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Original article

I_f blocking potency of ivabradine is preserved under elevated endotoxin levels in human atrial myocytes

Susanne Scheruebel [a], Chintan N. Koyani [b], Seth Hallström [c], Petra Lang [a], Dieter Platzer [a], Heinrich Mächler [d], Karl Lohner [e], Ernst Malle [b], Klaus Zorn-Pauly [a,*], Brigitte Pelzmann [a,*]

[a] Institute of Biophysics, Medical University of Graz, Harrachgasse 21, A-8010 Graz, Austria
[b] Institute of Molecular Biology and Biochemistry, Medical University of Graz, Harrachgasse 21, A-8010 Graz, Austria
[c] Institute of Physiological Chemistry, Medical University of Graz, Harrachgasse 21, A-8010 Graz, Austria
[d] Division of Cardiac Surgery, Medical University of Graz, Auenbruggerplatz, A-8010 Graz, Austria
[e] Institute of Molecular Biosciences, Biophysics Division, University of Graz, Schmiedlstrasse 6, A-8042 Graz, Austria

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ABSTRACT

Lower heart rate is associated with better survival in patients with multiple organ dysfunction syndrome (MODS), a disease mostly caused by sepsis. The benefits of heart rate reduction by ivabradine during MODS are currently being investigated in the MODI_fY clinical trial. Ivabradine is a selective inhibitor of the pacemaker current I_f and since I_f is impaired by lipopolysaccharide (LPS, endotoxin), a trigger of sepsis, we aimed to explore I_f blocking potency of ivabradine under elevated endotoxin levels in human atrial cardiomyocytes. Treatment of myocytes with S-LPS (containing the lipid A moiety, a core oligosaccharide and an O-polysaccharide chain) but not R595 (an O-chain lacking LPS-form) caused I_f inhibition under acute and chronic septic conditions. The specific interaction of S-LPS but not R595 to pacemaker channels HCN2 and HCN4 proves the necessity of O-chain for S-LPS–HCN interaction. The efficacy of ivabradine to block I_f was reduced under septic conditions, an observation that correlated with lower intracellular ivabradine concentrations in S-LPS- but not R595-treated cardiomyocytes. Computational analysis using a sinoatrial pacemaker cell model revealed that despite a reduction of I_f under septic conditions, ivabradine further decelerated pacemaking activity. This novel finding, i.e. I_f inhibition by ivabradine under elevated endotoxin levels in vitro, may provide a molecular understanding for the efficacy of this drug on heart rate reduction under septic conditions in vivo, e.g. the MODI_fY clinical trial.

1. Introduction

Sepsis, a microbial induced inflammatory response, encompasses a spectrum of illness that ranges from systemic infection to multiple organ dysfunction syndrome (MODS) [1]. Mortality due to septic cardiac dysfunction has been encountered in critically ill patients [2] where an increased heart rate may act as an independent risk factor [3]. Patients with lower heart rate in the early phase of MODS have better survival rates than those with higher heart rate [3].

Sepsis is induced by lipopolysaccharide (LPS, endotoxin), a major component of the outer cell wall of gram-negative bacteria. Notably, LPS from wild type bacteria usually is a mix of S-form LPS and varying proportions of so-called R-form LPS [4]. S-form LPS consists of three entities: the lipid A moiety, that harbours the endotoxic activity of the entire molecule, the core oligosaccharide and the O-polysaccharide chain, that is absent in R-form LPS [5,6]. Irrespective of their structural components, both LPS forms are capable to initiate sepsis by triggering the inflammatory response [7]. Besides initiating the inflammatory response, S-form LPS directly affects ionic channels of immune, neuronal and cardiovascular cells [8–10], the latter include channels conducting the pacemaker current I_f. This current is a mixed Na^+/K^+ inward current carried by pacemaker channels comprising four different homo- or heteromeric isoforms of hyperpolarization-activated cyclic nucleotide-gated (HCN 1–4) channels [11]. I_f plays an important role in the regulation of heart rate [12] by contributing to the slow diastolic depolarization phase that determines the firing rate of spontaneous action potentials of sinoatrial node cells [13]. Moreover, in response to autonomic transmitters, I_f contributes to the chronotropic regulation of heart rate [13]. Previously, we reported I_f loss-of-function in human atrial myocytes after chronic S-LPS treatment, an observation that in turn might be responsible for reduction of heart rate variability during sepsis [14,15]. Meanwhile it was shown that S-form LPS also acutely impairs I_f [16,17] and that the polysaccharide part (O-chain) of the LPS molecule [16] is necessary for reduction of pacemaker channel activity.

Beta-blocker administration has been shown to reduce mortality in MODS [18,19]. However, negative inotropic effects of beta-blockers

* Corresponding authors. Tel.: +43 316 380 4145; fax: +43 316 380 9660.
E-mail addresses: klaus.zornpauly@medunigraz.at (K. Zorn-Pauly), brigitte.pelzmann@medunigraz.at (B. Pelzmann).

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restrict its use in the majority of patients. Therefore, ivabradine may be considered as an alternative therapeutic approach to reduce heart rate. This drug is a pure heart rate lowering agent that selectively inhibits I_f [20]. Ivabradine blocks pacemaker channels in a use-dependent way being more effective at higher heart rate while its action declines during bradycardia [21,22]. Blocking of pacemaker channels by ivabradine requires three steps: diffusion through the cell membrane, opening of respective channels and intracellular binding of the drug to the channel pore [20,23]. Heart rate reduction by ivabradine has been found to be beneficial in the treatment of cardiovascular disease, since it lowers heart rate without adversely affecting other cardiovascular functions [24].

Currently, the MODI_fY trial carefully investigates potential benefits of heart rate reduction by ivabradine in sepsis and MODS [19]. Since S-LPS substantially inhibits I_f and ivabradine is administered in septic patients (MODI_fY trial), we hypothesized that I_f reduction by S-LPS might interfere with ivabradine action on I_f. From a clinical perspective, the efficacy of I_f inhibition by ivabradine under septic conditions is of special therapeutic relevance.

In this study we investigated the effect of S-LPS and R595 on human atrial I_f under acute and chronic septic conditions as well as the interaction of both endotoxins with HCN channels. Next, we focused on the effect of endotoxins on (i) I_f reduction by ivabradine and (ii) intracellular ivabradine concentrations. Finally, using a computer simulation model we tried to explore the efficacy of ivabradine on deceleration of sinoatrial pacemaking activity under septic conditions.

2. Material and methods

A detailed Materials and methods section on isolation of cardiomyocytes [25–27], cell culture [28], myocyte treatments, patch-clamp measurements [14,29,30], immunoprecipitation [31], Western blot and immuno-dot-blot [32], qPCR [33], determination of ivabradine concentrations by HPLC [34] and sinoatrial pacemaker cell modeling [35] is available under the online supplemental section.

3. Results

3.1. Representative recordings of I_f of human atrial myocytes

Fig. 1 shows representative traces of I_f under control (Fig. 1A), ivabradine (Fig. 1B) and septic conditions (mimicked *in vitro* by LPS incubation, Figs. 1C and D) elicited by hyperpolarizing voltage steps from −40 to −130 mV. I_f was considered to be present when current

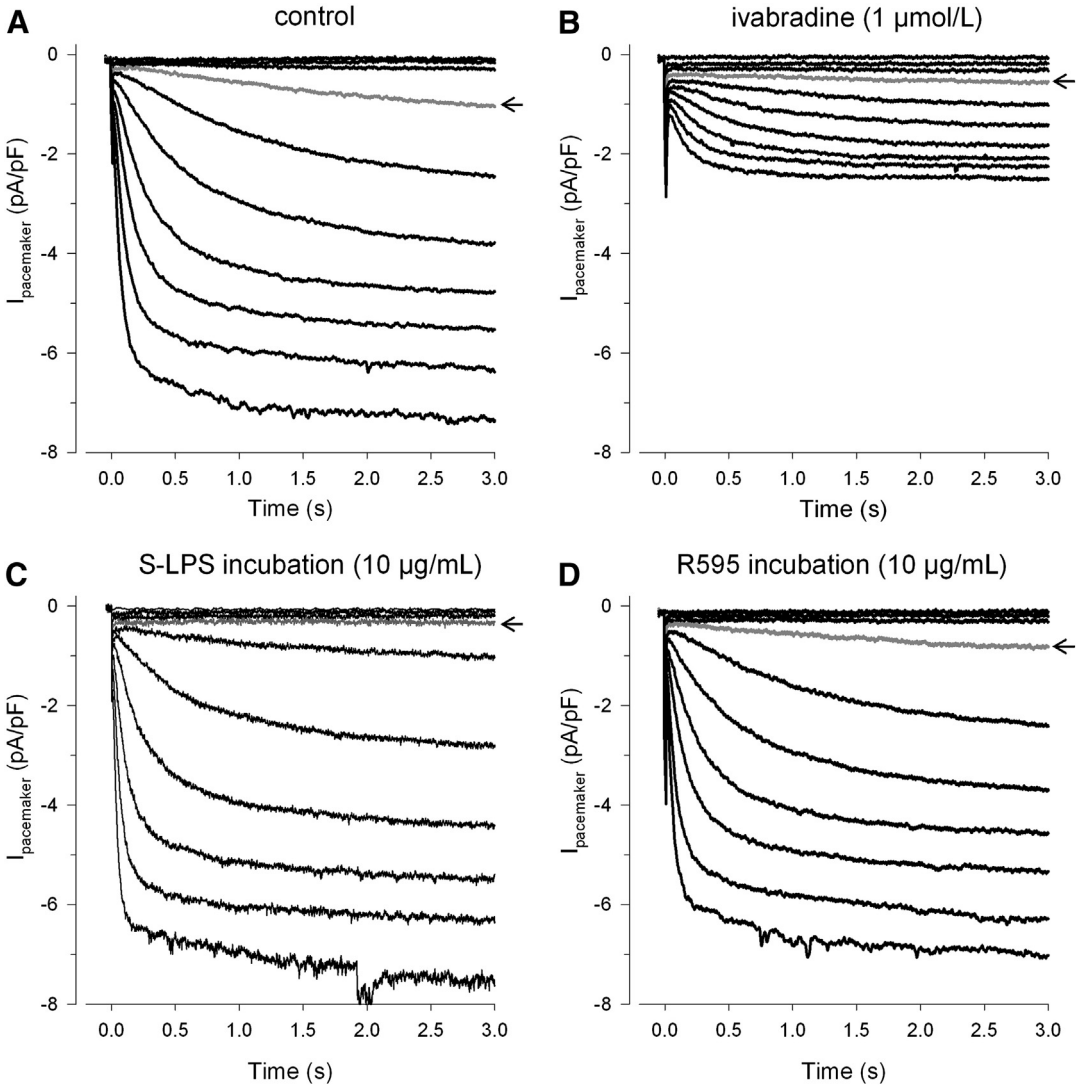

Fig. 1. Representative pacemaker current recordings of human atrial myocytes. Pacemaker currents (pA) were recorded by hyperpolarizing voltage steps ranging from −40 to −130 mV (10 mV increment, holding potential −40 mV, 3 s duration) and normalized to cell capacitance (pF) for a control cell (A) and for cells treated with indicated concentrations of ivabradine (7 min) (B), S-LPS (6 h) (C) or R595 (6 h) (D). Arrows indicate current at −70 mV.