LEADING ARTICLE

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Micro-Electrode Arrays in Cardiac Safety Pharmacology

A Novel Tool to Study QT Interval Prolongation

Thomas Meyer,[1] *Karl-Heinz Boven*,[1] *Elke Günther*[2] and *Michael Fejtl*[1]

1 Multi Channel Systems MCS GmbH, Reutlingen, Germany
2 Natural and Medical Sciences Institute, Tübingen University, Reutlingen, Germany

Abstract

Drug-induced QT interval prolongation is now a major concern in safety pharmacology. Regulatory authorities such as the US FDA and the European Medicines Agency require *in vitro* testing of all drug candidates against the potential risk for QT interval prolongation prior to clinical trials. Common *in vitro* methods include organ models (Langendorff heart), conventional electrophysiology on cardiac myocytes, and heterologous expression systems of human ether-a-go-go-related gene (hERG) channels. A novel approach is to study electrophysiological properties of cultured cardiac myocytes by micro-electrode arrays (MEA).

This technology utilises multi channel recording from an array of embedded substrate-integrated extracellular electrodes using cardiac tissue from the ventricles of embryonic chickens. The detected field potentials allow a partial reconstruction of the shape and time course of the underlying action potential. In particular, the duration of action potentials of ventricular myocytes is closely related to the QT interval on an ECG. This novel technique was used to study reference substances with a reported QT interval prolonging effect. These substances were E4031, amiodarone, quinidine and sotalol. These substances show a significant prolongation of the field potential. However, verapamil, a typical 'false positive' when using the hERG assay does not cause any field potential prolongation using the MEA assay. Whereas the heterologous hERG assay limits cardiac repolarisation to just one channel, the MEA assay reflects the full range of mechanisms involved in cardiac action potential regulation. In summary, screening compounds in cardiac myocytes with the MEA technology against QT interval prolongation can overcome the problem of a single cell assay to potentially report 'false positives'.

1. Cardiac Safety Pharmacology

According to the latest guidelines from the US FDA and European Medicines Agency, safety pharmacology for human pharmaceuticals is supposed to include *in vitro* assays to assess the potential for a drug to cause delayed ventricular repolarisation (QT interval prolongation). Results from nonclinical studies to address a potential for QT interval prolongation contribute to the design and interpretation of clinical investigations.[1] Therefore, careful nonclinical investigation of the ability of pharmaceuticals to prolong the QT interval should be carried out to assess potential risk for humans.[2]

As QT interval prolongation can cause arrhythmia and death, safety pharmacology done in this respect is essential.

Guideline S7B from the International Conference on Harmonisation (ICH) draft consensus guidelines for the registration of pharmaceuticals for human use[3] is driven by a concern for the need for the safety of drugs in humans; it is a step-by-step process, leading from essential investigations of vital functions to a case-by-case process, where specific studies are stimulated by previous knowledge from the literature or the pharmaceutical class or previous investigations on the compound to be tested. The individual properties and intended use of the pharmaceuticals influence the design of the studies in preclinical development of pharmaceutical compounds.[4,5]

Three new categories of safety pharmacology studies have been defined ('core battery', follow-up and supplemental studies).

Safety pharmacology core battery studies are supposed to include experiments to detect life-threatening drug adverse effects. The purpose of the safety pharmacology core battery is to investigate the effects of the test substance on vital functions. In this regard, the cardiovascular, respiratory and central nervous systems are usually considered to be the vital organ systems that should be studied in the core battery. Follow-up and supplemental studies are included when adverse effects may be suspected. The guideline clearly requires an assay for QT interval prolongation in the core battery. These supplemental studies could involve organotypic models like Langendorff heart or even *in vivo* ECG studies.

The most common electrophysiological mechanism of QT interval prolongation is basically the inhibition of the ventricular repolarisation. Ventricular repolarisation is composed of various potassium currents. However, the main component is physiologically referred to as the delayed rectifier potassium channel (I_{Kr}) and from the point of view of molecular biology as human-ether-a-go-go related gene (hERG). Ventricular action potential duration is also influenced by calcium currents (mostly L-type) and the slow (I_{Ks}) and ultra rapid (I_{Kur}) components of potassium currents.

2. Classical Measurement Techniques

The classical techniques used to measure QT interval prolongation include patch-clamp experiments of either native or heterologously hERG expressing cells. Patch-clamping of native cardiac cells is a well accepted way to study cardiac action potential parameters; however; it is time consuming and requires a skilled operator.

A second approach is based on whole heart experiments, e.g. the Langendorff heart. This is a very sophisticated and reliable experiment; however, it is expensive and time consuming. The high amounts of test compound necessary to perform an experiment make it unsuitable for screening purposes. A technique that lies between single cell and whole organ approaches is represented by papillary muscle or Purkinje fibre preparations. Again the preparation is technically difficult and expensive.

A channel based assay such as hERG expression in xenopus oocytes is a process which can be automated, but it lacks the accuracy of native cells.

In contrast micro-electrode assay (MEA)-based approaches allow automation and are based on cardiac cells. Moreover, they are on a higher complexity level than single-cell based assays, as networks of cells are monitored. The MEA system has been described and used by various authors as a cell based sensor system in drug development (for an overview see Offenhausser and Knoll[6] and Stenger et al.[7]).

3. Micro-Electrode Assay (MEA) Design and Function

3.1 Tissue Culture

The animal model based on embryonic chicken ventricular cells is not the standard model, but it was established and validated in conjunction with MEAs in 1990.[8] The major advantage of this model is that cells become spontaneously active, which enables the user to study drug-induced changes on this endogenic rhythm. The cells are able to couple and form a syncytium. The MEA technology allows data acquisition and allows the user to calculate conduction velocity and signal propagation patterns. A future goal is to use a multitude of electrodes to gain higher throughput.

The cardiac tissue used in the MEA technology is taken from the ventricles of embryonic chickens. The chicken embryos are excised from the egg after 10–12 days in the incubator and the heart is re-

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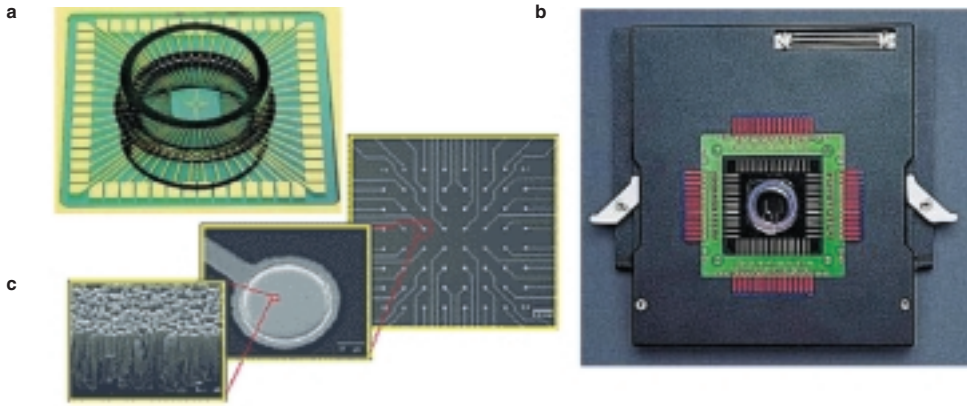

Fig. 1. (**a**) A micro-electrode array (MEA) with attached culture dish; (**b**) MEA1060 amplifier with 60 channels for upright microscopes; and (**c**) a view of the MEA electrode at different magnifications, revealing the fractal structure of the actual titanium nitride recording electrode.

moved. Subsequently, the ventricle is isolated, minced and digested with 0.05% trypsine (original activity 10 400 U/mg) in Dulbecco's Modified Eagle's Medium (DMEM). After 8 minutes of digestion the supernatant is removed and discarded before addition of 3.5mL of trypsine solution. Every 8 minutes the supernatant is collected in ice cold DMEM with 10% fetal bovine serum and replaced by fresh trypsine solution. After 4–5 digestion cycles the heart is completely digested. Cells were pelleted and plated in high density on the electrode field of the polyethylenimine coated MEAs. All chemicals are purchased from Sigma (Deisenhofen, Germany). The MEAs are obtained from Multi Channel Systems MCS GmbH (Reutlingen, Germany). For the tests recording was carried out 2–5 days after preparation.

3.2 Electrophysiological Recordings and Analysis

Recordings are carried out with an MEA1060 System. This system allows simultaneous recording of 60 channels[9,10] with a sampling rate of up to 50 kHz. The amplifier that is used has a gain of 1000 and a bandwidth from 0.1–3 kHz.

Data acquisition and on-line analysis are achieved using MC Rack software also obtained from Multi Channel Systems. Further off-line data analysis is done by Cardiomyocyte Tools (UGert, University of Freiburg, Germany),[11] a customised analysis software tool box based on MATLAB (The MathWorks, Natick, Massachusetts, US). The MEA System features either titanium nitride electrodes with a 30µm diameter, spaced by 200µm (type A MEAs) or gold electrodes with a diameter of 100µm diameter, spaced by 700µm (type B MEAs). The noise level on type B MEAs was lower, due to the increased size of the electrodes. Electrode layout, amplifier and a MEA of type A is shown in figure 1.

3.3 MEA Performance

Using our techniques, ventricular myocytes can be cultured directly onto the MEA. These cells are able to proliferate and form a spontaneously beating syncytium. In figure 2a the proliferating myocytes are shown. Even though we have not conducted a specific proliferation assay we have evidence that cells are proliferating. We have observed that the cell density increases over time, and that these cultures start beating spontaneously after 2–3 days; a property that is typical of cardiac myocytes.

The recorded signal from the MEA consists of three components, reflecting the composition of the cardiac action potential.[12,13] The rapid negative component reflects the influx of sodium through voltage-dependent sodium channels. This is the initial negative slope of the field action potential (fAP) signal. This can be seen in detail in figure 2 (c1 and c2). The spontaneous diastolic depolarisation currents of cardiac pacemaker cells are not detected, due to the integrative nature of the MEA electrodes. These currents can only be detected if a pacemaker

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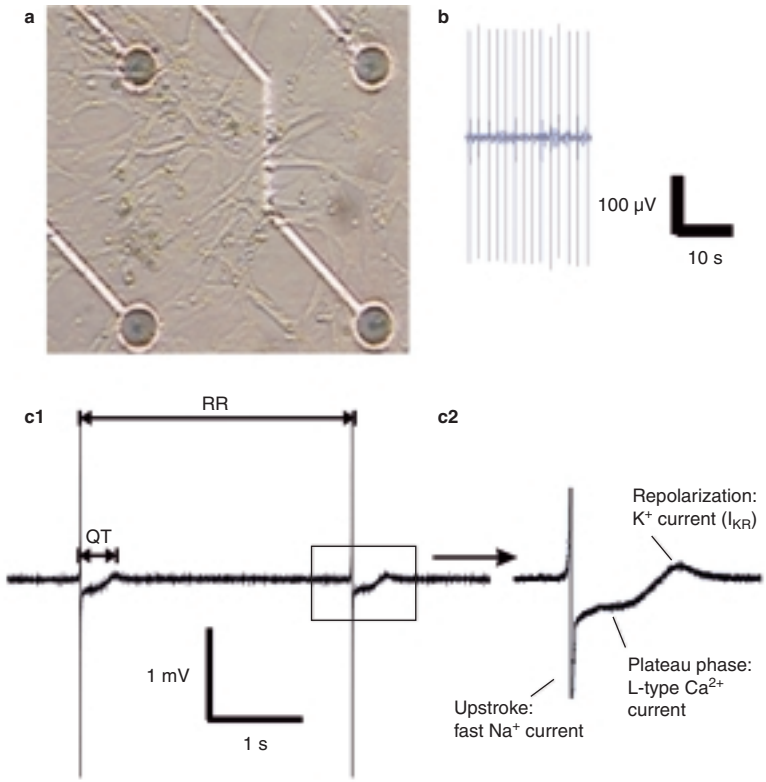

Fig. 2. (**a**) Ventricular myocytes cultured on a micro-electrode array (MEA) [200 × magnification]. The cells form a functional syncytium within two days of culture. (**b**) A signal from one MEA electrode. A spontaneous rhythmic activity occurs after 2–3 days in culture. (**c1**) Two action potential signals recorded from a single electrode of the MEA. The time between two field action potentials (fAP) is defined as RR. (**c2**) The components of the fAP are characterised as evoked by sodium influx (rapid component), calcium influx (plateau) and repolarising potassium currents (I_{KR}) [wave].

cell fortuitously occurs above a recording electrode. However, the plateau, typical of cardiac action potential correlates with a negative plateau in the fAP. The repolarisation is reflected in the positive wave component at the end of the fAP signal. All signal components are characterised by the respective channel blockers.[14-17] The amplifier lower cut-off frequency is set to 0.1 Hz. This enables the recording of even slow signal components like a plateau phase of about 100–200ms. Thus, the negative plateau cannot be equalled, but correlated to the plateau phase of a transmembrane action potential.

The repolarising current is frequently referred to as the 'T-wave' due to the similarity of fAP signals to ECG data.[15,18]

All data shown are recorded by just one out of the 60 available electrodes of an MEA. In figure 3b, a typical recording, done by the MC Rack recording software, is shown as a screen shot. The position of the fAP signals refers to the position of the electrodes on the MEA. Figure 3a shows a close-up of a MEA recording field. In the MC Rack screen shot the signal components outlined in red were automatically detected by the spike detector feature of the MC Rack recording software

4. Study of Reference Substances

Using the novel technology of MEA, QT interval prolongation induced by three drugs, E4031, amiodarone, quinidine and sotalol, known to cause this

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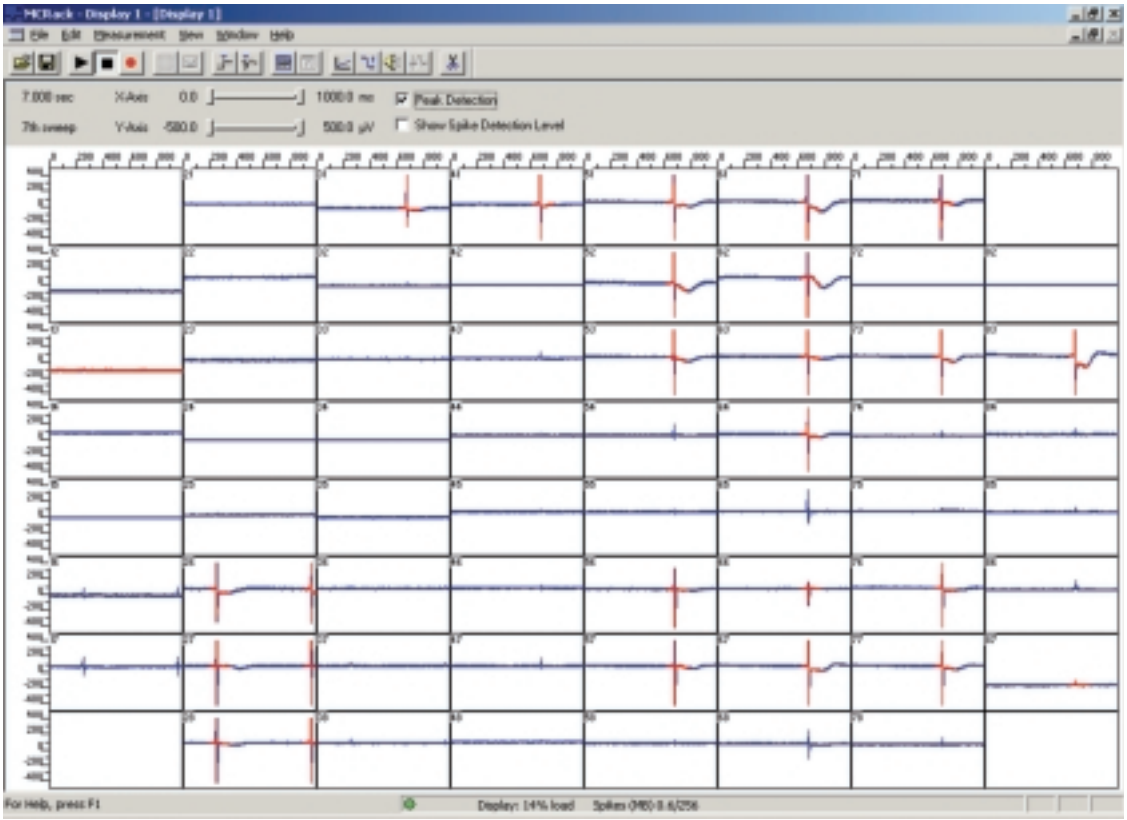

Fig. 3. A screen shot of the recording software (MC Rack): each data window represents the recording from one electrode. The traces indicated in red reflect spike detection by the MC Rack spike detector tool. There are two independent pacemakers causing rhythmic activity on 36 channels.

effect was assessed using an appropriate concentration range. Moreover, verapamil, a substance normally reported as causing 'false positive' results with single-cell assays was also tested.

4.1 Methodology

To start, spontaneously contracting myocytes were recorded in absence of any drug (control conditions). During recording the bath temperature was set to 38°C and pH was buffered at 7.4 using HEPES buffered DMEM. The temperature was initially measured in the bath for calibration purposes. It was found that in order to obtain a bath temperature of 38°, the temperature controller needed to be adjusted to 40°C. There was no continuous superfusion. However, the medium was changed with every new drug concentration. Media changes alone were found to have no effect.

Dose response curves of the various drugs were recorded in cumulative manner from the lowest to highest concentrations. The recording cycle started 3 minutes after the addition of the drug and lasted for 2 minutes. The 3-minute interval was sufficient for the wash-in of the drug and sufficient data could be recorded in 2 minutes for the analysis of arrhythmia and interspike interval duration. Data analysis of cardiac action potentials was performed on the last 40 seconds of the 2-minute recording and allowed the analysis of around 40–50 signals. This is enough for averaging and the control of signal consistency. However, if in that interval there was any technical disturbance whatsoever, the other recorded data backed up the datapoint.

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In the case of verapamil and amiodarone, where there was no effects observed, cells were challenged with quinidine as a positive control at the end of the dose increase.

Data analysis was performed in a semi-automated fashion. The files and the traces used for analysis were selected manually. The measurement of fAP durations from the initial negative peak to the maximum of the repolarising positive wave component was automated. All statistical numbers given refer to independent pacemakers. So two independent pacemakers on one culture are counted as 2. There were never more than two pacemakers per culture. At least two preparations were used for every drug. Errors reflect standard deviations (SD).

4.2 Results

4.2.1 Field Action Potential (fAP) Prolongation Induction by Reference Substances

Whereas the interpretation of the components of fAPs might be primarily of scientific interest, there is a major need of an assay for cardiac repolarisation. As mentioned above, cardiac repolarisation is reflected by the 'T-wave' of the fAP. Inhibition of the repolarising potassium channels results in a dual effect on the fAP: (i) a decrease in the amplitude of the wave; and (ii) an increase in the time interval from the initial sodium spike to the maximum value of the T-wave. The second effect reflects an increase of fAP duration (fAPD). As this is measured in ventricular myocytes, fAPD correlates closely with the QT interval in the ECG.

To study this effect E4031, a specific inhibitor of the hERG channel[19] was used. This substance caused a significant prolongation of fAPD in submicromolar concentrations. E4031 at a concentration of 100 nmol/L caused an increase in fAPD of 67% (n = 5, ± 4.7% SD). Clinically used drugs (mostly antiarrhythmics) were effective in the low micromolar range (see figure 4 for details).

The effect of quinidine, sotalol, E4031, amiodarone and verapamil is shown in figure 4a. Sample traces of the quinidine recording are shown in figure 4b. The absence of an fAPD prolonging effect of verapamil will be discussed in the section 4.2.2. According to the data shown in figure 4a, the reference substances of various chemical and pharmaceutical classes show a dose-dependent fAPD prolonging effect. This supports the idea that the MEA system can be used as a tool for screening for substances with a potential QT interval prolonging effect.

4.2.2 Verapamil and fAP Prolongation

Verapamil is a known antagonist of the L-type calcium channel.[20,21] The IC_{50} for L-type Ca channels is around 100 nmol/L. However, at 150 mmol/L verapamil acts as a potent inhibitor of hERG channels. Verapamil is a notable example of a false positive:[22-24] it blocks ERG K^+ channels, but is reported to have little potential to trigger torsade de pointes.[2] The dual effect of verapamil on L-type calcium channels and the hERG channel even abolishes early afterdepolarisations, which represent the cause of torsade de pointes.[24-26]

In a pure hERG assay verapamil shows inhibition of hERG currents, whereas action potential duration in native cells is not affected. MEA recordings show only a minor shortening of action potential duration on a dose scale from 1 nmol/L up to 3 μmol/L. However, positive controls show significant action potential prolongations (quinidine, E4031, and sotalol). Similar effects are reported for amiodarone.[27] Whereas amiodarone blocks hERG channels, torsade de pointes is extremely rare with this agent.[28-30] Native cardiac myocytes show just a minor action potential prolongation at high doses.

The conclusion of these data is that a one-channel assay does not reflect the complex regulatory mechanisms that underlie the cardiac action potential. There is a significant risk of losing potential drug candidates due to false-positive results from a safety pharmacological test based solely on heterologously expressed hERG channels. As such, cardiac safety should be done with cardiac cells.

4.2.3 Tachycardia due to Early Afterdepolarisation

Whereas in itself, QT interval prolongation on an ECG does not cause any harm to the patient, it increases the risk of fatal arrhythmias.[31-33] These arrhythmia include torsade de pointes, a form of tachycardia which is characterised by twisting of the QRS complex. Torsade de pointes are evoked by early afterdepolarisations (EAD). These EADs are the primary key to understanding the fatal adverse effects of a drug.[34-37] Whereas the risk of torsade de

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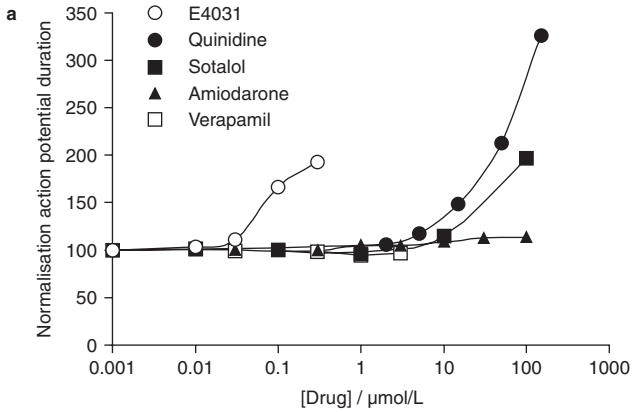

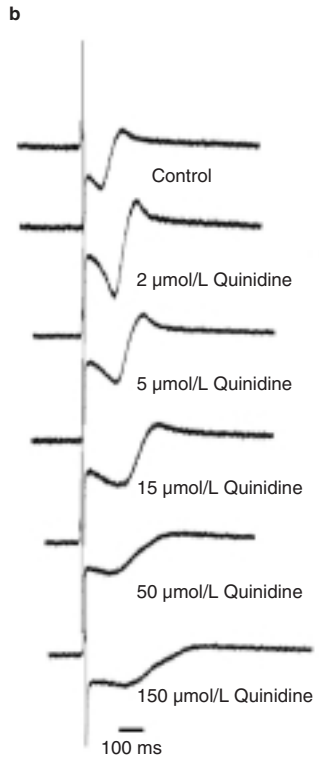

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Compound	n	Average SD (%)
Quinidine	8	1.5
Sotalol	5	7.3
E-4031	5	4.7
Amiodarone	5	8.3
Verapamil	5	6.8

Fig. 4. (**a**) Dose response curves of E4031, quinidine, sotalol, amiodarone and verapamil. The field action potential duration was normalised to 100% under control conditions. Values reflect mean values from at least four independent experiments. All error bars were less than 10% (SD) and are not displayed here. Drug concentration is indicated on a logarithmic scale. (**b**) Sample traces of a quinidine challenged culture. (**c**) A summary of statistical data

pointes and EAD is massively increased by quinidine[34,38] and sotalol,[39,40] just a small minority of patients is affected in the case of amiodarone.[41,42] In the assay system using the MEA, a functional ventricular syncytium is challenged by the drug. This enables the test system to perform EADs and even a kind of 'tachycardia on the chip'. These effects have been observed in a total of eight independent experiments using E4031, quinidine and sotalol. However, similar experiments using amiodarone or verapamil did not show this effect in any experiment. A typical example of EADs evoking additional action potentials is shown in figure 5a, whereas a torsade de pointes-like effect, caused by E4031, is shown in figure 5b.

5. Discussion

Extracellular recordings using MEAs have been used to detect various parameters of interest in cardiac safety pharmacology. The correlation of fAP data and action potential as well as ECG data has been shown by various authors.[12,15,18,43]

The upstroke component is according to the groups of Egert in Freiburg and Hescheler in Cologne based on the signal propagation.[17] It is not detected at the origin of a propagating signal (pacemaker). It seems to run in front of a signal propagating over the electrode field. However, this is still a matter of discussion.

We demonstrated that the crucial parameter of QT prolongation is reflected in fAP prolongation, and is detected in MEA recordings from cardiac

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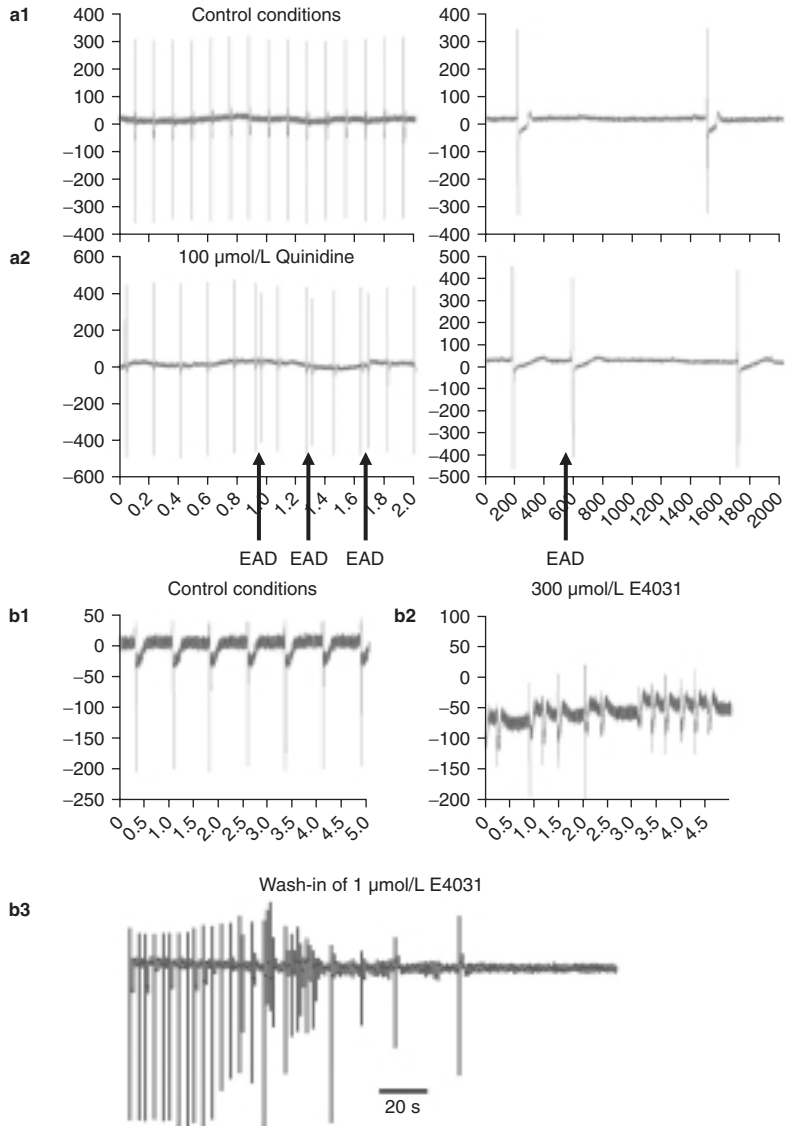

Fig. 5. Under control conditions a rhythmic activity of the cardiac preparation is observed (a1 & b1), whereas preparations challenged by quinidine show a change in the rhythmic activity. There are 15 fAP signals in 20s in a1 as well as in a2. In a2 early after depolarizations (EAD) occur three times as indicated by the arrows. Details are seen on the stretched time scale on the right. Figure b2 shows arrhythmia based on extra depolarizations caused by 300 nmol/L E4031. In b3 the loss of rhythm during the wash-in of 1 µmol/L E4031 is shown. At this high concentration spontaneous activity comes to a complete stop. In all parts of the figure voltage (µV) vs time (ms) is plotted.

myocytes. This enables the user to utilise MEA as a tool for screening for adverse drug effects.

The standard methods for *in vitro* testing for the potential for QT interval prolongation *in vivo* include assays based solely on hERG channel inhibition. This is suitable for rapid screening; however, there is the danger of retrieving false positives (like those seen with verapamil). This assay is very limited due to high specialisation, as interactions of various channels are not taken into consideration. On the other hand there are organ models like the Langendorff heart, which are very reliable, but time

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consuming and not suitable for high or medium throughput.

The use of cardiac tissue on MEAs closes the gap between these two systems. Even though the cells are of avian origin and embryonic, all reference substances revealed the expected results. Verapamil would not have been considered harmful from the result obtained with this assay.

The second major advantage of the MEA system is the ability to detect arrhythmia. This information is much closer to the medical needs than QT prolongation. It is the tachycardia and torsade de pointes, caused by EAD that cause fatalities. EADs do not happen after every 'regular' depolarisation, but only after about 20–30% of the fAPs analysed. This is still sufficient to detect this arrhythmia.

QT prolongation itself is just a more or less reliable indicator for the probability of torsade de pointes.[44-48] However, similar to clinical trials, arrhythmia was not observed in all experiments. Even in one experiment EADs occur just on a random basis. However, high statistical numbers should solve this problem. Whereas statistical significance often cannot be realised in clinical trials due to a lack of sufficiently high numbers of patients included in the study, it can be done in *in vitro* assays. The conclusion of these data is that extracellular recording systems are a reliable and easy to handle alternative in cardiac safety pharmacology. They will not replace organ systems; however, they allow rapid screening in an organotypic model. The 'model' should be further developed to allow better prediction of drug effects in humans. The second direction for the future is to increase the throughput and to automate multiple steps of the experiment.

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Correspondence and offprints: Dr *Thomas Meyer*, Multi Channel Systems GmbH, Aspenhaustr. 21, Reutlingen, 72770, Germany.
E-mail: meyer@multichannelsystems.com

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