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Appraisal of state-of-the art

Cardiac tissue slices with prolonged survival for in vitro drug safety screening

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ABSTRACT

Introduction: We have recently introduced the use of mammalian cardiac tissue slices for in vitro drug testing purposes. Here we show how this method can be applied for long-term studies in safety pharmacology.
Methods: In freshly prepared cardiac slices from guinea-pig or rat ventricle, extracellular field potentials (FP) and intracellular action potentials (AP) were recorded in response to electrical stimulation using the 4-channel heart slice screening system 'Synchroslice'. To assess viability of the slices on consecutive days after preparation, drug effects on FP/AP parameters, like duration and latency, were monitored.
Results: In the presence of the potassium channel blocker E4031 (1 μM), FP and AP duration (FPD and APD) were significantly increased (FPD, 39.0%; APD, 28.1%) in guinea-pig ventricular slices. Similar changes were observed 24–28 h after slice preparation (FPD, 48.6%; APD, 25.4%). Furthermore, AP duration was reduced in the presence of the calcium channel blocker nifedipine (10 μM) on the day of preparation (40.5%) and 24–28 h later (38.7%). In contrast, in the presence of the potassium channel blocker 4-aminopyridine (30 mM) AP duration was prolonged 4.95 and 4.19-fold, 2–8 h and 24–28 h after preparation, respectively. Finally, FP propagation was repeatedly slowed down by the gap junction blocker carbenoxolone (30 μM), as revealed from FP onset latency increases observed on three consecutive days (2–8 h after preparation, 93.0%; 24–28 h, 76.8%, 48–56 h, 61.7%).
Discussion: Freshly isolated cardiac slices reproduced established physiological and pharmacological responses for more than 24 h after preparation. Thus, cardiac slices can be used for several days after preparation which makes them a robust model for electrophysiological studies. We propose that cardiac slices can become a versatile tool in heart research and risk assessment of drugs.

1. Introduction

Tissue slices from different organs have been utilized for several decades as in vitro models to study organ physiology and function. Initially, tissue slices were cut with a razor blade free-hand, but this technique generated rather thick slices (>0.5 mm; Buja & Willerson, 1981). Today, automated slice techniques allow cutting thin tissue slices (100–350 μm) in a rapid and reproducible way (Parrish, Gandolfi, & Brendel, 1995). Tissue slices from different organs including brain, kidney, liver, lung, and pancreas are well established models for electrophysiological and pharmacological studies (Colbert, 2006; Edwards, Konnerth, Sakmann, & Takahashi, 1989; Vickers & Fisher, 2004). Compared to isolated cells, advantages of using tissue slices are the preservation of tissue structure, the opportunity to omit enzymatic digestion, and that any bias for a particular cell type during the isolation procedure is avoided. By selecting the appropriate thickness, the diffusion distance in the slice can be controlled, which does not only ensure sufficient tissue oxygenation but also proper penetration of drugs to their target. In addition, a large number of preparations can be obtained from a single organ. Moreover, tissue slices remain viable for considerable periods of time when maintained under appropriate conditions.

Despite the advantages of this in vitro technique, acute slices from cardiac tissue are less frequently used than slices from other organs. This most likely is due to notorious difficulties with the slicing procedure of living heart tissue. Because the myocardium displays a high degree of elasticity, the blade supposed to cut slices may just push the tissue forward away instead of cutting it. Thus, during the slicing procedure with a vibratome, the advance speed of the blade and its oscillation amplitude and frequency have to be set to adequate values within narrow limits. These difficulties can be overcome by embedding embryonic, neonatal or even adult heart tissue into "low-melting" agarose (Halbach et al., 2006; Pillekamp et al., 2005). However, the extra material surrounding the heart tissue 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 the integration of embryonic stem cell-

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derived cardiomyocytes into living heart tissue in studies of cardiac regeneration (Habeler et al., 2009; Halbach et al., 2007; Pillekamp et al., 2007). Furthermore, Burnashev and co-workers demonstrated that patch clamp experiments are feasible in newborn rat heart muscle slices yielding similar features of cardiac Na^+ currents and inward rectifying K^+ currents as in single myocytes (Burnashev, Edwards, & Verkhratsky, 1990). However, resting membrane potential was clearly less negative, suggesting room for improvement of heart slice preparation before this model can be employed in routine pharmacological experiments.

We have recently demonstrated that cardiac slices from adult mammals can be used for pharmacological and physiological investigations (Bussek et al., 2009). In that study, we analyzed the spread of excitation within a slice, conduction velocity and drug effects on intracellular action potential (AP) and extracellular field potential (FP) parameters. We could demonstrate that drug effects on both extracellular and intracellular signals were comparable to other in vitro heart models. Here we show that cardiac slices from guinea pig or rat heart can be used for the assessment of drug effects over at least three consecutive days when kept under appropriate cardioplegic conditions after preparation.

2. Methods

All animal experiments were carried out in accordance with the Helsinki guidelines for the care and use of experimental animals and were approved by local authorities.

2.1. Animals

Ventricular heart slices were prepared from male guinea pigs of 307.4 ± 12.7 g (n = 23) body weight and Wistar rats of 231.7 ± 17.5 g body weight (n = 17) that were obtained from a commercial source (Charles River, Sulzfeld, Germany, or Harlan Winkelmann, Borken, Germany).

2.2. Preparation of ventricular cardiac slices

Animals were anesthetized 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, composed of (in mM), NaCl 126.7, KCl 5.4, $MgCl_2x6H_2O$ 1.05, $CaCl_2x2H_2O$ 1.8, $NaH_2PO_4xH_2O$ 0.42, $NaHCO_3$ 22, Glucose 5, for 1 min, until the blood was completely removed. This was followed by a 1-min perfusion with high potassium (20 mM) Tyrode's solution (HK^+) composed of (in mM) NaCl 106.7, KCl 20, $MgCl_2x6H_2O$ 1.05, $CaCl_2x2H_2O$ 1.8, $NaH_2PO_4xH_2O$ 0.42, $NaHCO_3$ 22, Glucose 5, supplemented with 2,3-butanedione monoxime (BDM) 15, to inhibit electrical activity and contractile activation. A tissue piece (10 × 4 mm) of the left ventricle was glued to an agarose block (5% agar in glucose free Tyrode's solution) with histoacryl tissue adhesive. The agarose block was then fixed to the cutting stage of a vibratome. Vertical transmural slices were cut in cold (4 °C) oxygenated HK^+ solution containing 15 mM BDM using a stainless steel blade advanced at 0.03 mm/s, oscillating at an amplitude of 1 mm and a frequency of 51 Hz.

For extracellular field potential (FP) and intracellular action potential (AP) recordings, 350 μm-thick slices were prepared. Slices were transferred to a preincubation chamber and kept in HK^+ at room temperature for at least 1 h before recording to allow recovery. Slices designated for recording on consecutive days were stored in HK^+ supplemented with 15 mM BDM at 12 °C in a temperature-controlled incubator.

2.3. Recording and electrical stimulation

For FP recordings, slices were fixed in a chamber and continuously superfused with oxygenated Tyrode's solution (2 ml/min) at 34 °C. After a 30 min equilibration period, FPs were recorded simultaneously in up to 4 heart slices using the multiple slice evaluation system Synchroslice (Lohmann Research Equipment (LRE), Castrop-Rauxel, Germany). Under visual control, concentric bipolar stimulation electrodes (stainless steel, LRE) and monopolar tungsten platinum recording electrodes (Thomas Recording, Giessen, Germany) were advanced until contact with the slice surface. FPs were elicited by 400 μs single biphasic electric pulses of appropriate amplitude (100 to 800 μA, peak-to-peak) at a repetition rate of 1/s. Data acquisition (sampling rate 10 kHz per channel), electrical stimulation, and application of drugs to the superfusion fluid with an 8-channel Teflon® valve system were controlled via an automated software program ("Synchroheart", LRE) which was also used for on-line and off-line data analysis.

For intracellular recordings, slices were mounted in an organ bath and superfused at a constant rate of 8 ml/min with oxygenated Tyrode's solution at 37 °C. The slices were stimulated with a concentric bipolar stimulation electrode (see above). Intracellular APs were recorded with conventional glass micropipettes (inner tip diameter < 1 μm, impedance 10–20 MΩ when filled with 2.5 M KCl). The signals were amplified (Intra 767 amplifier, World Precision Instruments Inc., Sarasota FL, USA), digitized and analyzed by a PowerLab 2/26 analog to digital converter using CHART5 software (ADInstruments GmbH, Spechbach, Germany). For intracellular recordings with the Synchroslice system (LRE, Castrop-Rauxel, Germany), a Dagan 8100 amplifier was used on one channel of the 4-channel system. Intracellular data were digitized with the Synchroslice data acquisition system and analyzed with the Synchroheart software. A voltage step 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.

2.4. Application of compounds

All compounds used in this study were bath-applied after dilution from stock solutions prepared in dimethylsulfoxide (DMSO). The DMSO concentration in the final solution (<0.3%) did not have any effect on FP or AP parameters (data not shown).

To achieve drug concentration–effect relationships, FPs and APs were first recorded for at least 20 min under control conditions to assure stability of the signals. Then, drugs were cumulatively added to the superfusion solution (one concentration every 30 min).

N-[4-[[1-[2-(6-Methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl] phenyl]methanesulfonamide dihydrochloride (E-4031), nifedipine, 4-aminopyridine (4-AP) and carbenoxolone were purchased from Sigma-Aldrich (Deisenhofen, Germany).

2.5. Data analysis and statistics

Data are presented as means ± standard error of the mean (S.E.M) observations. FP and AP parameters were analyzed with "Synchroheart"

Table 1
Comparison of AP parameters in guinea pig left ventricular heart slices after 2–8 h and 24–28 h. RMP: resting membrane potential; APA: action potential amplitude; dV/dt_{max}: maximum rate of depolarization; APD_{50}, APD_{90}: action potential duration at 50% and 90% of repolarization, respectively. Mean values ± S.E.M., *p < 0.05.

	RMP (mV)	APA (mV)	dV/dt_{max} (V/s)	APD_{50} (ms)	APD_{90} (ms)
2–8 h	−87 ± 0.5	120 ± 0.4	210 ± 7	150 ± 2.2	176 ± 2.1
24–28 h	−87 ± 0.6	121 ± 0.9	200 ± 17	164 ± 4.0*	190 ± 3.6*

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Table 2
Comparison of FP parameters in guinea pig left ventricular heart slices after 2–8 h and 24–28 h. FPD: field potential duration; Q_{Na}: time difference between 1st and 2nd FP peak.

	FPD (ms)	Q_{Na} (μs)
2–8 h	195 ± 2.3	844 ± 56.5
24–28 h	188 ± 15.0	883 ± 112.8

software. In case of non Synchroslice recordings, AP parameters were analyzed with PowerLab 2/26 analog to digital converter and CHART5 software and peak detection module. Concentration–effect curves fitted to averaged data and EC_{50} values as well as statistical analysis were calculated with GraphPad Prism software version 4. Statistical comparison between means was made using two-tailed unpaired Student's *t*-test. Results were regarded significantly different if $p<0.05$.

3. Results

3.1. Action potential and field potential characteristics

In order to considerably reduce the number of laboratory animals and maximize experimental work efficiency, we validated the slice model for long-time usage. The following action potential (AP) parameters were measured: resting membrane potential (RMP), action potential amplitude (APA), maximum rate of depolarization (dV/dt_{max}) as an indirect measure for Na^+ conductance, and the action potential duration at 50% and 90% of repolarization (APD_{50}, APD_{90}). For field potential (FP) characterization, we measured the latency of the first peak (negative or positive, depending on signal shape), the time difference between 1st (negative or positive) and 2nd (positive or negative) FP peak (Q_{na}) as an indirect measure for Na^+ conductance, and the field potential duration (FPD). Recording experiments were performed not only on the day of preparation of the slices (i.e. 2–8 h after cutting) but also on the next day (24–28 h after cutting) or even 2 days after slice preparation (48–56 h after cutting). A comparison of basic parameters revealed that no significant changes occurred for RMP, APA, or dV/dt_{max}. In contrast to the other parameters, AP duration was slightly but significantly increased after 24–28 h ($p<0.05$; Table 1).

Similar to AP parameters, the majority of FP parameters appeared unchanged 24–28 h after slice preparation when compared to control values obtained 2–8 h after preparation (Table 2).

3.2. Drug effects on action potentials and field potentials

We performed pharmacological studies with the selective I_{Kr} potassium channel blocker E-4031 and the Ca^{2+} channel blocker nifedipine in guinea pig cardiac slices. In addition, the unselective K^+ channel blocker 4-AP and the unspecific gap junction blocker carbenoxolone (Cbx) were tested on rat ventricular cardiac slices. Data obtained 24–28 h after slice preparation (and 48–56 h in case of Cbx) were compared with the results from freshly prepared slices.

3.3. Effects of potassium channel blockade by E-4031

The class-III antiarrhythmic compound E-4031 has been developed to suppress abnormal heart rhythms. It predominantly blocks the rapidly activating K^+ channel hERG (I_{Kr}) and thereby prolongs the cardiac action potential and refractory period. E-4031 does not interfere with cardiac Na^+ channels and, hence, the rate of depolarization is not impaired (Davie et al., 2004; Ducroq et al., 2007). Due to absence of a Na^+ channel block conduction velocity remains unaltered. Class III antiarrhythmics prevent re-entrant arrhythmias because they prolong APD.

In the presence of 1 μM E-4031, APD_{90} was maximally prolonged from 168.7 ± 5.7 ms to 229.6 ± 9.8 ms with a log EC_{50} [M] of −7.6 ± 0.3 (n = 6) in freshly prepared guinea pig cardiac slices,

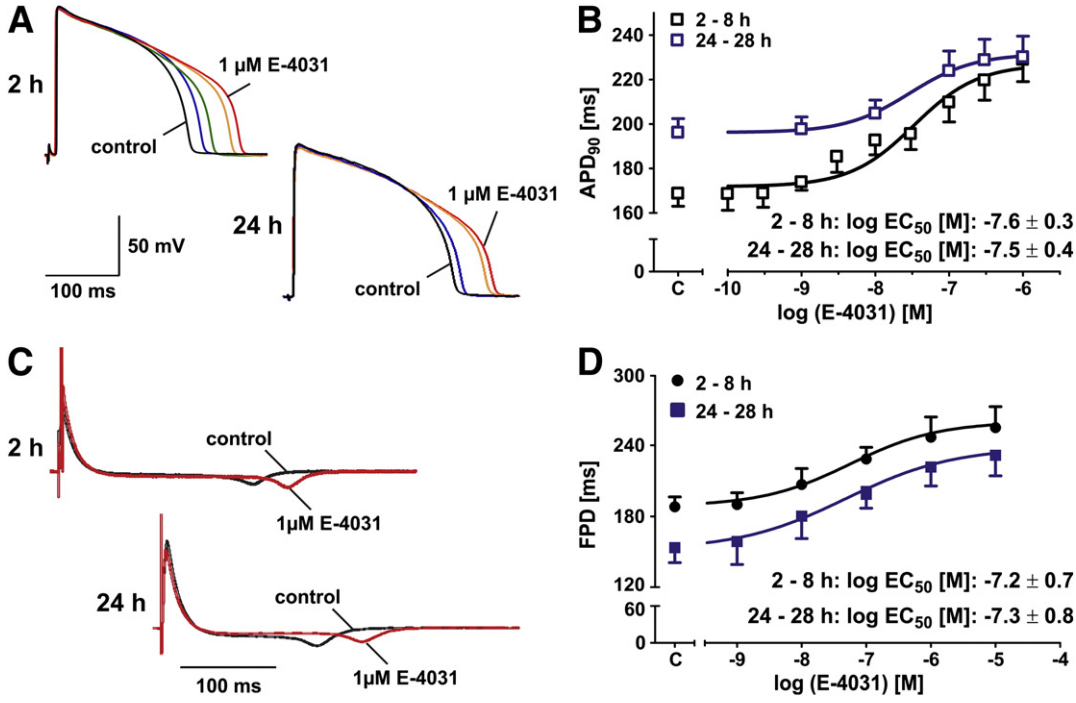

Fig. 1. Effects of E-4031 on intracellular action potentials and extracellular field potentials in acute and 24 h-old guinea pig ventricular cardiac slices. A: Action potential traces under control conditions and in the presence of increasing concentrations of E-4031 (10 nM, blue; 30 nM, green; 100 nM, orange; 1 μM, red). B: Concentration–effect curves for E-4031 on APD_{90} in freshly prepared guinea-pig left ventricular cardiac slices (black line) and in slices that were kept overnight at 12 °C (blue line). C: Field potential traces under control conditions (black traces) and in the presence of 1 μM E-4031 (red traces). D: Concentration–effect curves for E-4031 on FPD in freshly prepared guinea pig left ventricular cardiac slices (black line) and in slices that were kept overnight at 12 °C (blue line). Mean values ± S.E.M. from 3 to 8 slices. Log EC_{50} [M], logarithm of the molar concentration for the half-maximum effect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 3
Effect of E-4031 on intracellular action potentials in ventricular slices from adult guinea pigs, 2–8 h vs. 24–28 h. C: control; RMP: resting membrane potential; dV/dt_{max}: maximum rate of depolarization; APD_{90}: action potential duration at 90% of repolarization.

E-4031								
[mM]	2–8 h				24–28 h			
	RMP (mV)	dV/dt_{max} (V/s)	APD_{90} (ms)	n	RMP (mV)	dV/dt_{max} (V/s)	APD_{90} (ms)	n
C	−80.0 ± 2.5	164.8 ± 12.0	168.7 ± 5.7	7	−86.7 ± 1.1	170.3 ± 22.9	193.3 ± 6.2	8
0.001	−81.5 ± 3.6	147.7 ± 21.0	173.9 ± 3.6	7	−86.7 ± 1.1	165.3 ± 22.9	197.1 ± 4.9	8
0.010	−86.6 ± 3.9	146.8 ± 23.7	191.2 ± 5.5	5	−87.5 ± 1.5	148.2 ± 10.3	204.4 ± 5.3	8
0.100	−79.2 ± 3.6	145.3 ± 19.4	211.8 ± 8.5	7	−89.0 ± 0.9	151.0 ± 9.3	224.5 ± 7.9	8
0.300	−80.6 ± 3.6	147.4 ± 19.5	220.5 ± 8.8	7	−88.1 ± 1.3	165.6 ± 16.8	229.6 ± 8.3	8
1.000	−75.0 ± 4.8	150.9 ± 20.0	229.6 ± 9.8	7	−87.9 ± 1.3	173.3 ± 24.0	231.1 ± 8.4	8

and from 193.3 ± 6.2 ms to 231.1 ± 8.4 ms in 24–28 h-old slices (log EC_{50} [M]: −7.5 ± 0.4; n = 6; Fig. 1). The RMP and dV/dt_{max} remained unchanged at all concentrations in both preparations (Table 3).

In guinea-pig ventricular slices 2–8 h after preparation, E-4031 maximally increased FP duration from 187.8 ± 11.0 ms to 271.3 ± 11.0 ms with a log EC_{50} [M] of −7.2 ± 0.7. When tested 24–28 h after preparation, E-4031 maximally increased FPD from 152.1 ± 12.1 ms to 234.1 ± 17.1 ms with a log EC_{50} [M] of −7.3 ± 0.8 (Fig. 1).

3.4. Effects of calcium channel blockade by nifedipine

Nifedipine exhibits the common feature of class-IV antiarrhythmic drugs, it blocks L-type Ca^{2+} channels ($I_{Ca,L}$; Uehara & Hume, 1985; Zhang, Sawanobori, Hirano, & Hiraoka, 1997). As a therapeutic agent, nifedipine modulates the vascular smooth muscle tone, where the potency of nifedipine is increased due to more depolarized membrane potentials.

In the presence of nifedipine, APD_{90} was significantly shortened in a concentration dependent manner, the log EC_{50} [M] was −5.7 ± 0.2 (Fig. 2). Other parameters, like the RMP and dV/dt_{max}, were not significantly affected (Table 4).

3.5. Effects of the potassium channel blocker 4-aminopyridine (4-AP)

To show that its application is not limited to one species, we verified the usefulness of the slice technique in rat ventricular cardiac slices. Because cardiomyocytes in rat express other ion channels than in guinea pig heart, the K^+ channel blocker 4-aminopyridine (4-AP) can be tested. 4-AP non-selectively blocks several voltage-activated K^+ channels. It is used to treat selected neuromuscular disorders like multiple sclerosis (Solari, Uitdehaag, Giuliani, Pucci, & Taus, 2002). In the myocardium, it blocks I_{Kur} at micromolar concentrations and the transient outward current (I_{to}), responsible for the rapid repolarization (phase 1), at millimolar concentrations (Gillis et al., 1998; Li, Feng, Yue, Carrier, & Nattel, 1996; Wang, Fermini, & Nattel, 1995; Yue, Feng, Li, & Nattel, 1996). Furthermore, a non-selective blocking effect of the I_{Kr} was described (Mitcheson & Hancox, 1999; Ridley, Milnes, Zhang, Witchel, & Hancox, 2003).

The effect of 4-AP was tested in rat left ventricular slices. 4-AP had no effect at low concentrations (10 μM–1 mM) on APD_{20}, APD_{50}, or APD_{90} however it prolonged APD_{20}, APD_{50}, and APD_{90} at higher concentrations (3–30 mM; Fig. 3). The maximum rate of depolarization was decreased in the presence of higher concentrations of 4-AP (300 μM–30 mM; Table 5). Similar results were obtained 24–28 h after preparation, dV/dt_{max} was decreased and APD_{20}, APD_{50}, and APD_{90} increased at comparable concentrations as 2–8 h after preparation (APD_{20}, 2–8 h, log EC_{50} [M]: −1.7 ± 0.6, 24–28 h, log EC_{50} [M]: −1.4 ± 0.3).

3.6. Effects of the gap junction blocker carbenoxolone

In rat cardiomyocytes, AP repolarization is fast and masks the Ca-dependent plateau. Thus, different from guinea pig, the absence of a plateau phase in rat cardiac slices results in a lack of a clear signal component in the extracellular FP that allows depicting the end of the FP. We therefore analyzed FP latency shifts in acute rat cardiac slices in response to the application of the gap junction blocker carbenoxolone (Cbx). By blocking gap junctions, Cbx uncouples cardiomyocytes and prolongs action potential propagation (Rohr, 2004). As a consequence, in acute slices FP onset latency was increased by Cbx more than 2-fold. The mean FP onset latency increase in the presence of 30 μM Cbx was 193.0 ± 58.1% 2–8 h after preparation (n = 6; Fig. 4). This effect was almost unchanged 24–28 h (176.8 ± 47.5%; n = 6) and 48–56 h after preparation (161.8 ± 18.2%; n = 6; Fig. 4). The log EC_{50} [M] for the latency increase was −4.8 ± 0.3, 2–8 h after

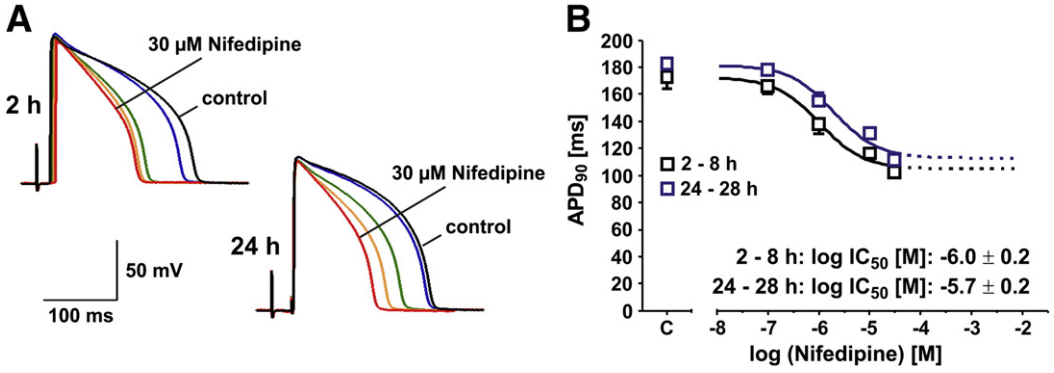

Fig. 2. Effects of nifedipine on intracellular action potentials in 24 h old guinea pig ventricular cardiac slices. A: Action potential traces under control conditions and in the presence of increasing concentrations of nifedipine (0.1 μM (blue), 1 μM (green), 10 μM (orange) and 30 μM (red)). Calibrations as indicated by the bars. B: Concentration–effect curves for nifedipine on APD_{90} in freshly prepared (2–8 h old) guinea pig left ventricular cardiac slices (black line) and in slices that were kept overnight at 12 °C (24–28 h old; red line). Dotted line represents an extrapolation of the concentration-response curve. Mean values ± S.E.M. from 5 experiments. Log IC_{50} [M], logarithm of the molar concentration for the half-maximum effect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 4
Effect of nifedipine on intracellular action potentials in ventricular slices from adult guinea pigs, 2–8 h vs. 24–28 h. C: control; RMP: resting membrane potential; dV/dt_{max}: maximum rate of depolarization; APD_{90}: action potential duration to 90% of repolarization.

Nifedipine								
[mM]	2–8 h				24–28 h			
	RMP (mV)	dV/dt_{max} (V/s)	APD_{90} (ms)	n	RMP (mV)	dV/dt_{max} (V/s)	APD_{90} (ms)	n
C	−88.5 ± 1.5	138.2 ± 25.0	178.2 ± 9.6	6	−86.7 ± 0.8	154.9 ± 20.1	182.7 ± 4.3	5
0.10	−90.2 ± 2.3	139.2 ± 35.5	172.5 ± 7.4	6	−88.8 ± 1.3	137.3 ± 17.8	178.2 ± 3.6	5
1.00	−91.3 ± 2.5	140.6 ± 34.6	147.3 ± 10.2	6	−87.5 ± 0.9	140.5 ± 11.4	155.1 ± 6.4	5
10.00	−92.6 ± 1.6	144.9 ± 23.7	122.5 ± 6.5	6	−87.3 ± 1.5	134.0 ± 8.9	131.2 ± 4.3	5
30.00	−88.9 ± 1.8	190.3 ± 49.0	105.4 ± 4.0	6	−88.8 ± 2.1	135.2 ± 15.9	111.6 ± 1.8	5

preparation. This also did not significantly change within the next 48 h, the log EC_{50} [M] was -4.4 ± 0.4, 24–28 h after preparation, and −4.5 ± 0.4 and 48–56 h after preparation.

4. Discussion

We have examined physiological and pharmacological properties in tissue slices of high structural integrity from adult guinea pig and rat hearts. When stored in high potassium Tyrode's solution supplemented with 15 mM BDM for up to 48 h, ventricular cardiac slices from guinea pig and rat hearts revealed mostly unchanged electrophysiological characteristics and responded to drug application in a similar way as freshly prepared slices. This suggests that cardiac slices represent a robust model for electrophysiological studies and thereby can become a versatile tool in heart research and risk assessment of drugs. Furthermore, since many slices can be obtained from a single heart applying the cardiac slice technique can help to reduce the number of animals used according to the 3R principle ('replace, reduce, refine') for experimental animal welfare.

4.1. Physiological characteristics

When judged by electrophysiological function, i.e. stability of AP shape, the heart slices remained stable in the storage solution for up to 24 h if kept overnight at 12 °C. Interestingly, we observed that APD_{90} was significantly longer in slices after 24 h as compared to freshly prepared slices. Possibly, the long-term exposure to BDM might prevent complete reversibility of effects on I_K. However, BDM is indispensable for storage of slices. BDM inhibits cross-bridge formation (Mulieri, Hasenfuss, Ittleman, Blanchard, & Alpert, 1989) and reduces oxygen consumption in cardiac muscle (Hebisch, Bischoff, & Soboll, 1993) and therefore it is commonly used to protect myocardial tissue during transport, storage and to prevent cutting injury. Nevertheless, slices seem to have typical physiological behavior, because deprivation of oxygen would cause APD shortening instead of prolongation (Ravens, Ravens, & Schafer, 1977).

4.2. Cardiac slices as an alternative model for physiological and pharmacological investigations

Drug-induced prolongation of the APD or FPD, corresponding to QT-interval in the electrocardiogram (ECG), is associated with a high risk of developing torsade de pointes arrhythmias and sudden cardiac death. The most common reason for this is the inhibition of I_{Kr} (Krikler & Curry, 1976; Moss, Schwartz, Crampton, Locati, & Carleen, 1985; Ravens, Wettwer, & Hala, 2004; Redfern et al., 2003; Rothman, 1980). Since this potentially lethal side effect is caused by a large number of substances, drug agencies 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 ECGs in whole animals and Langendorff hearts, recordings of action potentials in papillary muscles, Purkinje fibers and isolated cardiomyocytes, and measurements of ionic current in native myocytes or hERG channel expression systems. In the present study we attempted to evaluate the potential of cardiac tissue slices for physiological and pharmacological investigations to assess the risk factor for proarrhythmic potential of drugs.

4.3. Pharmacological validation of cardiac slices

Cardiac slices for pharmacological experiments were initially prepared from fetal, neonatal or adult hearts of mice because the small size of the organs facilitates cutting (Habeler et al., 2009; Halbach et al., 2006; Halbach et al., 2007; Pillekamp et al., 2005). However, mouse and human hearts differ substantially thus limiting the use

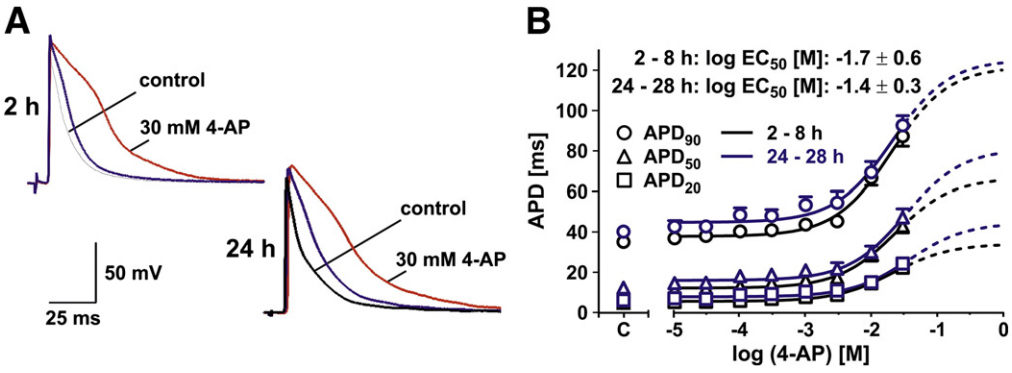

Fig. 3. Effects of 4-aminopyridine on intracellular action potentials in 24–28 h old rat ventricular cardiac slices. A: Action potential traces under control conditions and in the presence of increasing concentrations of 4-AP (300 μM (blue) and 30 mM (red)), respectively. B: Concentration–effect curves for 4-AP on APD_{20} (□), APD_{50} (Δ), and APD_{90} (○) in freshly prepared (2-8 h old) rat left ventricular cardiac slices (black symbols) and in slices that were kept overnight at 12 °C (24–28 h old; blue symbols). Dotted lines indicate extrapolation of the concentration–effect curves. Mean values ± S.E.M. from 3 to 9 experiments. Log EC_{50} [M], logarithm of the molar concentration for the half-maximum effect for APD_{20}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 5
Effect of 4-aminopyridine on intracellular action potentials in ventricular slices from adult rats, 2–8 h vs. 24–28 h. C: control; RMP: resting membrane potential; dV/dt_{max}: maximum rate of depolarization; APD_{20} APD_{50} and APD_{90}: action potential duration to 20, 50 and 90% of repolarization.

4-Aminopyridine												
[mM]	2–8 h						24–28 h					
	RMP (mV)	dV/dt_{max} (V/s)	APD_{20} (ms)	APD_{50} (ms)	APD_{90} (ms)	n	RMP (mV)	dV/dt_{max} (V/s)	APD_{20} (ms)	APD_{50} (ms)	APD_{90} (ms)	n
C	−86.6 ± 2.9	201.2 ± 27.5	4.4 ± 0.6	10.0 ± 1.3	34.6 ± 2.0	9	−81.8 ± 2.0	159.9 ± 23.6	5.7 ± 0.8	11.9 ± 1.2	39.8 ± 3.3	5
0.01	−87.8 ± 3.1	205.1 ± 25.9	4.8 ± 0.6	11.0 ± 1.2	36.4 ± 1.8	9	−82.7 ± 1.6	159.8 ± 28.1	6.7 ± 1.0	14.2 ± 1.3	42.2 ± 3.0	5
0.03	−84.6 ± 2.8	208.2 ± 25.3	4.9 ± 0.6	11.6 ± 1.3	37.6 ± 2.4	9	−83.0 ± 2.5	169.4 ± 33.5	6.4 ± 1.8	14.4 ± 2.0	42.3 ± 1.7	3
0.10	−84.1 ± 2.8	196.5 ± 25.4	5.4 ± 0.7	12.6 ± 1.2	39.9 ± 2.2	9	−82.8 ± 1.4	152.2 ± 21.4	8.4 ± 1.0	17.6 ± 1.5	48.0 ± 3.6	5
0.30	−83.5 ± 2.7	197.9 ± 26.9	6.4 ± 0.9	14.1 ± 1.2	40.4 ± 2.2	9	−83.2 ± 1.9	145.3 ± 33.4	8.8 ± 1.9	18.4 ± 2.5	47.5 ± 3.1	3
1.00	−83.0 ± 2.8	183.0 ± 28.2	7.3 ± 0.6	15.6 ± 1.0	43.2 ± 2.2	9	−81.8 ± 1.1	142.9 ± 17.0	10.0 ± 0.9	20.6 ± 1.7	52.9 ± 4.1	5
3.00	−81.8 ± 2.9	178.3 ± 27.1	8.3 ± 0.5	16.8 ± 1.0	44.9 ± 2.6	9	−81.4 ± 1.5	142.4 ± 31.6	10.5 ± 1.5	21.3 ± 3.0	54.0 ± 5.6	3
10.00	−81.7 ± 3.1	171.9 ± 23.2	14.1 ± 1.2	28.3 ± 2.9	66.3 ± 3.7	9	−81.1 ± 1.0	132.3 ± 17.9	14.6 ± 1.5	29.7 ± 2.9	69.0 ± 5.4	5
30.00	−81.4 ± 3.2	154.9 ± 22.8	21.8 ± 1.8	42.5 ± 3.8	42.5 ± 4.9	7	−74.0 ± 2.8	100.9 ± 26.8	23.9 ± 2.0	46.8 ± 4.3	92.2 ± 4.8	5

of mouse heart tissue as a model in safety pharmacology. 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 to APs (London, 2001).

In the present study, guinea pig hearts were chosen because they more closely resemble properties of human heart than mouse with respect to action potential duration (APD) and pharmacological responses (Wang et al., 1995). In addition, we also prepared slices from rat cardiac tissue to show that the technique is not limited to one species.

Freshly prepared and 24–28 h old slices from guinea pig and rat hearts responded to drugs in a similar manner as papillary muscles or isolated cardiomyocytes. In guinea pig cardiac slices, nifedipine induced APD shortening and E-4031 prolonged the APD. In rat ventricular cardiac slices, 4-AP prolonged APD whereas Cbx increased the latency.

The reduced APD in the presence of nifedipine is a consequence of L-type Ca^{2+} channel block and confirms previously published observations (Jurevicius, Muckus, Macianskiene, & Chmel-Dunaj, 1991; Zhang et al., 1997). Moreover, E-4031 as selective I_{Kr} blocker, prolonged APD in a concentration dependent manner, which is also in agreement with previously work (Davie et al., 2004).

4-AP prolonged APD when applied in millimolar concentrations in rat cardiac slices. This is supposed to be a consequence of I_{to} block by 4-AP in millimolar range (Gillis et al., 1998; Mitcheson & Hancox, 1999; Ridley et al., 2003; Wang et al., 1995). The apparent reduction of the rate of depolarization is most likely explained by spontaneous rundown since an inhibition of the fast activating transient outward current I_{to} should increase rather than decrease the depolarization rate.

In the Langendorff heart preparation, application of the gap junction blocker Cbx significantly reduces heart rate (Howarth & Qureshi, 2006). In rat ventricular slices, an increase in FP latency was observed in the presence of Cbx. This is most likely caused by a reduced electrical coupling of cardiomyocytes which results in a decelerated spread of excitation within the cardiac syncytium (Rohr, 2004).

The class-III antiarrhythmic E-4031 prolonged APD in guinea pig cardiac slices in a concentration dependent manner, but the effects were smaller compared to other animal models, e.g. rabbit (Abrahamsson, Duker, Lundberg, & Carlsson, 1993; Ducroq et al., 2007; Lu, Marien, Saels, & De Clerck, 2001) and dog Purkinje fibers (Gwilt et al., 1991; Knilans, Lathrop, Nanasi, Schwartz, & Varro, 1991; Lu et al., 2001; Németh et al., 1997; Wyse, Ye, & Campbell, 1993). This reduced sensitivity towards I_{Kr}-specific class-III antiarrhythmics in slices from guinea pig hearts compared to rabbit cardiac slices is due to differences in the contribution of I_{Kr} and I_{Ks} to repolarization in the two species, with rabbit being more sensitive to drug-induced prolongation than guinea pig because of the predominant contribution of I_{Kr} to final repolarization (Abrahamsson et al., 1993; Lu, Marien, Saels, & De Clerck, 2000; Sanguinetti & Jurkiewicz, 1990).

5. Conclusion

In our opinion, the feasibility of the cardiac slice technique for physiological and pharmacological investigations has been demonstrated by similarities in AP parameters compared to papillary muscles and equivalent drug effects on APD compared to well established models in safety pharmacology. Optimized cardioplegic conditions allow slice characteristics to be preserved for up to 28 h after preparation. We therefore propose that mammalian heart slices are a new alternative model for assessment of proarrhythmic drug

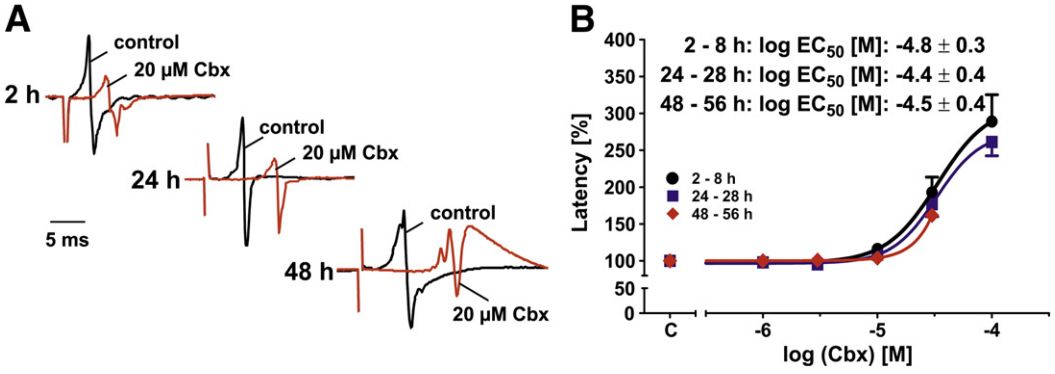

Fig. 4. Effect of Cbx on FP latency in rat cardiac slices. A: Field potential traces under control conditions (black) and in the presence of Cbx (50 µM, red). A comparison of Cbx effects in 2–8 h, 24–28 h, and 48–56 h old slices reveals similar FP latency shifts. Furthermore, concentration–effect curves for Cbx on FP latency (B) were also similar. Log EC_{50} [M], logarithm of the molar concentration for the half-maximum effect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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effects and that this technique can also be a useful tool for long-term pharmacological studies.

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