Tissue Slices from Adult Mammalian Hearts as a Model for Pharmacological Drug Testing

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Key Words

Action potential • Heart slices • Propagation • Heart physiology • Antiarrhythmic drugs • Safety pharmacology

Abstract

Aim: Isolated papillary muscles and enzymatically dissociated myocytes of guinea-pig hearts are routinely used for experimental cardiac research. The aim of our study is to investigate adult mammalian ventricular slices as an alternative preparation. **Method:** Vibratome cut ventricular slices (350 μm thick) were examined histologically and with 2-photon microscopy for fibre orientation. Intracellular action potentials were recorded with conventional glass microelectrodes, extracellular potentials were measured with tungsten platinum electrodes and multi-electrode arrays (MEA). **Results:** Dominant direction of fibre orientation was absent in vertical and horizontal transmural slices, but was longitudinal in tangential slices. Control action potential duration (APD_{90} , 169.9 ± 4 ms) and drug effects on this parameter were similar to papillary muscles. The L-type Ca-channel blocker nifedipine shortened APD_{90} with a half maximal effective concentration (EC_{50}) of 4.5 μM . The I_{Kr} blocker E4031 and neuroleptic drug risperidone prolonged APD_{90}

with EC_{50} values of 31 nM and 0.67 μM , respectively. Mapping field potentials on multi-electrode arrays showed uniform spread of excitation with a mean conduction velocity of 0.47 $\text{m} \cdot \text{s}^{-1}$. **Conclusion:** Slices from adult mammalian hearts could become a useful routine model for electrophysiological and pharmacological research.

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Introduction

Tissue slices from different organs including brain, kidney, liver, lung, and pancreas are well established models for electrophysiological and biochemical studies [1-3]. Compared with isolated cells, tissue slices possess several advantages, i.e. preserved tissue structure, no enzymatic digestion, no selection of cells during the isolation procedure. Tissue slices remain viable for considerable periods of time when maintained under appropriate conditions, and have been extensively used in biochemical and toxicological studies (for review, see [4]).

Despite these advantages, cardiac tissue slices are less frequently used than other organ slices, possibly because of notorious difficulties with the slicing procedure

in intact hearts due to the elasticity of the myocardium. For instance, the vibratome blade may push the tissue forward without actually cutting it unless the speed and amplitudes are set to optimal values. These difficulties can be overcome by embedding embryonic, neonatal or even adult heart tissue into “low melt” agarose [5, 6], however, the extra material surrounding the heart increases diffusion distance and hence may hamper oxygen and substrate supply to central cell layers. Nevertheless myocardial slices from fetal and neonatal hearts have been employed as scaffolds for integration of embryonic stem cell-derived cardiomyocytes into living heart tissue in studies of cardiac regeneration [7-9]. Burnashev et al. [10] demonstrated that patch clamp experiments are feasible in newborn rat heart muscle slices yielding similar features of cardiac sodium currents and inward rectifying potassium currents as in single myocytes, however the resting membrane potential was clearly less negative, suggesting room for improvement of heart slices before they can be employed in routine pharmacological experiments.

In the wake of risk stratification for identifying proarrhythmic potential of drugs, national and international drug registering agencies request numerous *in-vitro* tests for drug safety. Accepted models are intact hearts perfused according to the Langendorff technique [11], especially for rabbit heart [12], isolated structured tissues like perfused ventricular wedge preparation [13], trabecula or papillary muscles, and single, enzymatically dissociated cardiomyocytes. Pharmacological experiments in Langendorff hearts provide evidence of drug action under conditions close to *in-vivo* physiology, but on the other hand are time-consuming and expensive, because only a limited number of drug concentrations can be tested in the heart of one animal. The advantage of isolated cardiomyocytes is that numerous measurements in cells from a single heart are feasible, whereas lack of intercellular contacts or cell surface damage by enzymatic digestion may lead to erroneous conclusions about drug actions. Heart slices combine the advantages of whole organ and isolated cells, because they exhibit intact tissue structure and cellular contacts, yet a large number of preparations is obtained from a single heart. Furthermore, the thinness of each slice (350 μm) ensures good tissue oxygenation which may become limiting in other *in-vitro* heart preparations like papillary muscles [14].

In order to fulfill the need for an inexpensive model for cardiac drug testing we have used precision-cut slices of adult guinea-pig hearts, rat hearts, and human papillary muscles. The aim of our study was to validate this

method by providing electrophysiological and pharmacological data in comparison with conventional papillary muscles. Our results demonstrate that heart slices serve as a suitable model for reliable drug testing.

Materials and Methods

Preparation of slices

All animal experiments were carried out in accordance with the Helsinki guidelines (permission 24-9168.24-1-2002-8 of the Dresden Regierungspräsidium). Ventricular heart slices were obtained from male guinea pigs of 307.4 ± 12.7 g body weight and Wistar rats of 231.7 ± 17.5 g body weight (both from Charles River, Sulzfeld, Germany). Human cardiac slices were prepared from explanted hearts of transplant recipients with written permission of the patients.

Animals were anaesthetized with a mixture of 70% CO_2 and 30% O_2 . Guinea-pig and rat hearts were quickly removed and perfused on a Langendorff apparatus with oxygenated (5% CO_2 , 95% O_2) Tyrode's solution (composition in mM: NaCl 126.7, NaH_2PO_4 0.4, NaHCO_3 22, KCl 5.4, CaCl_2 1.8, MgCl_2 1.1, glucose 5, pH 7.4) for 1 min followed by a 1-min perfusion with high potassium (HK^+) solution (composition in mM: NaCl 120, KCl 20, CaCl_2 2, MgCl_2 1, HEPES 10, glucose 10, pH 7.4) for inhibition of electrical activity. Contractile activation was suppressed with 2,3-butanedione monoxime (BDM, 15 mM, Sigma Aldrich, Deisenhofen, Germany, [15]). A tissue piece (10 x 4 mm) of the left ventricle was glued with histoacryl tissue adhesive (Aesculap AG&Co. KG, Tuttlingen, Germany), corresponding to the slice direction, directly to an agarose block that was fixed on top of the cutting stage of a vibratome (Integralslice, Campden Instruments Ltd., UK). Vertical transmural, vertical horizontal and tangential slices, 350 μm thick (Figure 1), were cut in cold (4°C) oxygenated HK^+ solution with a steel blade at a speed of 0.03 $\text{mm}\cdot\text{s}^{-1}$, amplitude of 1 mm and vibration frequency of 51 Hz. The slices were transferred to a nylon net in a preincubation chamber filled with oxygenated HK^+ solution at room temperature. In order to prevent curling, the slices were held down by a grid (“slice holder”, SDH-27N/15, Harvard Apparatus, Holliston, MA, USA).

Slices structure

To investigate tissue slices histo-architecture, living slices were incubated for 1-2 h with the vital fluorescent dye CellTracker Green CMFDA (5-chloromethylfluorescein diacetate, Invitrogen, Karlsruhe, Germany, 2 $\mu\text{g}\cdot\text{ml}^{-1}$) prepared in oxygenated HK^+ solution containing 15 mM BDM. Living slices were then imaged with a fluorescent 2-photon microscope (Leica TCSMP2, Leica Microsystems GmbH, Germany) using an excitation wavelength of 850 nm, and emission wavelengths between 400-700 nm.

Heart slices were embedded in tissue freezing medium (Leica Microsystems Nussloch GmbH, Germany), immediately frozen in liquid nitrogen and stored at -80°C until use. Cryosections (10 μm) were prepared with a Cryostat Microtome (CM1900, Leica Microsystems GmbH, Germany). For

Fig. 1. Scheme of the heart slice preparation. A: The heart was sectioned into 4 mm thick transversal rings. B: Illustration of slicing direction in a piece of the left ventricular wall yielding vertical transmural, horizontal transmural and tangential slices.

Fig. 2. Fibre orientation in heart slices. A-C: 2-photon images of 350 μm thick living left ventricular tissue slices stained with CellTracker green, D-F: 10 μm thick cryosections from heart slices stained with haematoxylin-eosin. Note absence of dominant fibre orientation in vertical transmural (A, D) and horizontal transmural slices (B, E), and mainly longitudinal orientated fibres in tangential slices (C, F).

haematoxylin-eosin (H&E) staining, cryosections were washed with distilled water for 5-10 min and then stained for 8 min with haematoxylin, followed by a washing step with warm water for 10 min. After a short washing step with distilled water the slices were counter-stained for 6 min with eosin. Washing was followed by dehydration steps in 2 min intervals in 50%, 60%, 70%, 80% and 90% of ethanol.

Field potential recordings

Superfused chamber. Myocardial slices were fixed in a chamber and continuously superfused with oxygenated Tyrode's solution ($8 \text{ ml} \cdot \text{min}^{-1}$) at 37°C . Field potentials (FP) were recorded simultaneously in up to 4 heart slices using the multiple slice evaluation system Synchroslice (Lohmann Research Equipment, Castrop-Rauxel, Germany). Concentric bipolar stimulation electrodes (stainless steel, Lohmann Research Equipment, Castrop-Rauxel, Germany) and recording electrodes (tungsten platinum) were advanced on to the slices with micromanipulators under visual control until contact. Data acquisition (sampling rate 10 kHz per channel), electrical stimulation (1 Hz), and application of drugs to the superfusion fluid with an 8-channel Teflon-valve system were controlled via an automated software program. The same program was used for field potential analysis.

Multi-electrode array. In order to evaluate signal propagation within heart slices after electrical stimulation, a 60-channel multi-electrode array system (MEA60BC, Multi Channel Systems, Reutlingen, Germany) was used. Heart slices were positioned on the multi-electrode array (inter-electrode distance $200 \mu\text{m}$) and fixed with a slice holder. Slices were stimulated at a rate of 0.3 Hz with an external concentric bipolar electrode (see above) that was positioned by a micromanipulator onto the heart tissue (MM33, Märzhäuser Wetzlar GmbH & Co. KG, Wetzlar, Germany). Slices were superfused like described before. Propagation of field potentials was analysed with a self-programmed software based on MATLAB (The Mathworks, Natick, USA). Negative peaks of the FP (FP_{MIN}) (see Figure 4) were used for colour-coded mapping of excitation spread and for determination of conduction velocity between the outer electrodes on the two opposite sides of the array.

Intracellular recordings. Ventricular slices were mounted in an organ bath and superfused at a constant rate of $8 \text{ ml} \cdot \text{min}^{-1}$ with oxygenated Tyrode's solution at 37°C . The slices were stimulated (1 Hz) with a concentric bipolar stimulation electrode (see above). Intracellular action potentials were recorded with conventional glass micropipettes (inner diameter of the tip $< 1 \mu\text{m}$, resistances 10 - $20 \text{ M}\Omega$ when filled with 2.5 M KCl). The signals were amplified (Intra 767 amplifier, World

Fig. 3. Electrophysiological measurements in guinea-pig (left column) and rat heart slices (right column). A: Intracellular recordings of action potentials. B: Recordings of field potentials with higher resolution in the lower row. FPD = FP duration (FP_{MIN} to end of FP); FP_{MIN} = first negative peak in FP; (modified from [41]). C: APD₉₀ in ms measured after 2 h and 6 h of storage in HK⁺ solution (means ± S.E.M., n = 7-8). D: Tracings of intracellular action potentials from a guinea-pig heart slice at the stimulation frequencies of 0.5 Hz and 2 Hz.

Precision Instruments Inc., Sarasota FL, USA), digitized and analysed by a PowerLab 2/26 analog to digital converter and CHART5 software (ADInstruments GmbH, Spechbach, Germany). A sharp voltage drop to negative potentials detected impalement of cardiomyocytes. Signals were accepted when the resting membrane potential was more negative than -75 mV and the amplitude of the action potential was greater than 115 mV. After 20 min under control conditions drugs were cumulatively added (one concentration every 10 min) to the superfusion solution.

Solutions

All drugs were obtained from Sigma Aldrich, Deisenhofen, Germany. Stock solutions were prepared in dimethylsulfoxide (DMSO). E4031 (1-[2-(6-methyl-2pyridyl)ethyl]-4-(4-methylsulfonylamino-benzoyl) piperidine was a gift of Eisai (Ibaraki, Japan). Nifedipine was purchased from Sigma (Deisenhofen, Germany) and risperidone was a gift of Janssen-Cilag (Neuss, Germany). The concentration of DMSO in the final solution (< 0.3%) did not have any effect on action potential parameters (data not shown).

Statistics

Data are presented as means ± standard error of the mean (S.E.M) for n observations. Concentration-response curves (averaged data) and statistical analysis were calculated with GraphPad Prism software version 5 (GraphPad Prism Software Inc., San Diego CA, USA). A value of P < 0.05 was considered statistically significant.

Fig. 4. Two-dimensional spread of excitation in a guinea-pig heart slice. A: Overview of FPs of all 60 electrodes. B: Comparison of enlarged FPs from electrodes 54 and 32 shows a delay of FP_{MIN} by 1.6 ms, which corresponds to a propagation velocity of 0.35 m·s⁻¹. C: Colour-coded latency of excitation at individual electrodes on the MEA (squares). The excitation wave spreads uniformly from the lower right to the upper left corner. Numbers in the corners indicate the organization of the electrodes on the MEA in columns from left to right.

	RMP (mV)	APA (mV)	$V_{\max}$ ($V \cdot s^{-1}$)	APD ₂₀ (ms)	APD ₅₀ (ms)	APD ₉₀ (ms)
Heart slices (n = 59)	-84.4 ± 0.8	120.7 ± 0.7	159.4 ± 6.9	79.0 ± 3.1	142.7 ± 3.6	169.9 ± 4.0
Papillary muscle (n = 7)	-86.9 ± 1.0	124.3 ± 1.0	160.0 ± 9.0	91.0 ± 9.0	140.0 ± 11	159.0 ± 11
P	*	*	n.s.	n.s.	n.s.	n.s.

Table 1. Comparison of intracellular action potentials (APs) in left ventricular heart slices and right ventricular papillary muscles from adult guinea pigs [19]. RMP: resting membrane potential; APA: action potential amplitude; $V_{\max}$: maximum dV/dt of the upstroke; APD₂₀, APD₅₀, APD₉₀: action potential duration at 20%, 50% and 90% of repolarization, respectively. * P < 0.05 for differences between heart slices and papillary muscles.

Results

Preparation efficiency

Routinely we obtained 11 ± 1 slices per heart depending on the cutting direction. In tangential direction only 5-6 slices could be obtained, because of limiting wall thickness. Per heart 2 to 4 experiments were done and $80 \pm 7.8\%$ of all slices showed stable and physiological signals.

Heart slice structure

Two-photon imaging of living slices stained with the fluorescent dye CellTracker Green revealed no dominant fibre orientation in vertical and horizontal transmural slices (Figure 2A, 2B). In tangential slices (Figure 2C), however, mainly longitudinal fibre orientation was observed. Similar results were shown with hematoxylin-eosin staining (Figure 2D-F). For the electrophysiological characterization we chose transmural slices because of known transmural heterogeneity, and arbitrarily picked vertical transmural slices since defined spatial fibre alignment was absent in both transmural slice directions.

Action potential and field potential characteristics in cardiac slices

Intracellular action potentials (APs) and extracellular field potentials (FP) were recorded in the mid-myocardium at the centre of the slices (Figure 3A, 3B). The resting membrane potential of guinea-pig heart slices stimulated at 1 Hz was -84.4 ± 0.8 mV, AP amplitude was 120.7 ± 0.7 mV (n = 59). While action potentials from guinea-pig cardiac slices exhibited the prominent plateau phase characteristic for this species, and long action potential durations (APD₉₀ 169.9 ± 4.0 ms), APs from rat heart slices were of triangular shape and typically much shorter in duration (APD₉₀ 33.7 ± 1.8 ms, n = 5).

Fig. 5. Simultaneous registration of extracellular potentials in four different ventricular slices from a guinea-pig heart. Arrow indicates the field potential duration (FPD). FPD 1: 172 ms, FPD 2: 171 ms, FPD 3: 172 ms and FPD 4: 162 ms.

Since field potentials approximately reflect the first derivative of the intracellular action potential versus time, phases of rapid potential change are easily detected. Therefore, in guinea-pig slices an equivalent of APD could be determined because the steep phase of final repolarization yielded a clear upward “hump” indicating

Fig. 6. Effect of E4031 (100 nM) on guinea-pig FP and intracellular AP. A: Recording of FP under control condition and in the presence of 100 nM E4031. B: Recording of intracellular AP under control condition and in the presence of 100 nM E4031. Calibration as indicated by bars. C: Effect of 100 nM E4031 on field potential duration (FPD). Steady-state effect is reached 600 seconds after drug application.

the end of the FP, whereas in rat slices this was not possible because of the “tailing-off” of final repolarization (Figure 3B, upper tracings). All action potential parameters from guinea-pig heart slices were similar to published values in papillary muscles and are summarized in Table 1. Action potential duration (APD_{90}) did not change when guinea-pig heart slices were stored for 2 h (APD_{90} 169.5 ± 6.1 ms) or up to 6 h (APD_{90} 186.1 ± 7.6 ms) in HK^+ solution at room temperature (Figure 3C). In addition, APD_{90} of guinea-pig slices depended on stimulation frequency (Figure 3D), i.e. it shortened with increasing frequency from 170.4 ± 8.7 ms at 0.5 Hz to 142.4 ± 8.2 ms at 2 Hz ($n = 6$, $P < 0.05$).

Spread of excitation through the tissue slices was determined with MEA electrodes. Figure 4A gives an overview of FP recorded at electrodes covered by the heart slice, Figure 4B depicts enlarged tracings from electrodes 54 and 32 that are highlighted in Figure 4A. The delay in the first negative peak of the FP signal (FP_{MIN}) was used for calculation of the conduction velocity between the recording electrodes. The time delay between electrodes 54 and 32 which were 0.57 mm apart was 1.6 ms, corresponding to a conduction velocity of $0.35 \text{ m}\cdot\text{s}^{-1}$ at a stimulation frequency of 0.3 Hz. A colour code based on the time delay between stimulation artefact and the first negative peak termed “latency” was created for visualizing propagation of electrical signals within the slice (Figure 4C). The excitation wave spread uniformly from the lower right to the upper left corner. The mean propagation velocity was $0.47 \pm 0.15 \text{ m}\cdot\text{s}^{-1}$ ($n = 11$).

In an additional approach for measuring electrical activity in slices, we used a new setup (Synchroslice)

that permits recording of single FPs in four slices at the same time (Figure 5). The mean field potential duration (FPD) from guinea-pig cardiac slices measured in this device was 174.0 ± 5.1 ms (1 Hz, $n = 8$). To show the correlation between FPD and APD_{90} in guinea-pig ventricular slices, we recorded FPs (Figure 6A) and APs (Figure 6B) in different slices in the presence of 100 nM E4031. Field potentials were prolonged by $29.8 \pm 5.3\%$ ($n=8$) and APD_{90} by $26.7 \pm 4.7\%$ ($n=7$), $p > 0.05$. Steady-state effects were reached after 10 min (Figure 6C).

Pharmacology

For pharmacological validation of the slice model we have tested drug effects on intracellular APs from guinea-pig ventricular slices. Each of the 3 compounds represents a standard drug group: i.e. the L-type Ca^{2+} -channel blocker nifedipine [16], the selective I_{Kr} blocker E4031 [17], and the neuroleptic drug risperidone with known QT-interval prolongation [18]. Control APs recorded in heart slices were not different from APs recorded in guinea-pig papillary muscles [19]. Nifedipine (30 μM) shortened APD_{90} (Figure 7A) in slices from 145.1 ± 9.4 ms (control) to 83.1 ± 9.2 ms ($n = 7$). Time-matched control experiments showed stable APD_{90} for 100 min ($n = 6$, Figure 7B). The EC_{50} for the drug effect on APD_{90} was 4.5 μM for ventricular slices and 0.9 μM in papillary muscles suggesting a significantly higher sensitivity towards nifedipine of papillary muscles than of slices. E4031 (1 μM) significantly prolonged APD_{90} from 168.7 ± 6.0 ms to 228.9 ± 10.0 ms ($n = 7$). The EC_{50} was 31 nM in slices and 44 nM in papillary

Fig. 7. Effects of nifedipine, E4031 and risperidone on intracellular APs. Action potential tracings under control conditions and in the presence of increasing concentrations of A: nifedipine (1, 3, 10, 30 μ M), C: E4031 (1, 30, 100 nM and 1 μ M) and E: risperidone (1, 10 μ M). Calibrations as indicated by the bars. Concentration-response curves for B: nifedipine, D: E4031 and F: risperidone on APD₉₀ in left guinea-pig ventricular slices (filled circles) and in right ventricular papillary muscles (open squares), for nifedipine T. Christ, unpublished). Time-matched controls (TMC, open circles) in guinea-pig ventricular slices. Mean values $\pm$ S.E.M. from 4-11 experiments. Log EC₅₀, logarithm of the molar concentration for the half maximum effect. $P < 0.05$ for differences in EC₅₀ for the nifedipine effect between heart slices and papillary muscles.

muscles (Figure 7C, 7D). The neuroleptic drug risperidone prolonged APD₉₀ in slices from 182.5 ± 7.9 ms to 221.2 ± 10.5 ms ($n = 11$), the EC₅₀ values were 0.7μ M in ventricular slices and 0.6μ M in papillary muscles (Figure 7E, 7F).

We were also able to prepare slices from human papillary muscle specimen. Control APD₉₀ at 1 Hz was 351.1 ± 35.4 ms, after application of 1μ M E4031 APD₉₀ increased to 444.1 ± 46.7 ms ($n = 3$ slices from 2 patients, $P > 0.05$ Figure 8A). For comparison in intact human papillary muscles 1μ M E4031 prolonged APD₉₀ from 242.0 ± 15.0 ms to 356.0 ± 18.0 ms ($n = 5$ preparations from 5 patients, $P < 0.01$, Figure 8B).

Discussion

In the present study we have examined bioelectrical activity and drug effects in tissue slices of high structural integrity from adult guinea-pig hearts, rat hearts, and human papillary muscles. Intracellular action potential parameters, extracellular field potentials and spread of excitation correspond well with data reported in the literature. In addition, heart slices responded to standard drugs in the expected manner suggesting that they represent valid models for physiological and

Fig. 8. Effects of E4031 on APD from human papillary muscle slices. A: Action potential tracings under control conditions and in the presence of E4031 (1μ M). Calibrations as indicated by the bars. B: Effects of E4031 (1μ M) on APD₉₀ of human papillary muscle slices with a prolongation of 93.0 ± 14.2 ms (3 slices/ 2 patients) and of intact papillary muscle with a prolongation of 114.2 ± 18.9 ms (5 preparations/ 5 patients; T. Christ, unpublished).

pharmacological experimentation.

Preparation of slices

Cutting viable heart slices is challenging because of injury-induced contractile activation and movement. To avoid this problem, slices were prepared and stored in depolarizing HK⁺ solution for suppression of electrical activity, and this solution was further supplemented with BDM for suppression of contractions by inhibition of cross-bridge cycling within the contractile apparatus [20]. Though BDM has long been used for preventing cutting injury [21], it is not clear whether all of its additional effects including impairment of L-type Ca²⁺ channels [22] are completely reversible upon wash-out.

Unlike other groups [5, 6], we glued the heart tissue directly to an agarose block for cutting without embedding the tissue in low-melt agarose. This procedure allowed direct contact between the heart tissue and the superfusion solution in order to avoid the risk of inadequate oxygen supply due to an increased diffusion distance by the embedding material. In addition, oxygen consumption was decreased by lowering the temperature to 4°C during the cutting process.

Integrity of cardiac tissue in slices and slice viability

The heart slices exhibited intact fine structure, with mainly longitudinal fibre direction in tangential sections and lack of dominant fibre direction in the transmural slices. Heterogeneity in pharmacological responses across the ventricular wall [23] can result in ventricular tachyarrhythmias [24, 25]. Here we have arbitrarily chosen vertical transmural slices for further experiments, however, horizontal transmural slices should be equally well suited for detection of regional heterogeneity.

When judged by electrophysiological function, i.e. stability of AP shape, the heart slices apparently fared well in the storage solution for up to 6 h.

Cardiac slices for pharmacological experiments were initially cut from mouse fetal, neonatal or adult hearts because the small size of the organs facilitates cutting [5-8]. However, mouse and human hearts differ substantially. Beating rate is 10-fold higher in mouse than in human heart, action potentials are much shorter in mouse than in man, and different ion channels contribute [26]. Here we chose guinea-pig hearts because they more closely resemble human heart in action potential duration and pharmacological responses [27].

Physiological characteristics

The slicing procedure induces large areas of cutting injury that could greatly distort physiological characteristics. From the similarity in action potential parameters recorded from guinea-pig heart slices and papillary muscles (see Table 1) we conclude that the inflicted injury must have healed over. Moreover, the slices are probably well oxygenated because lack of oxygen causes marked shortening in action potential duration [28]. However, lower resting membrane potential and action potential amplitude than in papillary muscles could indicate residual tissue lesion. We can also not exclude that there are regional differences between right and left ventricle, although we could not find evidence for such a difference in the literature. The maximum upstroke velocity ($dV/dt_{\max}$) was in a similar range for both preparations, but lower compared to other published data [29-30]. The most likely explanation is that we have underestimated $dV/dt_{\max}$ because of the low sampling rate (10 kHz). Field potential duration (FPD) measured in guinea-pig heart slices is comparable with recorded APD.

The observed shortening in APD with higher stimulation frequency resembles the well established response in guinea-pig papillary muscle [31] and has been explained by incomplete deactivation of K⁺ currents during the short stimulation interval [32].

Extracellular multi-electrode array recordings

Propagation of excitation in heart slices as measured with the multi-electrode array was homogeneous under control conditions as described previously [5, 6]. The calculated conduction velocity corresponds to previously published data in guinea-pig ventricular muscle [33]. This finding suggests that cell-to-cell communications in the slices should be largely intact. However, in some preparation also non-linear propagation was observed. Possible explanations are inhomogeneities in fibre orientation or 3 dimensional tissue structure, or technical problems such as poor contact between the slice and the electrodes. To prevent the latter, a slice holder was used in general.

Pharmacological studies

Guinea-pig heart slices responded to drug application in a similar manner as papillary muscles or isolated cardiomyocytes. Nifedipine resulted in AP shortening, E4031 and risperidone prolonged the action potential duration [19, 34]. Interestingly, higher

nifedipine concentrations were required for AP shortening in slices than in intact tissue, whereas potencies of E4031 and risperidone were similar in both preparations. Although potency of dihydropyridine derivatives is known to depend on membrane potential [35], this phenomenon cannot explain the observed effects because depolarization of heart slices would enhance sensitivity rather than decrease it. We speculate that the reduced sensitivity towards nifedipine is due to interference with L-type Ca^{2+} channel block induced by BDM [22], which may not be completely reversible upon wash-out.

In human right papillary muscle slices control APD_{90} was similar as in our previous study with intact right ventricular papillary muscles of failing human hearts, and application of E4031 also prolonged APD_{90} [36]. Apparent differences in effect size cannot be judged properly because of the small number of experiments.

Future directions

Drug-induced prolongation of the QT interval in the electrocardiogram (ECG) is associated with a high risk of developing torsade de pointes arrhythmias and sudden cardiac death [37-40]. Since this potentially lethal side effect is caused by a large number of drugs, drug agen-

cies require rigorous tests for any new compound before approval (see <http://www.emea.europa.eu/pdfs/human/ich/042302en.pdf>). Standard methods for assessment of QT interval or equivalent parameters in cells and tissues are: recording of (i) ECGs in whole animals and Langendorff hearts, (ii) action potentials in papillary muscles, Purkinje fibres and isolated cardiomyocytes, (iii) ionic current in native myocytes or hERG channel expression systems. Because of the similarities in action potential parameters reported here we propose that mammalian heart slices could become an alternative model for such studies.

Acknowledgements

We thank Manuela Weisflog for excellent technical assistance. This work was supported by the German Federal Ministry of Economy and Technology BMWi (PRO INNO II program, grant KF0682501 SB8), the European Union ("normaCOR", Grant LSHM-CT-2006-018676), and the Oxford EP Abraham Cephalosporin fund (research grant to P.C.). P.C. holds a Junior Research Fellowship at Christ Church Oxford.

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