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Original article

Characterization of cardiac repolarization in the Göttingen minipig

Morten Laursen [a,b,*], Søren-Peter Olesen [b,c], Morten Grunnet [b,c], Tomas Mow [a], Thomas Jespersen [b]

[a] H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Copenhagen, Denmark
[b] The Danish National Research Foundation Centre for Cardiac Arrhythmia, Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 2, 2200 Copenhagen N, Denmark
[c] NeuroSearch A/S, Pederstrupvej 93, 2750 Ballerup, Denmark

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ABSTRACT

Introduction: The minipig represents an attractive experimental animal within cardiovascular research due to its extensive similarities to the human heart in terms of anatomy and physiology. Although minipigs have been used for cardiovascular research for decades no thorough characterization of the minipig cardiac electrophysiology has been performed. Therefore, we have for the first time characterized the minipig cardiac repolarization in a series of experiments ranging from mRNA quantification to *in vivo* studies. **Methods:** Göttingen minipigs were used throughout the study. Cardiac mRNA quantification was performed using quantitative PCR methods. For *ex vivo* experiments, hearts were excised using cardioplegic procedures and Langendorff and microelectrode action potential recordings were performed. Effects of temperature *in vivo* were recorded in anesthetized animals. **Results:** On the mRNA level the expression profile of major cardiac ion channel proteins in both atria and ventricle was very similar to what has been reported for humans. In both intact isolated heart and isolated endocardial strips the I_{Kr} blocker dofetilide increased action potential duration (APD). The I_{Ks} blocker HMR1556 increased APD and triangulation only when I_{Kr} was blocked with dofetilide. In the presence of I_{Kr} and I_{Ks} blockade a reduction of $[K^+]_e$ resulted in a marked increase in APD_{90} in isolated hearts. I_{K1} blockade with Ba^{2+} increased APD in whole heart and isolated endocardium. In isolated endocardium, β-adrenergic stimulation with isoprenaline resulted in an increase in APD and potential amplitude but a decrease in triangulation. There was a rate-dependent decrease in APD in both whole heart and isolated endocardium. *In vivo* and *ex vivo* investigations revealed a negative correlation between temperature and duration of cardiac repolarization. **Discussion:** Our results point toward the minipig being a promising species for cardiac safety research.

1. Introduction

The minipig represents an attractive experimental animal in cardiovascular research due to its extensive similarities to the human heart in terms of anatomy and physiology (Swindle & Smith, 1998) albeit there are some structural differences due to pigs being quadrupeds (Crick, Sheppard, Ho, Gebstein, & Anderson, 1998). Other experimental animal species including dogs, rabbits, guinea pigs and rats have hitherto been thoroughly investigated with respect to cardiac physiology and information about their electrophysiology is widely available (reviewed by Nerbonne & Kass, 2005). Even though minipigs have been used for cardiovascular research for decades (Svendsen, 2006) a thorough characterization of the minipig cardiac electrophysiology has not been performed previously.

So far, basic scientific studies have focused on *in vivo* experiments recording electrocardiograms (ECG's) and hemodynamics, revealing that minipigs have heart rates, hemodynamics and ECG parameters comparable to humans (Stubhan et al., 2008). Furthermore, minipigs respond to the 'gold standard' human QT prolonging compound Moxifloxacin (Malik, Garnett, & Zhang, 2010; Markert et al., 2009), indicating a similarity with human cardiac repolarization. Thus, minipigs are attractive as an experimental species for cardiac research and might constitute a valuable alternative to other large animals, such as dog. The pharmaceutical industry has a significant interest in delayed repolarization of cardiac ventricular action potentials, underlying a prolonged QT interval on surface ECG's. From a cardiac safety perspective this is important since the single most common cause of withdrawal or restriction of the use of drugs has been prolongation of QT intervals. A prolonged QT interval is not arrhythmogenic *per se* but can constitute the trigger for arrhythmias such as polymorphic ventricular tachycardia, which can develop into ventricular fibrillation and ultimately sudden cardiac death (Roden, 2004). Cardiac repolarization in mammals rely mainly on an outward

* Corresponding author. H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark. Tel.: +45 36433445; fax: +45 36438313.
E-mail address: morl@lundbeck.com (M. Laursen).

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flow of K^+ ions through various K^+ channels, in larger animals being observed mainly as transient outward currents ($I_{to1,\ slow\ and\ fast}$), and delayed ($I_{Kr}$ and I_{Ks}) and inward (I_{K1}) rectifiers (Nerbonne & Kass, 2005). Within the area of drug development the most extensively studied channel is with no doubt the hERG1 (Kv11.1a) underlying the I_{Kr} current (Sanguinetti, Jiang, Curran, & Keating, 1995). The obvious reason for this is that unintended inhibition of hERG1 channels is a dominating risk factor for inducing ventricular arrhythmias. Thus, hERG1 channels have received widespread attention across the pharmaceutical industry as the hERG channel is blocked by chemicals with diverse structures encompassing several therapeutic drug classes including antihistamines, psychiatrics, anti-arrhythmics, anti-microbials and gastrointestinal drugs (Sanguinetti & Tristani-Firouzi, 2006). Even though hERG1 channels have gained the majority of interest from the pharmaceutical industry it should be recalled that unintended inhibition or activation of any cardiac ion channel could potentially be pro-arrhythmic.

As a result, both academia and pharmaceutical companies have focused on cardiac electrophysiology and pharmacology of a wide range of experimental animals. However, the amount of literature describing the basal electrophysiology of the minipig is very limited. Therefore, we have for the first time characterized the minipig cardiac repolarization in an experimental range from mRNA quantification to *in vivo* experiments. Our results point toward the minipig being a promising species for cardiac safety research, as its cardiac electrophysiology resembles human and other large species with respect to the key parameters.

2. Materials and methods

2.1. Animals

Göttingen minipigs were purchased from Ellegaard Göttingen Minipigs, Denmark. All animal experiments in this study were conducted under the supervision of a veterinarian and in accordance with the Danish legislation of animal use for scientific procedures as described in the "Animal Testing Act" (Consolidation Act No. 726 of 9 September 1993 as amended by Act No. 1081 of 20 December 1995). The Danish "Animal Testing Act" fully and extensively covers the requirements included in the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Health Institute. Both male and female minipigs were considered sexually mature at the time of use.

2.2. In vivo electrophysiology

Fasted male minipigs (~10 kg, ~5 months) were anesthetized with an intramuscular injection of (in mg/kg): Tiletamine 1.09, Zolazepam 1.09, Xylazine 1.09, Ketamine 1.09 and Butorphanol 0.17. After intubation, minipigs were kept on inhalation-anesthesia with Sevoflurane (3–3.5%). To prevent dehydration an intravenous infusion of physiological saline (0.9% NaCl, 5–10 mL/kg/h) was given through the right ear vein during the experiment. A temperature probe was placed in the rectum to ensure a continuous recording of body temperature. ECG electrodes were connected with crocodile clips, and ECG paste was applied to improve the electrical signal. ECG signals were recorded with an Einthoven Goldberger module and a Wilson lead amplifier Type 701 and 702 (Hugo Sachs, DE).

The QT interval (measured from the onset of the QRS to the end of the T wave, where the repolarization phase ends or crosses the isoelectric line) was taken from the lead II ECG. Due to heart rate effects on the QT interval a study specific correction formula was calculated based on the Van de Water formula (Van de Water, Verheyen, Xhonneux, & Reneman, 1989). The heart rate corrected QT interval (QTc) was calculated as $QTc = QT - 0.2119 \times (RR - 550.5)$, where -0.2119 is the slope of the average QT − RR correlation recorded in the minipigs during anesthesia and 550 is the average RR interval.

Two sessions were conducted in each animal. In the first session the animal was cooled from the normal temperature (37 ± 0.5 °C) to 34 ± 0 °C using bags filled with ice and an air fan. In the second session the animal was warmed to 40 ± 0 °C from their normal temperature using a hot-air fan and a hot water-circulating heating pad. At the end of each session the animal was slowly re-warmed or re-cooled to the normal temperature before awakening from the anesthesia. The sessions for each animal were separated by at least 7 days. The QT interval was recorded as 30-second averages every 5 min. The data from all experiments was pooled to achieve a range of temperature from 34 to 40 °C.

2.3. Ex vivo electrophysiology

Fasted female minipigs (~15 kg, ~7 months) were anesthetized with an intramuscular injection of (in mg/kg): Tiletamine 1.09, Zolazepam 1.09, Xylazine 1.09, Ketamine 1.09, Metadone 0.17 and Butorphanol 0.17. When the pigs were deeply anesthetized, a venflon was introduced in both ears and a continuous infusion of Propofol (1.5 mL/kg/h) was started to secure a maintained surgical anesthesia. Animals were then intubated and ventilated with pure oxygen with a peak inspiratory pressure of 14 cm H_2O and an inspiratory to expiratory ratio of 1:2. The breathing frequency was 14 breaths/min.

2.3.1. Surgical procedures

Following this preparation, the animal was placed on its back and secured with an inflatable pillow. A midline incision was made covering and exceeding the sternum to secure entry to the thoracic cavity. Then, the entire sternum was cut using bone shears and the thorax forced open using a large thoracic clamp. At this point, continuous inflation of the lungs was secured by increasing the positive end expiratory pressure on the ventilator to about 4 cm H_2O.

The pericardial and pleural sacs were removed and the descending aorta and the cranial and caudal v. cava were identified. In rapid succession, the cranial and caudal v. cava and caudal aorta were clamped and the v. cavae cut proximal to both clamps. This ensured no further cardiac filling and inhibited runoff via the aorta. A large diameter hypodermic needle was introduced in the aorta near the exit from the heart. Via this needle an ice-cold cardioplegic solution was forced retrogradely via the aorta to the coronary arteries at 60–100 mm Hg. The cardioplegic solution was composed of the following (in mM): NaCl 108.2, KCl 31, $CaCl_2$–$2H_2O$ 1.8, $MgCl_2$–$6H_2O$ 1.0, NaH_2PO_4–H_2O 1.8, $NaHCO_3$ 25, and $C_6H_{12}O_6$–H_2O (D-glucose monohydrate) 55, pH 7.4. The perfusion fluid left via an incision in the right atrium. When cardiac arrest was apparent, the heart was excised and placed in ice-cold cardioplegic solution.

Pigs used for RNA analysis were not ventilated. Instead, hearts were rapidly excised and placed in ice-cold cardioplegic solution.

2.3.2. Isolated Langendorff heart preparation

The whole heart was mounted in a modified IH-5 Isolated Heart apparatus (Hugo Sachs, DE) and retrogradely perfused according to the Langendorff principle (Langendorff, 1895). The Hugo Sachs IH-5 Isolated Heart apparatus is designed for animals no bigger than rabbits. Therefore, it was modified to accommodate the minipig hearts. First, the perfusate inlet port was moved to the upper part of the bubble trap. This proved necessary since the high flow (~230 mL/min) caused bubbles to be sucked into the aorta leading to ischemia. Secondly, due to the very high coronary flow rate, a 200 μM industrial pore filter was introduced to allow recirculation of the perfusate when needed. 6 liter buffer reservoirs were used in parallel (to allow the use of different compound solutions).

The perfusion fluid was composed of the following (in mM): NaCl 118.1, KCl 4.7, $CaCl_2$–$2H_2O$ 2.5, KH_2PO_4 1.2, $MgSO_4$–$7H_2O$ 1.6,