± 1.9 mg, N = 6, respectively) were isolated from male adult Wistar rats (6–8 month-old) after euthanasia by exsanguination following cerebral concussion. The protocol for animal care and use was in accordance to the Brazilian laws regarding the use of experimental animals and was approved by the institutional Committee for Ethics in Animal Use (CEEA-CEUA/IB/UNICAMP, doc. number 773-1 and 2587-1C).

Atria were mounted horizontally (dorsal surface up) under 5 mN preload in a circular chamber containing modified Krebs–Henseleit solution (mM composition: 115 NaCl, 4.5 KCl, 1.2 KH_2PO_4, 1.5 $MgSO_4$, 25 $NaHCO_3$, 2.5 $CaCl_2$, 11 glucose, pH 7.4) at 36.5 °C, gassed with 95% O_2/5% CO_2 [8], to which 0.1 μM propranolol was added to block β-adrenoceptors. Experiments started after a 45 min stabilization period. Atrial electrograms were recorded with coated Ag–AgCl wire electrodes and atria were electrically stimulated with a pair of platinum wire electrodes, as described elsewhere [26].

For atrial myocyte isolation, the hearts were perfused via the aorta with Ca^{2+}-free Krebs–Henseleit solution at 37 °C containing collagenase I (0.6 mg/ml; Worthington Biochem, Lakewood, NJ, USA) for 15 min. Atria were then removed and incubated with the collagenase solution at 37 °C for additional 15 min. The tissue was rinsed and triturated in a cardioplegic solution [27]. Cells were stored at 4 °C and used within 4 h after isolation.

2.2. Tachyarrhythmia induction

Atria were field-stimulated with trains of biphasic rectangular voltage pulses with 1 ms total duration, and amplitude 20% higher than the threshold for arrhythmia induction, determined previously in each preparation. Electrical stimulation was applied only during the arrhythmia induction trials. Two stimulation protocols were used, with different effectiveness at tachyarrhythmia induction [8]: the low inducibility protocol (*Low*) was a single train of 50 pulses at 30 Hz, while the high inducibility protocol (*High*) consisted of a triple train at 67 Hz, with 3 blocks of 20 pulses separated by 1.3 s intervals. Only one induction protocol was used in each preparation, i.e., before and during exposure to drugs. A trial was considered as the single application of a given induction protocol, whereas an episode was the succession of 8–10 trials applied at 1 min intervals. The tachyarrhythmia induction index (TI) was the fraction of trials in an episode that were successful at evoking AT, which was identified as high rate (>10 Hz) oscillations of the electrographic signal after the electrical stimulation was interrupted, with duration greater than 10 s (Fig. 1). If the AT lasted longer than 2 min, it was reverted with the same stimulation protocol used for its induction. The interval between episodes was at least 15 min.

2.3. Experimental protocol

In the right atria, the stimulation protocol for arrhythmia induction was applied in the absence (control) and presence of increasing concentrations of pinacidil or CCh. In some cases, CCh was added to enhance TI prior to pinacidil exposure. Some experiments were performed in the presence of 1 μM atropine and/or 30 μM glibenclamide, to which atria were exposed for 30 min prior to TI determination. Pinacidil and CCh effects were assessed after 5–7 min exposure. The total duration of the experiments (including the stabilization period) did not exceed 3.5 h, during which AT induction was previously shown to be reproducible [20].

Solutions were prepared with salts of analytical grade. All drugs were from Sigma Chem Co. Stock solutions were kept at −20° and diluted immediately before use.

2.4. Atrial vectorelectrogram

The atrial vectorelectrogram was determined according to Zafalon et al. [26] from electrograms recorded simultaneously (1 kHz acquisition rate) at two leads oriented at 60° (equivalent to the D1 and D2 leads in the Einthoven's triangle). After signal filtering (100 Hz low-pass and

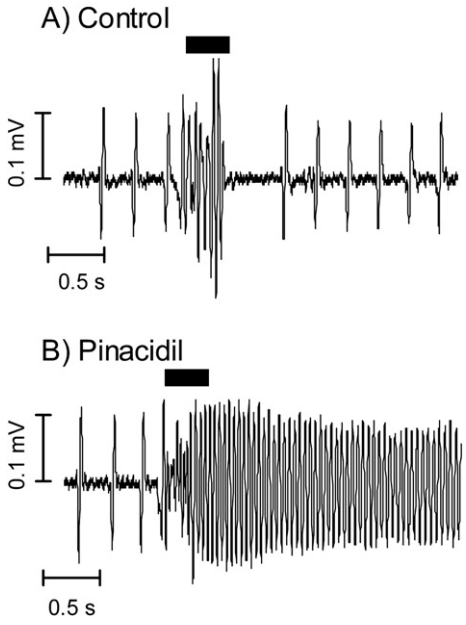

Fig. 1. Electrogram traces recorded from an isolated rat right atrium exposed to 1 μM atropine, in the absence (A) and in the presence of 20 μM pinacidil (B), using the high inducibility stimulation protocol. The black bars indicate the application of the last block of the stimulus train (the previous 2 blocks did not evoke arrhythmia, and were omitted for clarity).