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Effect of Cardioactive Drugs on Action Potential Generation and Propagation in Embryonic Stem Cell-Derived Cardiomyocytes

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Key Words
ES cells • EB • MEA • Drug testing • Arrhythmia

Abstract
Extracellular recordings of spontaneous electrical activity in contracting cardiac clusters differentiated from murine embryonic stem cells enable to study electrophysiological features of this *in-vitro* cardiac-like tissue as well as effects of pharmacological compounds on its chronotropy and electrical conduction. To test if the microelectrode array (MEA) system could serve as a basis for development of a pharmacological screening tool for cardioactive drugs, we used spontaneously beating outgrowths of three-dimensional ES cell aggregates ("embryoid bodies", EBs) plated onto substrate-integrated MEAs. The effects of the L-type Ca^{2+} channel antagonist verapamil and Na^+ and K^+ channel blockers (tetrodotoxin, 4-aminopyridine, and sparfloxacin) on the deduced interrelated cardiac network function were investigated. Application of 10^{-6} M verapamil led to arrhythmic spiking with a burst-like pattern; at a higher concentration (10^{-5} M) the drug caused a sustained negative chronotropy up to complete stop of beating. In the presence of tetrodotoxin a conduction block was observed. Since modulation of K^+ channel activity can cause anti- or proarrhythmic effects, the influence of K^+ channel blockers, namely 4-aminopyridine and sparfloxacin, was investigated. 4-aminopyridine (2x 10^{-3} M) significantly stabilized beating frequency, while the field potential duration (FPD) was concentration-dependently prolonged up to 2.7-fold. Sparfloxacin (3x10^{-6} M) stabilized the beating frequency as well. At a higher concentration of sparfloxacin (3x10^{-5} M), a significant prolongation of the spike duration was registered; application of the drug caused also early afterdepolarizations. The results demonstrate a suitability of the studied *in-vitro* cardiac cell model for pharmacological drug testing in cardiovascular research.

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Introduction

Drug-induced modulation of ion channel activities leading to anti- or pro-arrhythmic effects in cardiac tissue is important for safety in pharmacology. We used embryonic stem (ES) cell-derived cardiac clusters as an *in-vitro* model to study the modulatory effect of cardioactive drugs on chronotropy and action potential (AP) conduction. ES cells are derived from the inner cell mass of the mammalian blastocyst. These cells are immortal, pluripotent and can differentiate within aggregates ("embryoid bodies", EBs) into derivatives of all 3 primary germ layers, including cardiomyocytes which form spontaneously beating clusters [1]. During the *in-vitro* differentiation, cardiac-specific genes coding for proteins, e.g. receptors and ion channels (among them, sarcolemmal L- and T-type Ca^{2+} channels, Na^{+} and K^{+} channels) are expressed in a developmental continuum that closely recapitulates the developmental morphological and biochemical pattern from early (cardiac precursor cells) to terminally differentiated cells *in-vivo* (e.g., atrial-like, ventricular-like, sinus nodal-like, and Purkinje-like cells). Also the developmental succession of electrophysiological properties of EBs matches the sequence of electrophysiological changes described for the embryonic heart. Therefore, beating cardiomyocyte clusters within EBs could potentially serve as a feasible model for testing cardioactive drugs. However, up to date, the cardiac excitation process in terms of its physiological features, as well as drug responses within the beating multicellular aggregates have not yet been characterized in detail. For this reason, it would be important to develop a high spatial and temporal resolution tool for drug screening. It could be developed on the basis of the microelectrode array (MEA) system, which allows recording of field potentials (FPs) with the help of multiple electrodes [2]. As a first approach to evaluate the applicability of this system for pharmacological screening, we analysed in the present study spontaneous action potential (AP) generation and propagation by means of extracellular FPs and their pharmacological modification in ES cell-derived cardiac clusters. MEAs represent a novel tool for functional analysis of cell-cell interactions in electrogenic tissues, enabling to detect the origin, direction and propagation velocity of the excitation spread in the clusters of ES cell-derived cardiomyocytes [2-5]. Selection of cardiac clusters of different microarchitectures allows to study even their conduction properties similar to the atrio-ventricular (AV) conduction in the native heart tissue [6].

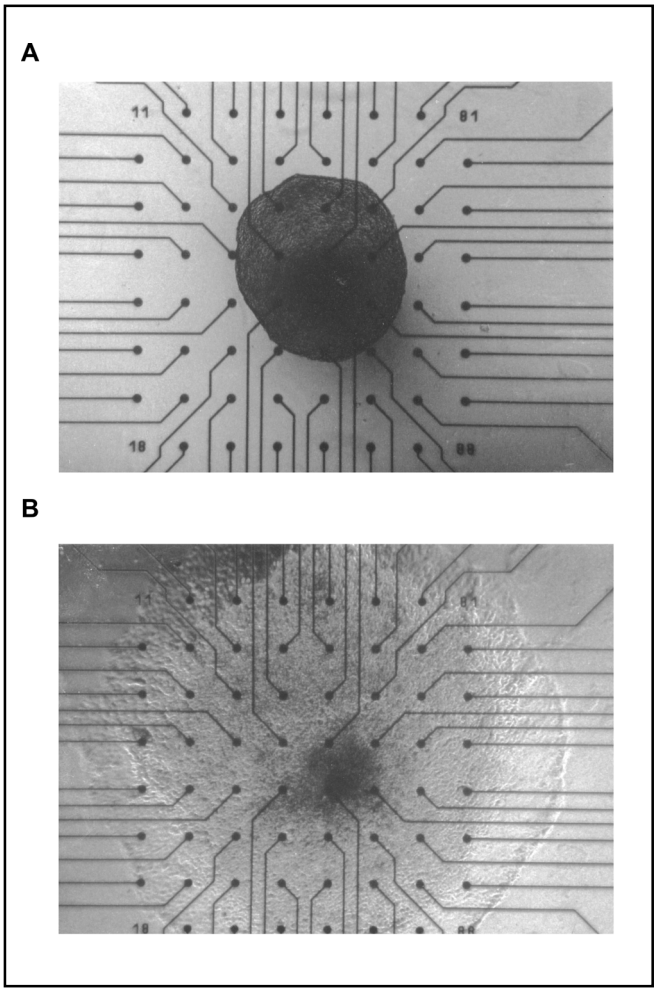

Fig. 1. EB plated onto a MEA. A: Freshly plated EB (after five days in suspension). B: The same EB three days after plating.

Materials and Methods

ES cell cultivation and differentiation into cardiomyocytes

Mouse ES cells of the line D3 [7] were cultivated on a feeder layer of mouse embryonic fibroblasts in the high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal bovine serum (FBS), 1% minimal essential medium (MEM), 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin (all from Invitrogen, Eggenstein, Germany), 0.05 mM β-mercaptoethanol (Serva, Heidelberg, Germany), and 1000 U/ml leukaemia inhibitory factor (LIF) (Invitrogen). EBs were produced with the hanging drop method as previously described [8, 9]. Briefly, the ES cells were cultivated in hanging

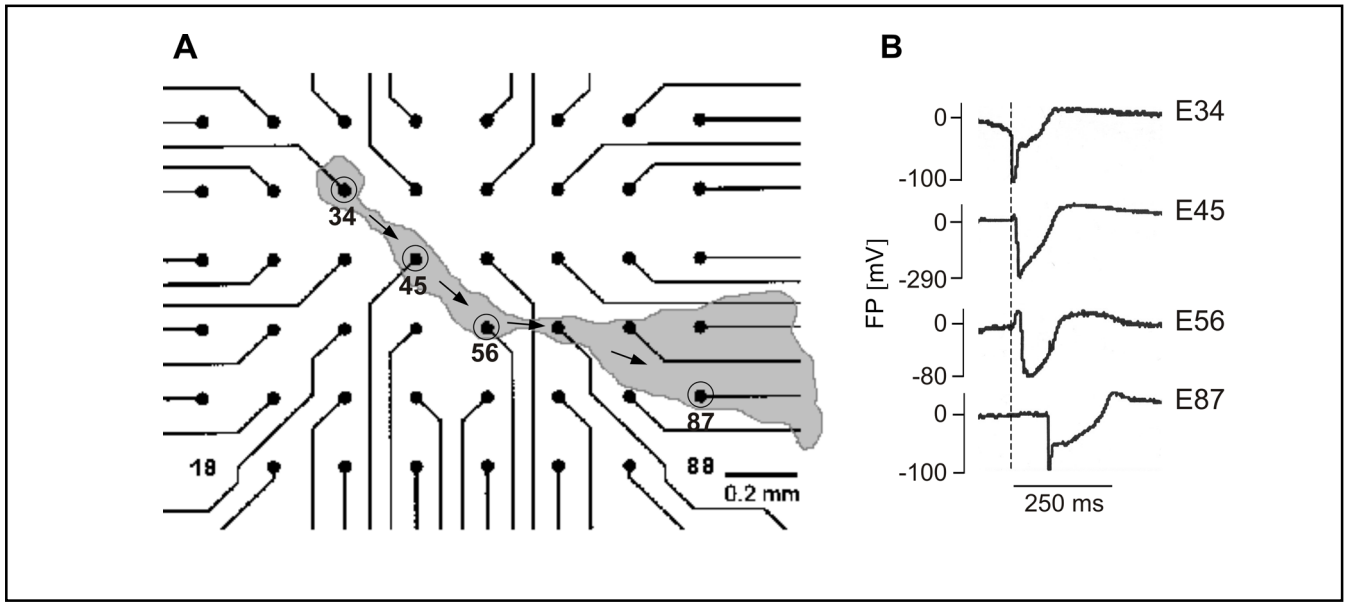

Fig. 2. FP recordings from a cardiomyocyte cluster within an EB plated onto a MEA. A: Elongated beating cell cluster at day 6 after plating the EB. Direction of contraction spread (microscopic observation) is shown by arrows; recording electrodes are marked by circles. B: Synchronous recordings from electrodes labeled in (A). The sequential increase of delay between spikes registered at electrodes 45, 56, and 87 *versus* electrode 34 (see positions of the negative peaks in respect to the dotted line) confirms the direction of the excitation wave indicated by arrows in (A).

drops (approximately 500 cells per 20 µl medium) for 2 days and then maintained as EBs in suspension for 5 days. Beginning from the hanging drop step, the culture medium (without LIF) containing 20% FBS was used. After 5 days in suspension, the EBs were plated onto the MEA dishes with DMEM for extracellular recordings.

Extracellular field potential recordings

The electrode matrix of the MEAs consisted of 60 TiN-coated gold electrodes with a diameter of 30 µm, arranged in eight columns and eight rows with a distance of 200 µm between adjacent electrodes [10]. EBs were fixed in the center of the MEAs with the aid of a gelatine drop measuring 200-300 µm in diameter (Fig. 1A). A gelatine „ring" placed around the electrode field, close to the electrodes, allowed a good tissue attachment after EB spreading on the MEAs. Long-term recordings and frequency and latency analysis were performed as previously described [6]. Briefly, continuous voltage signals were recorded on a 60-channel amplifier (Multichannel Systems, Reutlingen, Germany) and fed to threshold discriminators which delivered a single event for each spike. For low resolution during long-time recordings, both the continuous data and the event data were stored on computer using a CED1401 interface (Cambridge Electronic Design, Cambridge, UK) with SPIKE2 Software (CED). The sampling rate was 200-500 Hz and band-pass filters were set to 0.5-30 Hz. For on-line control, the analogue FP data as well as the frequency of beating, based on triggered events, were continuously displayed. For off-line analysis, the data were semi-automatically processed in SPIKE2 using our own macro scripts [6]. The origin, direction and velocity of AP propagation were estimated on the basis of latencies between electrical spikes at neighboring electrodes. For this purpose, the data were ridded of trigger errors: new event channels were created taking the minimum of a spike as the event time. Frequency and latency distributions were calculated for 250 events; distributions of the spike duration measured from the minimum of the FP (FP_{min}) to the maxium of the first positive deflection (FP_{max}) were calculated for 50 spikes. For high-resolution recordings of electrical signals in order to investigate the spike morphology, data were sampled at 2.0-5.0 kHz and analyzed using the MEA tools [11] programmed in MATLAB (The Mathworks, USA). All electrical recordings were combined with microscopic observations.

Cardioactive substances

Verapamil, 4-aminopyridine, and sparfloxacin were obtained from Knoll AG (Ludwigshafen, Germany). Tetrodotoxin (TTX) was purchased from Sigma-Aldrich (Muenchen, Germany).

The substances were pipetted into the medium and the MEA dish was gently swirled for approximately 5 sec before measurements were performed.

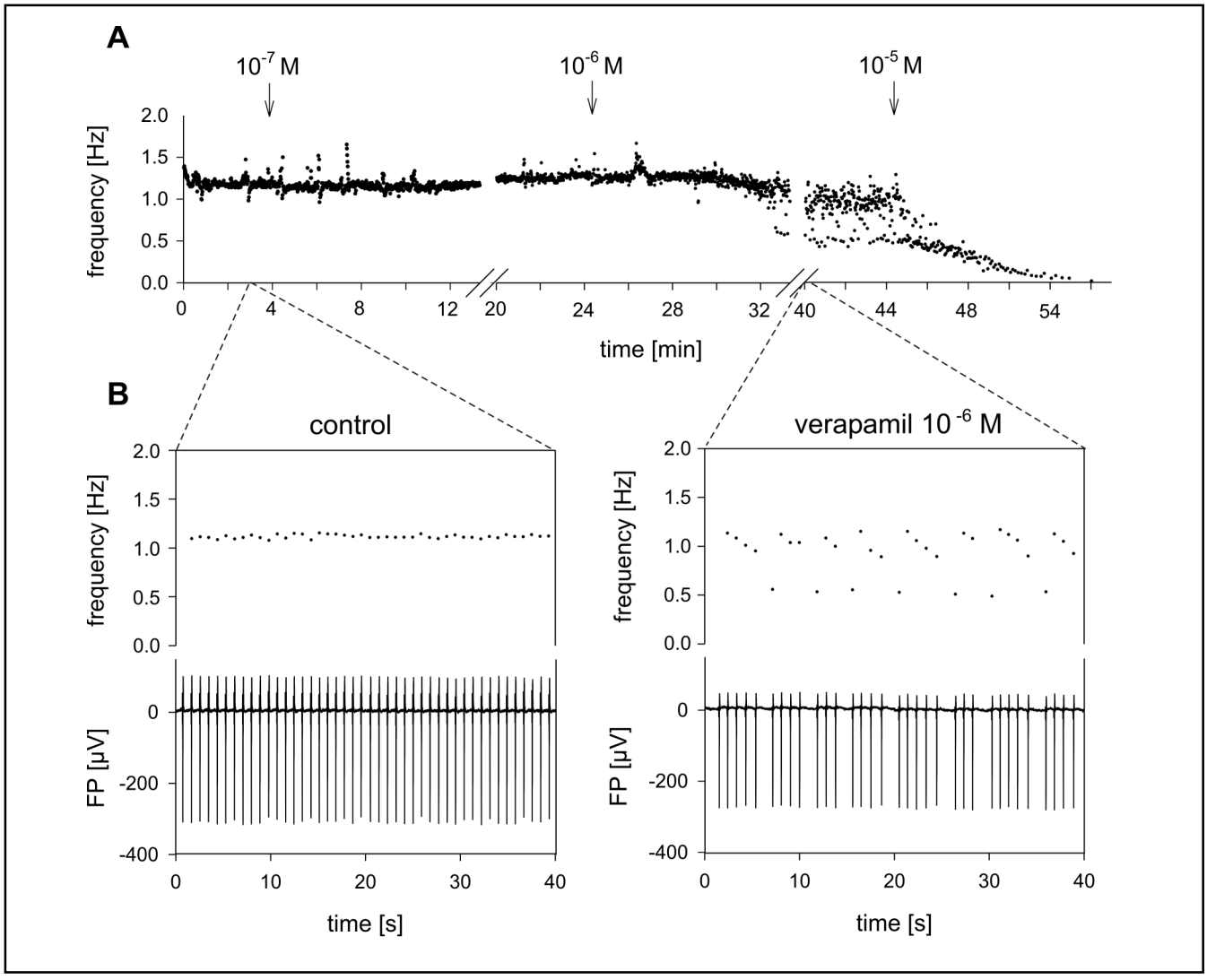

Fig. 3. Effect of verapamil on FP frequency. A: Long-term original trace of instantaneous beating frequency. Interruptions in the trace are due to switches to the high resolution registration system (see "Materials and Methods"). B, left panel: Regular spiking under control conditions. B, right panel: Irregular spiking after 15 min of incubation with 10^{-6} M verapamil, displaying a burst-like rhythm. Application of 10^{-5} M verapamil caused a decrease of the pacemaker activity until beating stopped completely (see (A)). The FP pattern and frequency of beating were homogeneously distributed throughout the whole contracting cluster.

Results

Cardiac differentiation in plated EBs

In outgrowing EBs plated on the MEAs (Fig. 1) we observed the earliest onset of beating 3-4 days after plating (7+3d to 7+4d), in accordance with previously reported data [6, 12]. Stable recordings from the contracting clusters were achievable up to day 8 after plating. The mean beating frequency was in the range of 0.5 to 5.0 Hz as also reported previously by our group [6]. In the course of differentiation, 1 to 3 beating areas of various microarchitectures typically appeared within individual EBs. We observed compact contracting clusters as well as more complex structures containing conductive narrow tissue strands. As an example, an elongated beating cell cluster is shown in Figure 2. Owing to a relatively slow excitation spread, propagation of contractions within the cluster could be clearly observed under microscope. Direction of the AP propagation, estimated upon FP recordings, was in agreement with the microscopic observations.

The huge variety in the microarchitecture of beating

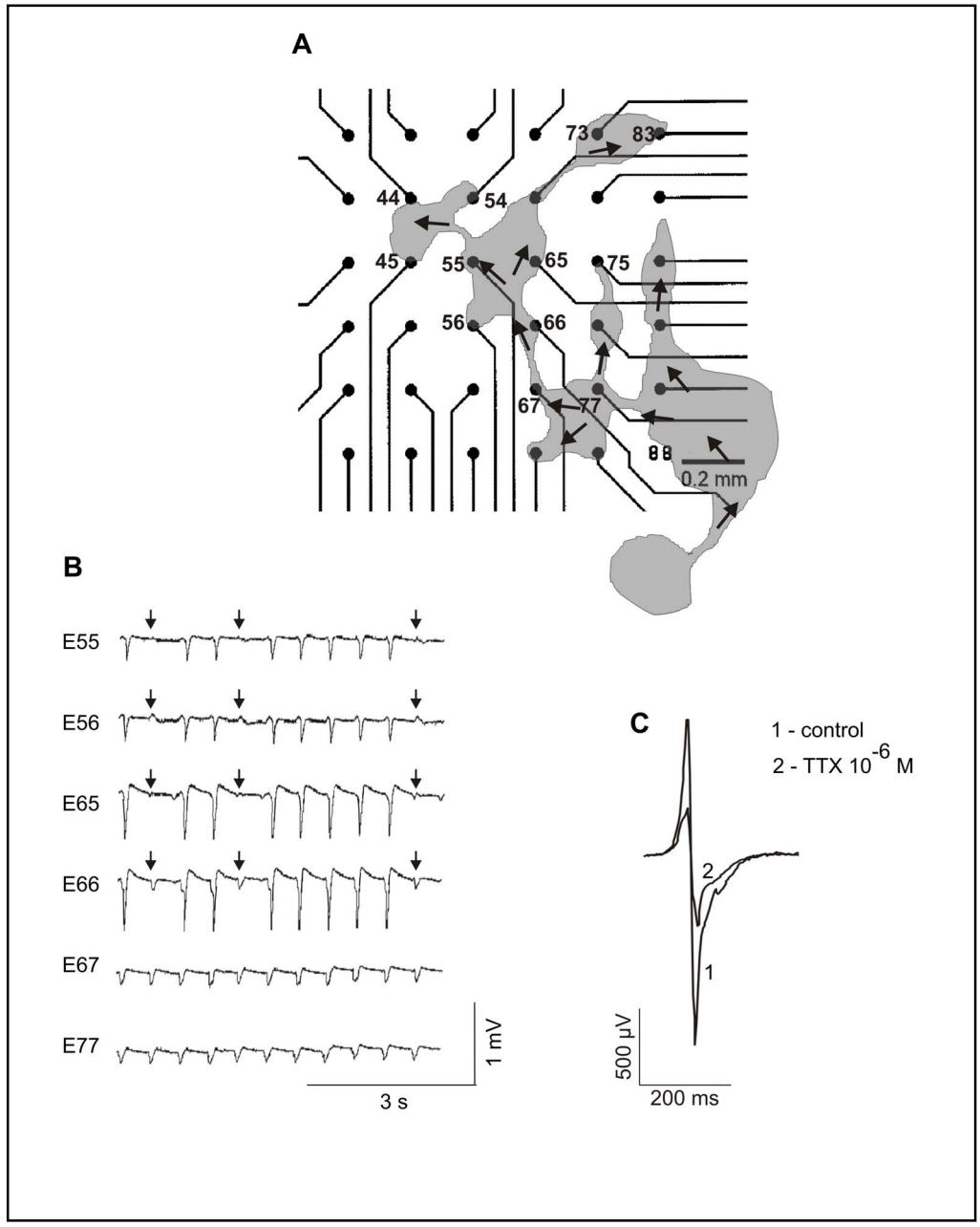

Fig. 4. Effect of TTX on AP generation and propagation. A: Cluster of cardiac cells within an EB, containing large contracting areas connected via narrow tissue strands. Arrows indicate the direction of the AP propagation. B: Synchronous recordings from electrodes 55, 56, 65, 66, 67, and 77 of the MEA preparation shown in (A) after application of TTX. Arrows indicate AP propagation failures. Under control conditions without TTX application no AP propagation failures were registered (data not shown). C: Slowing of the fast FP component after TTX application. The FP shape at one of the recording electrodes is shown for an experiment in which no conduction failures were registered.

areas allowed us to choose suitable EBs, depending on the experimental aim.

Effects of the Ca^{2+} channel antagonist verapamil on AP generation and propagation

Based on the relative specificity of the L-type Ca^{2+} channel antagonist verapamil to block conduction, especially in sinus nodal-like cells, we used verapamil for measurements of the spiking rhythmicity and electrical propagation.

We recorded electrical activity of beating clusters in EBs (n=3) without discontinuities in the conductive tissue. In two EBs, the cardiomyocyte clusters displayed a stable beating frequency at all recording electrodes. In the experiment presented in Figure 3, the beating frequency was about 1.2 Hz. Application of verapamil at a concentration of 10^{-6} M caused arrhythmic spiking of a burst pattern with a frequency spectrum of 0.5-1.2 Hz throughout the whole beating area (shown in Fig. 3 for one representative electrode). In another experiment, where the control beating frequency was non-patterned variable (1.0-1.8 Hz), the same verapamil concentration also led to burst-like spiking, but at a lower frequency (0.4-1.0 Hz) (data not shown). Localization of a

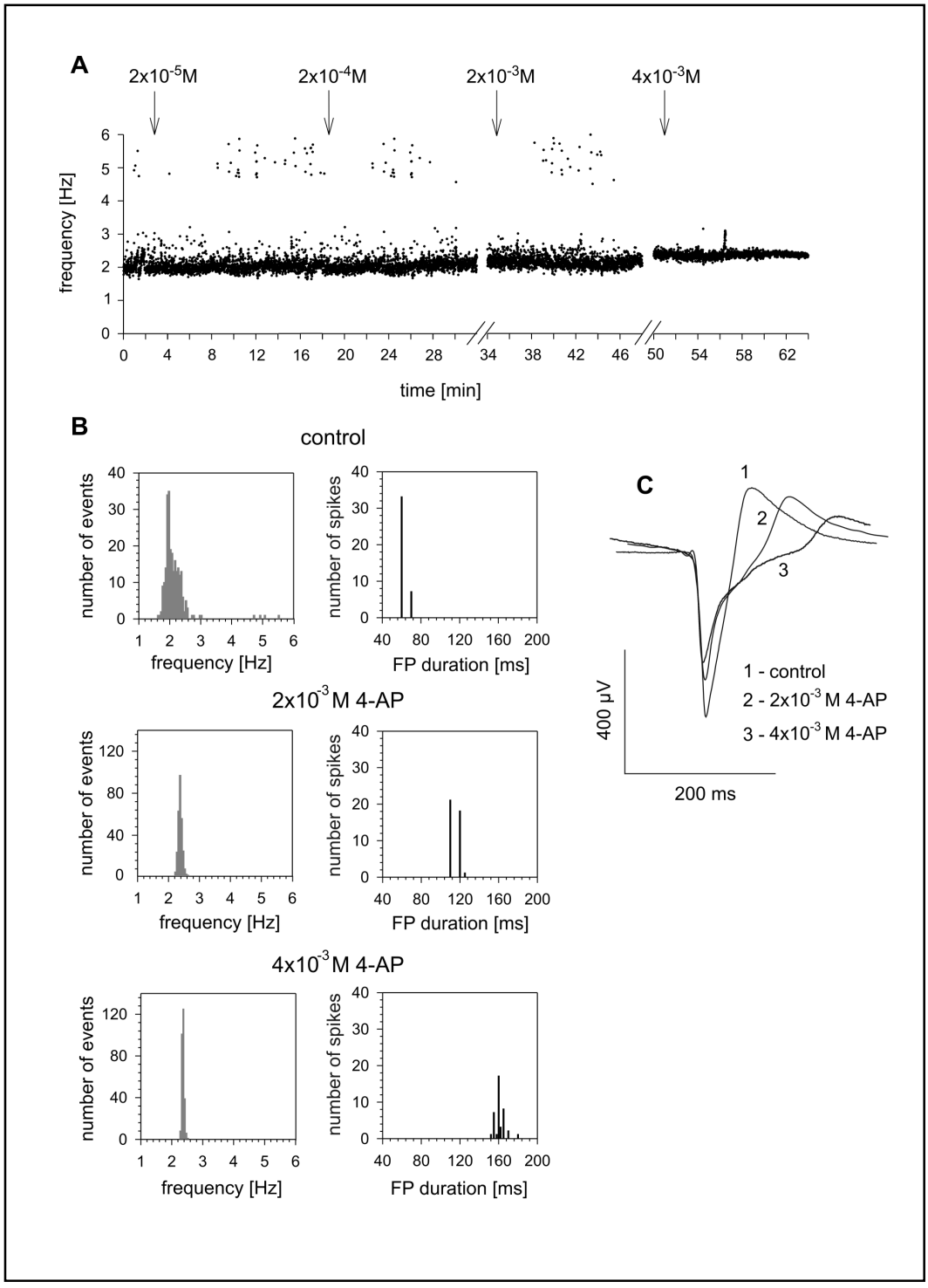

Fig. 5. Effect of 4-AP on beating frequency and spike duration. A: Long-term original trace of the instantaneous frequency. Explanation of interruptions in the trace see Figure 3. B: Distribution of instantaneous frequency (left panels) and spike duration (right panels) under control conditions and in the presence of 4-AP. As the spiking frequency was uniform throughout the whole beating cell cluster, its pattern is shown for one recording electrode. C: Change of the FP shape in the presence of 4-AP, shown for the same electrode as in (B).

pacemaker area and latency analysis confirmed absence of AP propagation failures in all EBs analyzed, suggesting an arrhythmic AP generation by the pacemakers. In line with the negative chronotropic and pro-arrhythmic effects known for verapamil from clinical reports [13, 14], a sustained negative chronotropy under 10^{-5} M verapamil until beating stopped completely after 12 to 15 min of incubation was observed in our experiments (exemplified in Fig. 3A).

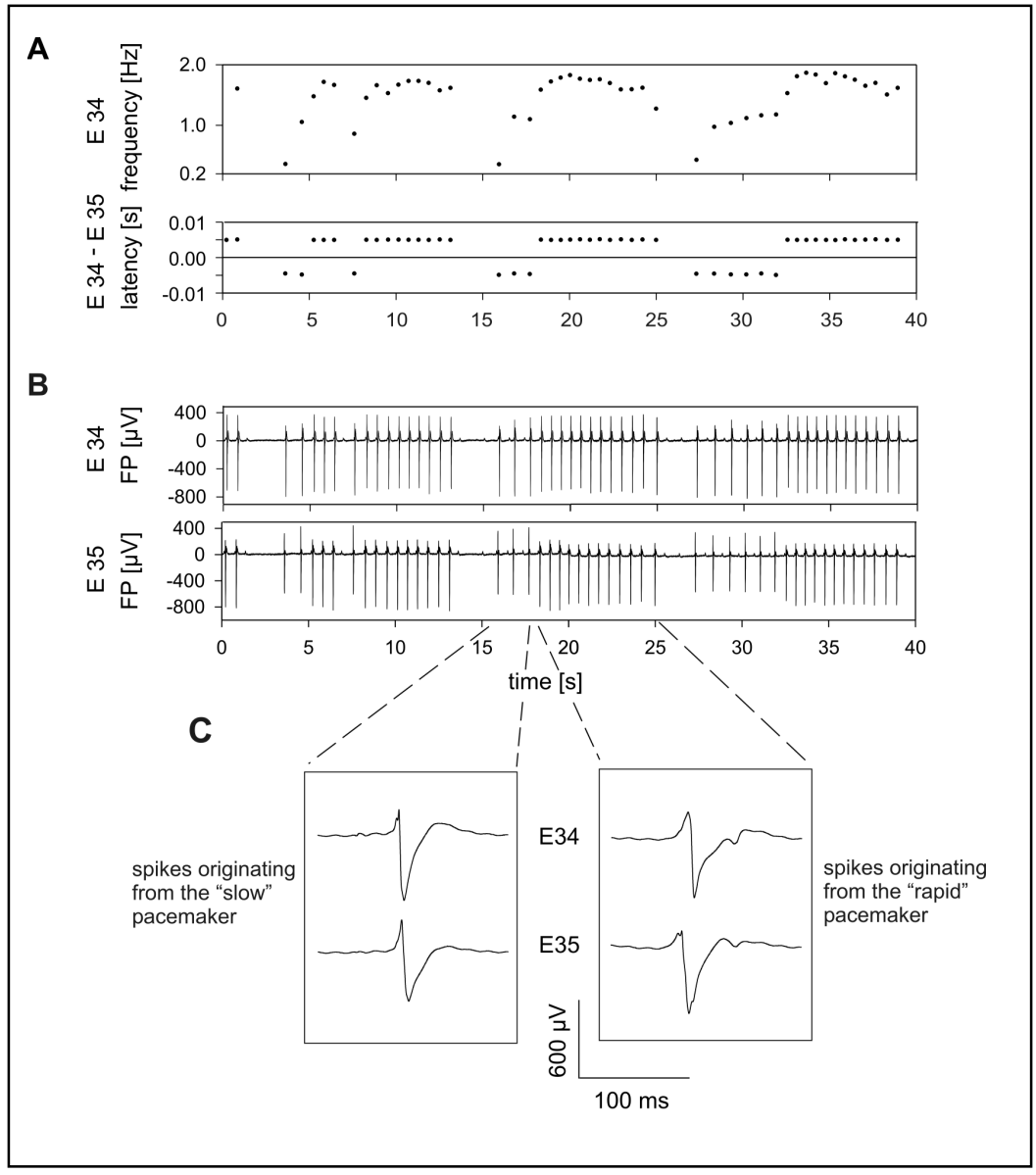

Fig. 6. Beating pattern in a cardiomyocyte cluster with multiple pacemakers. A, top panel: Irregular beating frequency displaying a burst-like rhythm pattern. The frequency pattern shown for the electrode 34 was the same at all recordings electrodes. A, bottom panel: Alternation of latencies between the neighboring electrodes E34 and E35, reflecting the switch between two different pacemakers underlying the FPs shown as original traces in (B) and (C). The panels in (C) show in higher time resolution individual spikes originating from the alternative pacemakers. The dotted lines indicate time periods in (B) during which spikes of the respective type were registered.

Effects of the Na^+ channel blocker tetrodotoxin

Since the sodium inward current (I_{Na}) is the main current underlying the fast impulse propagation in myocardium and the His-Purkinje system, we studied effects of TTX on spontaneous AP frequency and conduction (n=4). In a cardiomyocyte cluster containing multiple narrow tissue strands (Fig. 4A) TTX caused a concentration-dependent intermittent AP propagation block. The maximal percentage of non-propagated APs accounted to approximately 25% at TTX concentrations $\geq 10^{-7}$ M (Fig. 4B). Beating frequency of the pacemaking area localized in the vicinity of the electrodes, showing the earliest onset of the APs, remained unchanged. In the other three EBs, in which microarchitecture discontinuities in beating areas could not be found, no failure in electrical impulse propagation was registered (data not shown). In none of the contracting cell clusters investigated TTX had a noticeable effect on beating frequency. In line with the effect of the drug in native embryonic cardiomyocytes [3, 15], inhibition of the first (fast) component of FPs after application of 10^{-6} M TTX was observed (Fig. 4C).

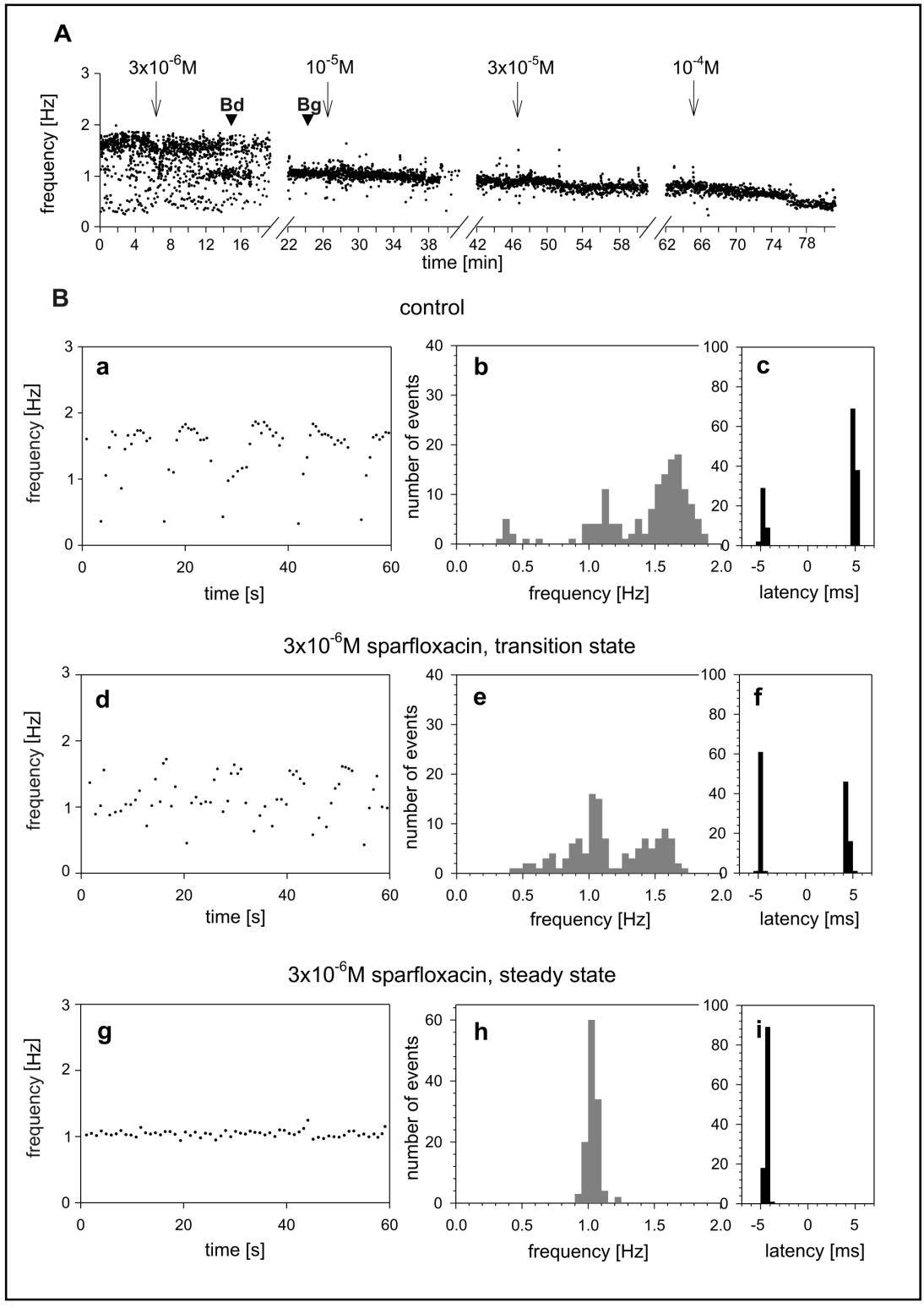

Fig. 7. Effect of sparfloxacin: stabilization of beating frequency. Data are derived from the preparation shown in Figure 6. Spiking frequency as a long-term trace (A) and shown for time windows of 60 s (B: a, d, g). Explanation of interruptions in the trace (A) see Figure 3. Distribution of frequency and distribution of latency between electrodes E34 and 35 under control conditions (B: b, c, see also Fig. 6) and in the presence of sparfloxacin (B: e, f, h, i). The data indicate that the time-dependent decrease in the spike portion originating from the „rapid" pacemaker during ongoing drug application led to stabilization of the beating frequency.

Effects of K^+ channel blockers

Blocking of HERG channels by K^+ channel inhibitors serves as a basis for treatment of arrhythmias [16]. However, in addition to their beneficial effect, they can substantially lengthen cardiac repolarization, potentially leading to life-threatening arrhythmias, e.g. torsades de pointes and ventricular tachycardia (VT). To assess the effect of K^+ channel blockers in our *in-vitro* system, we

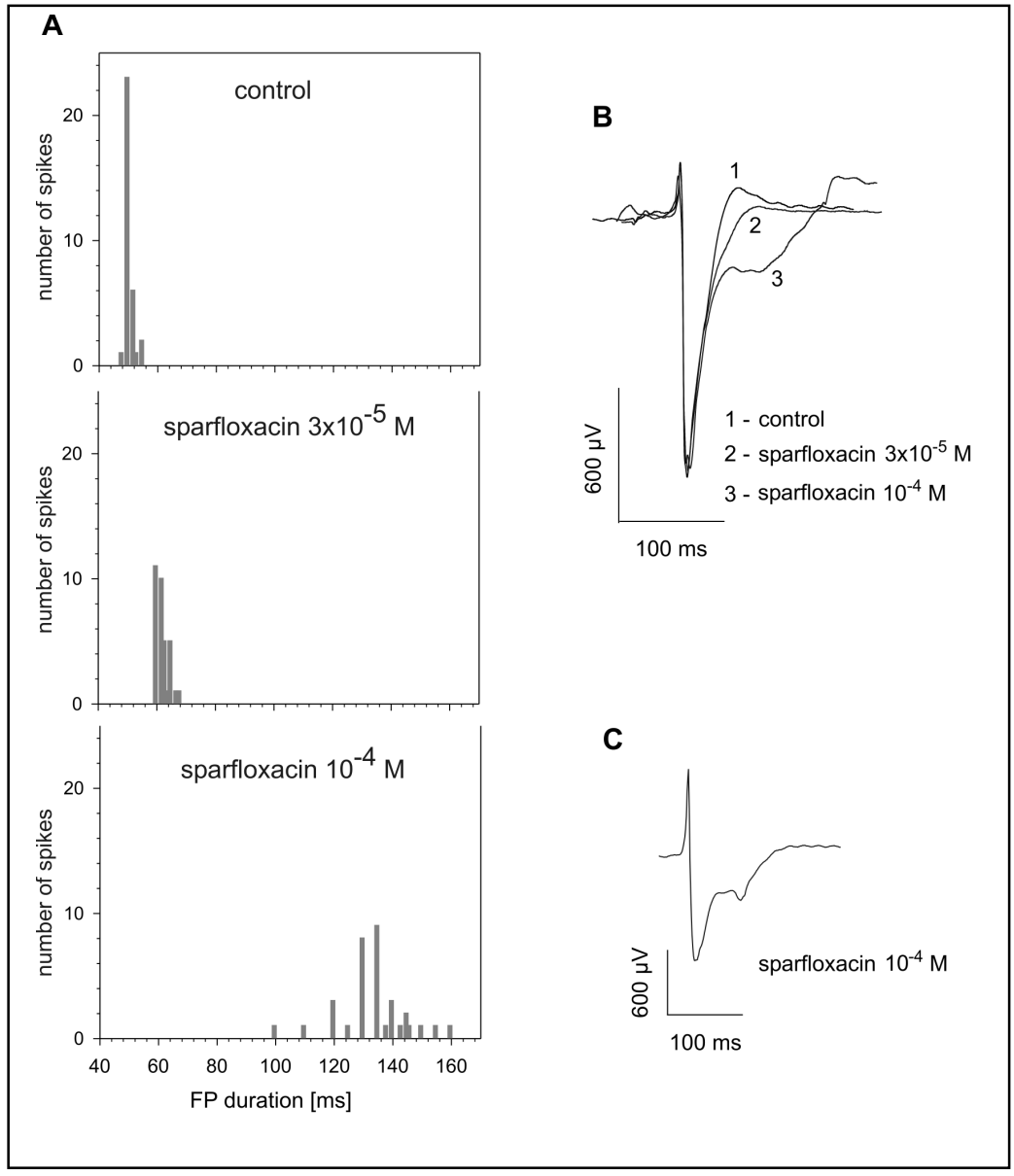

Fig. 8. Effect of sparfloxacin on spike duration and incidence of EADs. A: Distribution of the spike duration under control conditions and in the presence of different sparfloxacin concentrations. For 10^{-4} M sparfloxacin, the calculation is based on spikes occurring before appearance of EADs. B, C: Concentration-dependent effect of sparfloxacin on the spike shape. For 10^{-4} M sparfloxacin, trace 3 in (B) represents a spike without EAD, and (C) displays a spike of the EAD pattern.

studied the effect of the unspecific K^+ channel blocker 4-aminopyridine (4-AP) [17] first. To test whether blockage of K^+ channels affects beating frequency in our experimental model, 4-AP was applied to four EBs. In the EBs displaying arrhythmia under control conditions (n=3), $4x10^{-3}$ M 4-AP stabilized the spiking frequency. As exemplarily shown in Figure 5, a control frequency of 1.8 Hz to 6 Hz was registered; its value was stabilized at 2.2-2.5 Hz after application of 4-AP (A, B (left panels)). In the other EB, which displayed rhythmic beating under control conditions, no effects of 4-AP on the beating frequency were noticed (data not shown). In line with previously described effects of K^+ channel blockers on AP duration, spike duration in all above-mentioned experiments was concentration-dependently prolonged (in the EB exemplified up to 2.7-fold, i.e. from approximately 60-70 ms to 152-180 ms, see Figure 5B, right panels, C). In all EBs examined, the spiking pattern was the same at all recording electrodes.

Additionally, we used the HERG channel antagonist sparfloxacin [18], which has been reported to cause conduction blocks or arrhythmia-like ventricular fibrillation [19]. Sparfloxacin ($3x10^{-6}$ M) was applied to EBs (n=2) displaying a burst-like beating behavior under control conditions, underlined by the activity of two alternative pacemakers. In the experiment shown in Figures 6 and

7, the range of the bursting frequency under control conditions was 0.4-1.8 Hz. During application of $3x10^{6}$ M sparfloxacin the spiking frequency was gradually stabilized and reached the value of approximately 0.9-1.2 Hz at the steady-state (Fig. 7). Latency and frequency analysis revealed that this effect correlated with reduction of the pacemaker activity controlling the rapid burst phase. Similar to the 4-AP action, sparfloxacin caused a significant prolongation of the spike duration (Fig.8A, B, exemplified for one of the EBs). Moreover, after about 12 min of incubation in the presence of 10^{-4} M sparfloxacin, a spike shape deformation indicating early afterdepolarization (EAD) appeared in 70% of spikes (Fig. 8C). In the other EB the drug caused a very similar effect with the only difference that the EAD was visible already under $3x10^{-5}$ M sparfloxacin (data not shown). Our data are in line with the known side effect of sparfloxacin, i.e. prolongation of the electrocardiographic QT interval [20] and the finding that sparfloxacin lengthens the AP duration and provokes EADs in rabbit Purkinje fibers [21].

Discussion

The *in-vitro* model of ES cell-derived cardiac tissue in combination with the high-resolution MEA mapping technique can serve as a cost-effective screening tool for drug effects and safety in pharmacology. The present study aimed to get an impression of the suitability of this tool in respect to evaluation of basic electrophysiological features as chronotropy, arrhythmia, and conduction in the presence of cardioactive substances. In our study we demonstrate that in line with clinical observations murine ES cell-derived cardiomyocyte clusters display typical modifications of excitability and conduction under drug application. We show that the MEA system can be successfully used for detection of anti- and pro-arrhythmic effects of pharmacological substances and their action on conduction characteristics of the excitable tissue. Our data demonstrate that the cardiac-like tissue in EBs displays profound electrophysiological features similar to those of primary cultures of murine embryonic cardiomyocytes [12, 15]. This is in accordance with the evidence that the same mechanisms generate the APs and that the same ionic currents may underlie the integrated electrophysiological properties in these two systems [12]. We have shown earlier a linear correlation between the extracellular spike duration and AP duration simultaneously measured in contracting ES cell-derived cardiac clusters [15]. Moreover, the first, fast occurring negative deflection of the FPs has been demonstrated to represent the activity of the Na^{+} channels (unpublished data), whereas the second, slow FP deflection reflects the plateau phase of the APs [15], which is maintained by the activity of the L-type Ca^{2+} channels [22, 23]. Thus, owing to its high temporal and spatial resolution, the MEA-system enables investigation of the FP morphology in a close relation to the cardiac APs, e.g. characteristics of the repolarisation phase.

Different ion channels are of high clinical relevance in respect to anti- and pro-arrhythmic effects of cardioactive drugs. Some blockers of the ion channels, such as verapamil, are used in *in-vitro* studies, in which they exert typical primary and side effects known from clinical observations. Verapamil is used clinically to treat supraventricular (SV) arrhythmias involving transmission in the AV node, because of its slowing effect on a fast ventricular rate associated with atrial fibrillation [24]. However, it has been suggested that verapamil can promote adverse cardiovascular effects, especially in coronary heart disease and in the failing heart, probably due to its pro-ischemic, negative inotropic and pro-arrhythmic effects [25, 26]. Moreover, excessive concentrations of verapamil may cause sinoatrial (SA) nodal asystole and varying degrees of AV blocks [27]. In line with these side effects we could demonstrate arrhythmic beating of cardiac clusters within EBs in the presence of this drug, up to the complete excitation block. These observations are in accordance with previous data from our group which showed that propagation blocks could be induced in regularly spiking EBs after application of the Ca^{2+} channel antagonist nimodipine [6].

The sodium inward current (I_{Na}) is the main current underlying the fast impulse propagation in the heart. It was shown that application of TTX results in prolongation of cardiac conduction time [28, 29]. Applying TTX, we observed intermittent AP propagation blocks in a complex cardiac cluster containing narrow tissue strands. However, we did not register failures in electrical impulse propagation in clusters without tissue discontinuities. In contrast to the Ca^{2+} channel blockers, TTX had no effect on the beating frequency, suggesting that this drug did not influence the pacemaker activity. Importantly, we registered a reduction of the fast components of the spikes after TTX application. This is in line with previous MEA recordings from our group (Zhongju Lu and co-workers, unpublished data) in native murine embryonic hearts.

The blocker of I_{to} channels, 4-AP, is capable to abolish ventricular flutter [30]. On the other hand, 4-AP

has previously been demonstrated to prolong the AP duration in normal and diseased hearts [31], hereby potentially exerting pro-arrhythmic effects, such as induction of VTs [30, 32]. Accordingly, in our experiments, 4-AP prolonged the FP duration. Also sparfloxacin has been reported to be occasionally pro-arrhythmic, due to increase of the QT interval [33]. In line with the latter, we observed a prolongation of the spike duration and sparfloxacin-induced EADs, presumably due to its blocking effect on HERG channels.

Taken together, our data demonstrate that electrophysiological properties and drug responses of the ES cell-derived cardiac tissue match clinical observations of anti- and pro-arrhythmic effects of the drugs known to influence excitation/transmission in the heart *in-vivo*. We suggest that the *in-vitro* model investigated in this study could serve as a basis for a future high-throughput screening of cardioactive drugs and their potential side effects, i.e. conduction failures, induction of EADs and arrhythmias.

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References

1 Hescheler J, Fleischmann BK, Lentini S, Maltsev VA, Rohwedel J, Wobus AM, Addicks K: Embryonic stem cells: a model to study structural and functional properties in cardiomyogenesis. Cardiovasc Res 1997;36:149-162.

2 Reppel M, Pillekamp F, Lu ZJ, Halbach M, Brockmeier K, Fleischmann BK, Hescheler J: Microelectrode arrays: a new tool to measure embryonic heart activity. J Electrocardiol 2004;37 Suppl:104-109.

3 Hescheler J, Halbach M, Egert U, Bohlen H, Fleischmann BK, Reppel M: Determination of electrical properties of ES cell-derived cardiomyocytes using MEAs. J Electrocardiol 2004;37 Suppl:110-116.

4 Reppel M, Pillekamp F, Brockmeier K, Matzkies M, Bekcioglu A, Lipke T, Nguemo F, Bonnemeier H, Hescheler J: The electrocardiogram of human embryonic stem cell-derived cardiomyocytes. J Electrocardiol 2005;38(4 Suppl):166-170.

5 Stett A, Egert U, Guenther E, Hofmann F, Meyer T, Nisch W, Haemmerle H: Biological application of microelectrode arrays in drug discovery and basic research. Anal Bioanal Chem 2003;377:486-495.

6 Igelmund P, Fleischmann BK, Fischer IR, Soest J, Gryshchenko O, Bohm-Pinger MM, Sauer H, Liu Q, Hescheler J: Action potential propagation failures in long-term recordings from embryonic stem-cell derived cardiomyocytes in tissue culture. Pflugers Arch 1999;437:669-679.

7 Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R: The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. J Embryol Exp Morphol 1985;87:27-45.

8 Maltsev VA, Rohwedel J, Hescheler J, Wobus AM: Embryonic stem cells differentiate in vitro into cardiomyocytes representing sinusnodal, atrial and ventricular cell types. Mech Dev 1993;44:41-50.

9 Wobus AM, Wallukat G, Hescheler J: Pluripotent mouse embryonic stem cells are able to differentiate into cardiomyocytes expressing chronotropic responses to adrenergic and cholinergic agents and Ca^{2+} channel blockers. Differentiation 1991;48:173-182.

10 Nisch W, Bock J, Egert U, Hammerle H, Mohr A: A thin film microelectrode array for monitoring extracellular neuronal activity in vitro. Biosens Bioelectron 1994;9:737-741.

11 Egert U, Knott T, Schwarz C, Nawrot M, Brandt A, Rotter S, Diesmann M: MEA-Tools: an open source toolbox for the analysis of multi-electrode data with MATLAB. J Neurosci Methods 2002;117:33-42.

12 Banach K, Halbach MD, Hu P, Hescheler J, Egert U: Development of electrical activity in cardiac myocyte aggregates derived from mouse embryonic stem cells. Am J Physiol Heart Circ Physiol 2003;284:H2114-H2123.

13 Ferlinz J, Citron PD: Hemodynamic and myocardial performance characteristics after verapamil use in congestive heart failure. Am J Cardiol 1983;51:1339-1345.

14 Yusuf S, Held P, Furberg C: Update of effects of calcium antagonists in myocardial infarction or angina in light of the second Danish Verapamil Infarction Trial (DAVIT-II) and other recent studies. Am J Cardiol 1991;67:1295-1297.

15 Halbach M, Egert U, Hescheler J, Banach K: Estimation of action potential changes from field potential recordings in multicellular mouse cardiac myocyte cultures. Cell Physiol Biochem 2003;13:271-284.
16 Varro A, Biliczki P, Iost N, Virag L, Hala O, Kovacs P, Matyus P, Papp JG: Theoretical possibilities for the development of novel antiarrhythmic drugs. Curr Med Chem 2004;11:1-11.
17 Firek L, Giles WR: Outward currents underlying repolarization in human atrial myocytes. Cardiovasc Res 1995;30:1-38.
18 Kang J, Wang L, Chen XL, Triggle DJ, Rampe D: Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K+ channel HERG. Mol Pharmacol 2001;59:122-126.
19 Chiba K, Sugiyama A, Satoh Y, Shiina H, Hashimoto K: Proarrhythmic effects of fluoroquinolone antibacterial agents: in vivo effects as physiologic substrate for torsades. Toxicol Appl Pharmacol 2000;169:8-16.
20 Morganroth J, Talbot GH, Dorr MB, Johnson RD, Geary W, Magner D: Effect of single ascending, supratherapeutic doses of sparfloxacin on cardiac repolarization (QTc interval). Clin Ther 1999;21:818-828.
21 Adamantidis MM, Dumotier BM, Caron JF, Bordet R: Sparfloxacin but not levofloxacin or ofloxacin prolongs cardiac repolarization in rabbit Purkinje fibers. Fundam Clin Pharmacol 1998;12:70-76.
22 Kecskemeti V, Kelemen K, Marko R, Knoll J: Drugs affecting the calcium-dependent slow depolarization mechanism of the cardiac cell membrane. Adv Myocardiol 1982;3:205-214.
23 Morad M, Tung L: Ionic events responsible for the cardiac resting and action potential. Am J Cardiol 1982;49:584-594.
24 Aronow WS: Atrial fibrillation. Heart Dis 2002;4:91-101.
25 Schwinger RH, Bohm M, Erdmann E: Negative inotropic properties of isradipine, nifedipine, diltiazem, and verapamil in diseased human myocardial tissue. J Cardiovasc Pharmacol 1990;15:892-899.
26 Packer M: Pathophysiological mechanisms underlying the adverse effects of calcium channel-blocking drugs in patients with chronic heart failure. Circulation 1989;80:IV59-IV67.
27 Husaini MH, Kvasnicka J, Ryen L, Holmberg S: Action of verapamil on sinus node, atrioventricular, and intraventricular conduction. Br Heart J 1973;35:734-737.
28 Amitzur G, Schoels W, Visokovsky A, Lev-Ran V, Novikov I, Mueller M, Kraft P, Kaplinsky E, Eldar M: Role of sodium channels in ventricular fibrillation: a study in nonischemic isolated hearts. J Cardiovasc Pharmacol 2000;36:785-793.
29 Duff HJ, Sheldon RS, Cannon NJ: Tetrodotoxin: sodium channel specific anti-arrhythmic activity. Cardiovasc Res 1988;22:800-807.
30 Miyashita T, Kubota I, Yamaki M, Watanabe T, Yamauchi S, Tomoike H: 4-aminopyridine inhibits the occurrence of ventricular fibrillation but not ventricular tachycardia in the reperfused, P6olated rat heart. Jpn Circ J 2000;64:602-605.
31 Gillis AM, Geonzon RA, Mathison HJ, Kulisz E, Lester WM, Duff HJ: The effects of barium, dofetilide and 4-aminopyridine (4-AP) on ventricular repolarization in normal and hypertrophied rabbit heart. J Pharmacol Exp Ther 1998;285:262-270.
32 Wu MH, Su MJ, Lee SS, Young ML: The electrophysiological effects of antiarrhythmic potential of a secoaporphine, N-allylsecoboldine. Br J Pharmacol 1994;113:221-227.
33 Anderson ME, Mazur A, Yang T, Roden DM: Potassium current antagonist properties and proarrhythmic consequences of quinolone antibiotics. J Pharmacol Exp Ther 2001;296:806-810.