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Appraisal of state-of-the-art

Ion currents of cardiomyocytes in different regions of the Göttingen minipig heart

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ABSTRACT

Introduction: The Göttingen minipig is a promising model for pharmacological safety assessment and for translational research in cardiology. We have examined the main ion currents in cardiomyocytes of the minipig heart.
Methods: Cardiac cells were isolated from different cardiac regions (endo-, mid- and epicardial left ventricle and right ventricle) from Göttingen minipigs and examined using the whole cell patch clamp technique combined with pharmacological interventions.
Results: The inward rectifier (I_{K1}), the delayed rectifier (I_K), with the rapid and slow components, (I_{Kr}, I_{Ks}) and the L-type Ca^{2+} channel ($I_{Ca,L}$) were identified in the different regions of the heart, whereas the Ca^{2+}-independent transient outward current (I_{to1}) was observed in only a few cells. I_{K1} was similar in the cardiac regions with a slightly lower value in the epicardial cells. I_{Ks} was smaller in epi- and endo-cardial regions.
Discussion: The equivalents of the main human cardiac ion currents are present in the minipig cardiomyocytes with the exception of the Ca^{2+}-independent I_{to1}. The study provides further evidence that the minipig is a valid model for investigating cardiovascular pharmacology.

1. Introduction

The Göttingen minipig has been recognized as a relevant laboratory animal for decades (Beglinger, Becker, Eggenberger, & Lombard, 1975). As a cardiovascular model animal, it has obvious advantages compared to the other porcine models, due to its smaller size, associated with easier handling and reduced costs for animal husbandry. The minipig cardiovascular system has a similar anatomy compared to humans, enabling translational research to human clinical applications (Swindle & Smith, 1998). Previously, the Göttingen minipig has been used for cardiovascular monitoring and pharmacological testing (Stubhan et al., 2008; Markert et al., 2009) and disease models have been developed, including chronic heart failure (Schuleri et al., 2008). Thus, the minipig is considered an alternative to rodents, dogs and non-human primates in pharmacological characterization and safety assessment (Svendsen, 2006; Van der Laan, Brightwell, Mcanulty, Ratky, & Stark, 2010; Authier et al., 2011). Although a large amount of electrophysiological data from hearts of other species is available (cf. Schram, Melnyk, & Nattel, 2002; Nerbonne & Kass, 2005), very little information has been presented regarding the cardiac electrophysiology of the minipig. In a recent study, Laursen, Olesen, Grunnet, Mow, and Jespersen (2011a) used conventional microelectrode techniques and surface electrodes to record action potential duration in isolated retrogradely perfused hearts and in isolated endocardial muscle. They demonstrated effects of temperature, beating frequency and β-adrenergic stimulation on the action potential duration, with similar general characteristics as those reported for other species. Using K^+ channel blockers and mRNA expression analyses, they also provided indirect evidence for the presence of the main K^+ currents. In further work they characterized the pro-arrhythmic properties in comparison to the dog heart (Laursen, Grunnet, Olesen, Jespersen, & Mow, 2011b). However, for a complete characterization of the cardiac electrophysiology, information is also needed regarding the properties of the different ion currents at the cellular level. In the present investigation, we have therefore used whole cell patch clamp of myocytes isolated from different regions of the minipig cardiac ventricle to address the question: which ion currents are functional in the minipig cardiac cells? We provide electrophysiological evidence for the presence of key ion currents in the minipig heart and describe their properties in different cardiac regions.

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The results provide further evidence that the Göttingen minipig is a valid model for cardiovascular pharmacology.

2. Material and methods

2.1. Animals and cardiac preparation

Male Göttingen minipigs (Ellegaard Göttingen Minipigs A/S, Denmark) 6–8 months of age were used. The project was approved by the local animal ethical committee and the procedures were performed in accordance with the Swedish Animal Protection law and European conventions. Intramuscular injection of ketamine (30 mg/kg) was used to induce anaesthesia followed by sodium thiopental (5–8 mg/kg) and atropine (0.02 mg/kg) intravenously. A tracheal tube (7.0 mm internal diameter) was inserted and connected to a Servo Ventilator 300 (Siemens, Solna, Sweden). The animal was ventilated with a tidal volume of 200 ml, 25 breaths/min and a positive end-expiratory pressure (PEEP) of 8 cm H_2O. During open chest surgery, the aorta was clamped and cold (4 °C) Plegisol® (Hospira, Lake Forest USA) cardioplegic solution was infused antegradely into the coronary arteries. Exit of the cardioplegic solution was via an incision in the right atria at the onset of the cardioplegic infusion. Following cardiac arrest, the hearts were excised and immediately immersed in cold Plegisol and stored for approximately 1.5 h at 4 °C.

2.2. Isolation of ventricular myocytes

Ventricular myocytes were enzymatically isolated from wedges of the left and right ventricular free wall. A wedge was cannulated via a coronary artery and the tissue was perfused for 10 min with Ca^{2+}-free oxygenated solution (containing in mM: KCl 80, KH_2PO_4 30, $MgSO_4$ 5, Taurine 20, Creatine 5, Succinate 5, Glucose 10, Na_2ATP 5, albumin 0.0175%, Hepes 5, nitrilotriacetic acid (NTA) 5, pH 7.0, total osmolarity 285 mOsmol) at 37 °C, followed by perfusion for 30 min with the same solution without NTA and with Worthington collagenase Type-2 (~200 U/ml). The right ventricle (Right) and left endocardial (Endo), middle (Mid) and epicardial (Epi) sections of the wall were selected by dissection under a microscope: following extirpation for use of the outer and inner layer of the myocardium, residual portions of outer and inner layers discarded and the midsection was then selected. A small amount of CaCl2 (0.18 mM) and 2% Bovine Serum Albumin were added to the collagenase solution and the tissues were gently agitated on a shaker tablet for 45–60 min at 37 °C. Samples of supernatant containing free cells were collected and transferred to Minimum Essential Medium Eagle (MEM, Sigma-Aldrich).

2.3. Whole cell patch clamp of isolated myocytes

The whole-cell variant of the patch-clamp technique was used to record ionic currents and action potentials using an Axon switch-clamp amplifier. Experiments were performed at 36 °C. The resistance of the glass pipette was 1 to 3 MΩ when filled with an internal pipette solution (containing in mM: KCl 150, $MgCl_2$ 5, Hepes 5, pH 7.2). The cell capacitance was determined using 10 mV hyperpolarizing pulses applied from a holding potential of −10 mV. The Tyrode cell perfusate contained (in mM): NaCl 150, KCl 5.4, CaCl2 2.0, MgCl2 2.05, glucose 5.5 and Hepes 10, pH 7.4. Voltage and current signals were stored on a personal computer with in-house Linux-Mandrake-Comedi recording and analysis programs. In current clamp mode, action potentials were elicited by 3 ms depolarizing pulses through the pipette. The resting membrane potential, the action potential overshoot and the action potential duration (APD) were evaluated. APD was analyzed by determining the time to reach 25%, 50% and 90% repolarization (APD_{25}, APD_{50} and APD_{90}). In voltage clamp mode, individual currents were isolated using specific voltage protocols in combination with standard pharmacological blockers (see below). The currents investigated were: L-type Ca^{2+} current ($I_{Ca,L}$), Na^+ current (I_{Na}), Ca^{2+}-independent transient outward K^+ current (I_{to1},), inward rectifier current (I_{K1}) and the delayed rectifier current (I_K).

2.4. Calcium current ($I_{Ca,L}$) of isolated myocytes

From a holding potential of −40 mV, $I_{Ca,L}$ was elicited by imposing a series of 300 ms steps increasing with 5 mV in amplitude from −35 mV to a maximal potential of +40 mV with 5 s intervals. The $I_{Ca,L}$ amplitude was determined as the difference between peak current and the current level at the end of the pulse. The decay of $I_{Ca,L}$ with time (t) was fitted by an exponential function with an amplitude (A) and a time constant (τ): $I_{Ca,L} = y0 + A * (1\text{-exp.}(-t/\tau))$.

2.5. Potassium currents (I_{K1}, I_{to1}, I_K) of isolated myocytes

In these experiments the L-type Ca^{2+} current was blocked with 5 μM Nifedipine. From a holding potential of −50 mV, the inward rectifier K^+ current (I_{K1}) was elicited using 200 ms voltage steps in the range −120 mV to −20 mV with 3 s intervals. The current amplitude was determined at the end of the 200 ms step. We also examined the presence of a Ca^{2+}-independent transient outward K^+ current (I_{to1}) by making 300 ms steps in the range −40 mV to +60 mV from a holding potential of −70 mV and a pre-pulse step to −45 mV with 10 s intervals.

The delayed rectifier (I_K) was examined using an initial 3 s step in the range −20 mV to +60 mV from a holding potential of −50 mV, followed by a repolarizing step for 5 s to −25 mV or −50 mV. The protocol was applied in 20 s intervals. The step current was determined as the increase in current developed at the end of the 3 s step. The tail current was determined as the difference between the beginning and the end of the current transient during the 5 s returning step. The slow component, I_{Ks}, was measured during blockade of I_{Kr} by 10 μM E-4031 (Tocris Bioscience) or 1 μM Dofetilide (Hangzhou Uniwise Int. Co., Ltd.) added to the superfusate, and I_{Kr} was measured during blockade of I_{Ks} by 30 μM Chromanol 293B. The decay of I_K tails with time (t) was fitted by a single exponential function with an amplitude (A) and a time constant (τ): $I_K = y0 + A * \exp.(-t/\tau)$.

2.6. Sodium currents (I_{Na}) of isolated myocytes

The sodium currents, $I_{Na,}$ were of a magnitude and speed which were, even at room temperature, too large to control in voltage-clamp mode at the experimental settings applied here. Therefore data are not reported. I_{Na} was initiated at around −55 mV and the relaxation time constants were in the range of 2–10 ms (n = 16).

2.7. Statistics

All values are presented as mean ± SEM. Unless otherwise stated, the number of animals is given in parenthesis. Statistical comparisons were made using Student's *t*-test or when appropriate analysis of variance. Calculations and curve fitting were performed using Sigmaplot and SigmaStat for Windows (SPSS Science, Chicago, IL).

3. Results

3.1. Action potentials

Representative recordings of action potentials from cells isolated from different regions are depicted in Fig. 1 (pacing frequency of 0.5 Hz). Action potentials had a similar morphology across the left ventricular wall and between right and left ventricle, with a prominent phase 2 plateau, but without a prominent spike-and-dome morphology, suggesting the lack of a transient outward potassium current (Panel A). Action potential duration at 25, 50 and 90% repolarization was similar in cells from the different regions (Panel B).