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ORIGINAL PAPER

Drug profiling using planar microelectrode arrays

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Abstract Microelectrode arrays (MEAs) with evenly distributed multiple sensor spots have been designed for specific applications. Using the MEAs, we determined the relative profiles of potassium channel openers (KCOs) on cultured embryonic Sprague-Dawley rat cardiac myocytes. KCO, pinacidil (PIN), cromakalim (CROM), SDZ PCO400 (SDZ), or its vehicle, was added to the myocytes cumulatively. The action potential signal shapes in the presence of PIN and SDZ show that the changes in voltage over time and the magnitudes of the associated voltage change were reduced concentration-dependently. CROM affected sodium influx more than PIN and SDZ. The comparisons of changes in the rate of beating and propagation speed in the presence of KCOs were made using their corresponding pD_2 values (the negative log of EC_{50}). All KCOs caused concentration-dependent reductions in the rate of beating and propagation speed, with SDZ being the most potent. In addition to the signal shapes, rate of beating, and propagation speed, the origin of excitation and the excitation pattern inside the culture can be also extracted. The results show that the present system can differentiate the effects of different KCOs on myocytes. It might be possible to utilise the MEA as a means to classify drug action based upon a combined interpretation of the three different datasets gained from the extracellular recordings. The combination of these observations might be used as 'drug signatures' when profiling drugs in the future.

Keywords Microelectrode array · Potassium channel openers · Pharmacology · Bioassay · Cardiac myocytes

Introduction

Pharmacological bioassays, such as drug screening, have been carried out using a variety of in vitro or in vivo methods for many decades. These methods have undoubtedly provided us with a wealth of information regarding the effects of drugs on cells or tissues and ultimately the mechanisms of action of different drugs. For the recording of cell signals, the traditional patch-clamp system has been invaluable in the study of electrophysiological responses at the level of ion currents of cultured cells under different physiological conditions. Current biological investigations, however, are exploring a number of alternative strategies, such as the use of microelectronic devices known as microelectrode arrays (MEAs). This concept is based on the integration of biological cells on microchips in order to detect the changes in extracellular signals. The rationale is that microelectronic fabrication technologies have evolved so rapidly over the past couple of decades that it is now possible to construct microelectrode recording devices for interfacing with individual cells forming components of larger syncytia. There are many advantages of utilising

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these extracellular electrophysiological devices. Unlike the invasive patch-clamp recording method, cells cultured on the microelectronic device can be used for a number of experiments as long as they remain infection-free and the treatment given is reversible. Furthermore, the cells remain undamaged and signals from a plurality of cells can be recorded simultaneously. This is important when the view of a population of cells, as opposed to a single cell, is desired. The MEA system has been designed with these advantages in mind.

The reliability of these devices has always been an issue in the past. Through a great deal of improvement over the past few years, the currently available devices and amplifier systems have much higher signal-to-noise ratios and are much more reliable. Furthermore, these systems are sensitive enough to allow the recording of extracellular signal shape changes, which can be correlated to drug effects. At present, cell-integrated microelectronic devices are used to study different aspects of cellular physiology. A number of MEA systems exist and they have been used to study neuronal [1–4] and heart [5–7] functions. Reviews of the use of cell-based biosensors and the application of MEAs in drug discovery have been provided by Pancrazio et al. [8] and Stett et al. [9], respectively.

Our previous studies have demonstrated the potential application of cultured embryonic cardiac myocytes–integrated field effect transistor arrays in pharmacological bioassay and, in particular, for the interpretation of the recorded signal shapes [10–12]. Parallel to this project, an MEA system that uses 64-channel planar gold microelectrodes for signal recording has been developed [6, 13]. These studies have paved the way for the development of the current system with improved signal-to-noise ratio as well as reliability [14]. The present planar metal microelectrodes, which are the size of a single cell body, enable high input impedance recordings and reliable interpretations of the signal shapes. We have previously reported that our custom-made MEA system provides a load-free voltage measurement without distortion of the extracellular recorded signal shapes [14].

The characterisation of electrophysiological recordings of embryonic heart activity using the MEA has been described [15]. The present proof-of-principle study further explores the use of our MEA system to study a pharmacologically relevant condition involving ATP-sensitive potassium channels (K_{ATP} channels) in the heart. The presence of K_{ATP} channels, or more precisely sulphonylurea receptors, in the heart has been demonstrated in rats using binding studies [16]. The K_{ATP} channels have important implications in normal and pathophysiological cardiac conditions [17, 18] such as ischemic preconditioning [19, 20], arrhythmia [21, 22], myocardial infarction [23], myocardial necrosis and injury [24, 25], and cardiac hypothermia [26]. These channels are selectively activated by a group of compounds known as potassium channel openers (KCOs) [27]. The pharmacology of KCOs and K_{ATP} channels in the heart has been reviewed [28–30].

On the basis of recordings obtained for the effects of KCOs on cultured cardiac myocytes (i.e. beating frequency, excitation velocity, and signal shape, and in particular the combination of these parameters) we aim to show the value of the MEA as a rapid drug screening and function-determining system.

Experimental

Solutions and reagents

The standard culture medium was Ham's F10 medium, containing 10% (v/v) foetal bovine serum, 0.5% (v/v) insulin–transferrin–selenite solution, 6 mM L-glutamine, and a 2% (v/v) mixture of penicillin and streptomycin (5,000 U/ml penicillin and 5 mg/ml streptomycin) adjusted to pH 7.2.

The stocks of KCOs (10 mM) were made using 40% (v/v) ethanol in distilled water. The required concentrations of all the KCOs (0.1–100 μM) were made using the culture medium. In this study, different KCOs were used: (1) pinacidil [PIN; (±)-*N*-cyano-4-pyridyl-*N*-1,2,2-trimethylpropylguanidine monohydrate], (2) SDZ PCO400 [SDZ; (-)-(3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(3-oxocyclopent-1-enyloxy)-2*H*-1-benzopyran-6-carbonitrile], and (3) cromakalim [CROM; (±)-*trans*-6-cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ol].

Cell culture

Hearts of embryonic day 16–19 Sprague-Dawley rats were prepared according to previously published protocols [5, 31]. In the present protocol, however, 0.05% trypsin–EDTA solution was used for the trypsination of the heart, and each centrifugation cycle was set at 5 min. The myocytes were plated at a density between 800 and 1,200 cells per square millimetre (effective MEA surface 38.5 mm^2) and were incubated at 37 °C and 5% CO_2 for 5–6 days before use. The Ham's F10 culture medium was replaced every second day.

Microelectrode arrays

The MEAs were manufactured on glass wafers (Borofloat 33, SCHOTT GLAS, Mainz, Germany) using standard silicon technology. The planar 64-channel gold MEAs (8×8) were designed with diameters of either 10 or 20 μm at a pitch of 100 or 200 μm. In order to use the

MEA several times, the chip surface was passivated by an oxide–nitride–oxide layer deposited by plasma-enhanced chemical vapour deposition consisting of 500 nm SiO_2, 500 nm Si_3N_4, and 100 nm SiO_2. Details of the fabrication and encapsulation processes have been previously described [6, 13, 32].

The MEA chips were cleaned and coated with fibronectin before culturing cells onto them as previously described [6].

Recording setup

We used a custom-made, 64-channel amplifier system in which microelectrodes were coupled directly to the inputs of high-impedance operational amplifiers (OPA124U, Burr-Brown Products from Texas Instruments, USA, common mode input impedance 14 TΩ, preamplifier gain 10.22), then coupled to a main amplifier (gain 100) providing an overall gain of 1,022. The high-pass performance of the recording system was only dependent on the size, material, surface condition, and cleanliness of the microelectrodes providing a large bandwidth recording system [14]. As a result of this ultimate high-impedance input [33], the bandwidth (3-dB cutoff) of our MEA system was enhanced to 0.4 Hz–3.9 kHz for electrodes with a diameter of 20 μm. This performance enables reliable recordings of distinct signal shapes of extracellularly recorded action potentials originating from individual cells. Most importantly, recordings with our current setup can be done with cheap-to-fabricate, reusable, planar gold microelectrodes, which can be designed in sizes smaller than the soma of individual cells. Data were sampled at 10 kHz per channel, with simultaneous acquisition across all channels (PCI 6071E, National Instruments, Hong Kong) using the MED64 conductor 3.1 software (Alpha MED Sciences, Japan). An extracellular Ag/AgCl electrode, which was set to ground potential, served as a reference electrode. A more detailed description of the data acquisition has been published previously [6, 13, 32]. A poly(methyl methacrylate) incubation chamber was attached to the preamplifier headstage in order to prevent excessive evaporation and any air movement over the MEA during experimentation.

Pharmacology

KCOs are classified into several major groups: benzopyrans (e.g. CROM, SDZ), cyanoguanidines (PIN, P1075), pyridines (e.g. nicorandil), thioformamides (e.g. aprikalim), pyrimidines (e.g. minoxidil sulphate), and benzothiadiazines (e.g. diazoxide). Since the benzopyran and cyanoguanidine derivatives are the KCOs most commonly investigated, these two groups were chosen to be used in the present study. Increasing amounts (0.1–100 μM) of prewarmed PIN, SDZ, or CROM, or the corresponding concentrations of the vehicle (ethanol 0.0004–0.4%) were added cumulatively; i.e. no recovery under drug-free conditions was allowed. The subsequent amount was only added after the preceding amount of each drug had elicited its effect. Responses of at least 10 s were recorded by the MEA under control or KCO-treated conditions. The actions of the KCOs were expressed as EC_{50} values (the concentration of an agonist that is required to produce the half-maximal response).

Signal analysis

The effects of drugs on cardiac myocytes were expressed in terms of changes in the extracellular signal shape, the rate of beating, and the propagation speed of excitation.

Signal shape

Owing to the position and the type of electrogenic cells on an electrode, different extracellular signal shapes of single-cell action potentials can be obtained. This is possible only if the electrode dimensions are smaller than the soma size as is the case with the present MEA system. The cell–sensor contact can then be described by the contact of a single cell membrane to a single sensor spot. For the situation where the microelectrode dimension is larger than the cell soma, field potentials of more than one cell are usually recorded with one electrode [9, 15, 34].

It is known that the extracellular signal shapes from cardiac myocytes are composed of several signal components [9, 10, 32, 35]. In Fig. 1, several extracellular signal shapes of the embryonic rat cardiac myocyte culture are shown, with the shape in the centre resembling an ideal signal shape as a composite of all signal components:

1. The fast up-spike, which is capacitively coupled to the electrode, is related to the depolarisation of the cellular membrane. The amplitude of this peak is proportional to the first derivative of the time-dependent membrane voltage $V_M(t)$.
2. The fast down-spike is related to the sodium ion currents through the small cleft between the membrane and the sensor surface. The amplitude of this signal component is proportional to the sodium current.
3. The slow negative signal component is mainly the result of calcium influx.
4. The slow positive signal is the result of the repolarising potassium efflux.

The amplitudes and timescales of these different signal components are mainly determined by the type of cell present on the microelectrode; therefore, the composite

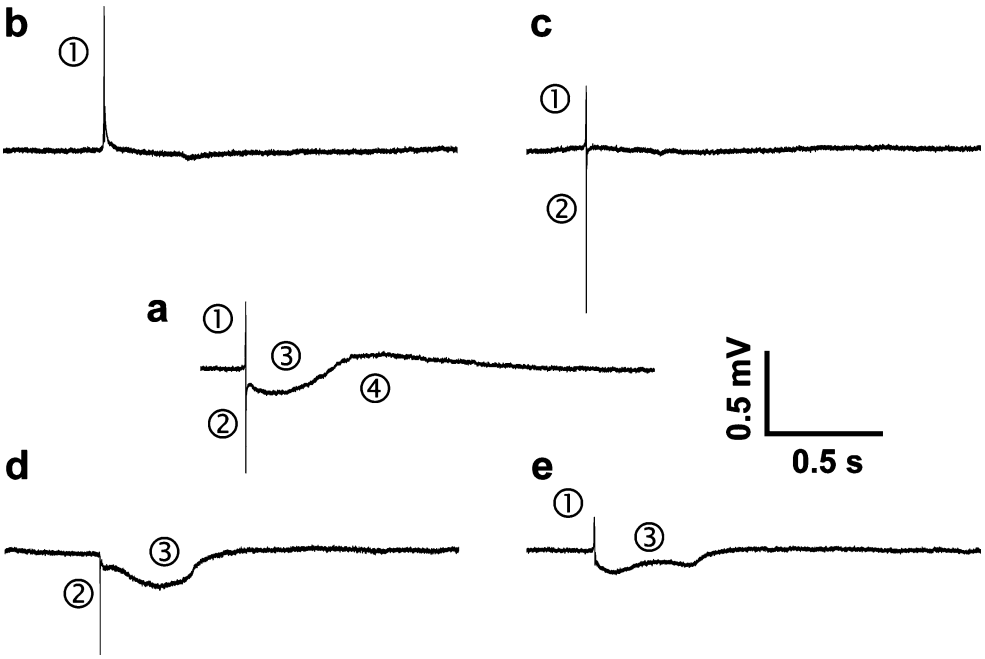

Fig. 1 Examples of a variety of extracellular action potential signal shapes recorded by the present microelectrode array (MEA) system in the absence of the drug. The signal in *a* resembles an ideal signal shape, where all components, i.e. *1* depolarisation, *2* sodium influx, *3* calcium influx, and *4* potassium efflux, are present. Signals shown in the *four corners* are a variety of different signal shapes, with one or more of these components being diminished or enhanced. These differences are mainly due to the different types of cardiac myocyte present on the respective electrode

signal shapes can vary widely when primary cultures of the whole heart are used (Wrobel et al., unpublished data). In Fig. 1, one or more components of different signal shapes are either diminished or enhanced depending on the cell that is being recorded. In a typical 64-channel control recording, a huge variety of extracellular signal shapes were found to be similar to what was found in an earlier version of our recording system [6]. In the present study, we tried to classify different signals into different versions presented in Fig. 1. From the 244 control recordings obtained from six MEA chips, the relative percentages of these signal shapes were found to be 33% (signal a), 34% (signal b), 11% (signal c), 7% (signal d), and 15% (signal e). However, the relative contributions of the respective signal components (Fig. 1, signal a, components 1–4) varied from electrode to electrode, resulting in subtypes of the basic signal shapes. The classification is therefore not straightforward and smooth transitions between these basic signal shapes in Fig. 1 can generally be found.

It has previously been shown that changes in these signal shapes can be related to the underlying mechanism of a particular drug administered [9, 10, 35]. Although the classification of signal shapes is complex, comparisons of all individual signal shapes before and after administration of different drugs may be used as a type of 'physiological signature' of their respective drug actions.

Rate of beating and propagation speed

In principle, it is possible to localise the origin, the route of excitation, and the corresponding waveform pattern [6, 35, 36] in addition to the rate of beating (beats per minute, bpm) and propagation speed (millimetres per second, mm/s).

The means of the rate of beating and propagation speed from all available channels of each MEA chip were calculated before obtaining the final means of all chips within the same treatment group. The control rate of beating and propagation speed were expressed as means with 95% confidence limit (95% CL). As cardiac myocytes on different MEA chips would have different rates and propagation speeds owing to the inability to guarantee identical cell numbers and myocyte-to-fibroblast ratios, changes in these responses in the presence of KCOs were normalised against their respective control, which was taken as 100%. Comparisons of the changes in rate of beating (n=4–6 chips) and propagation speed (n=3–5 chips) between KCO-treated groups were made using their corresponding mean pD_2 values (the negative log of the EC_{50} values) ± the standard error of the mean.

To visualise the signal propagation of the action potentials on the MEA, we developed a software routine in Matlab® 6.5 (Mathworks, USA) that converts the recordings of all 64 electrodes at the given sampling frequency into video files with an 8×8 matrix. We applied a drift and offset correction to the data of each microelectrode to ensure a constant zero level. A value of 50% grey shading corresponded to voltage amplitudes $V_{MEA}\approx 0$ mV as well as black and white (blue and red, respectively) corresponded to the lowest and highest recorded values of V_{MEA}. All V_{MEA} amplitudes obtained in-between were correlated to linearly interpolated grey-scale values (or colours). Before processing for the next time step was

initiated, the current 8×8 matrix was monitored and stored as a frame in the video file.

Subsequently, five additional values were interpolated and extrapolated between neighbouring electrodes in order to obtain a better visualisation of the signal propagation. To reduce the file size of each video, only every tenth recorded V_{MEA} data point was converted.

Figure 2 shows a series of screenshots from one such video of the signal propagation at different times. The spatiotemporal dynamics of the signal propagation can be observed in the corresponding movie (see supplementary material).

Results and discussion

This study provided a quick and simple means of determining the relative effects of some well known KCOs on cardiac myocytes cultured on the MEA.

Effects of KCOs on signal shapes

Owing to the position and the type of cardiac myocyte on each electrode, different signal shapes of extracellularly recorded action potentials were observed even in the absence of drugs (Fig. 1).

Drugs that have an ability to affect ion channels (e.g. Na, K, Ca) can affect all manners of cellular physiology, ranging from simple contraction and relaxation to metabolism. Cations are extremely important to the functions of the heart, and many diseases are the direct result of these ion channels not functioning properly; for example, sodium channel and calcium channel blockers are used in the treatment of arrhythmia (irregular beating of the heart). The variations seen in the present recordings are very typical, and we should be able to observe similar percentages of the contribution from each of these shapes for any microelectrode chips. By analysing the signal shapes in the absence and presence of a drug (i.e. comparing the effects obtained from the same channel before and after treatments), we will be able to identify how much the resultant signal has changed, as a function of the ionic currents, in the presence of this drug.

The present results show that the effects of these KCOs on signal shapes were concentration-dependent (Fig. 3). All three drugs caused reductions in the rate of change of voltage over time as well as the magnitude of the associated

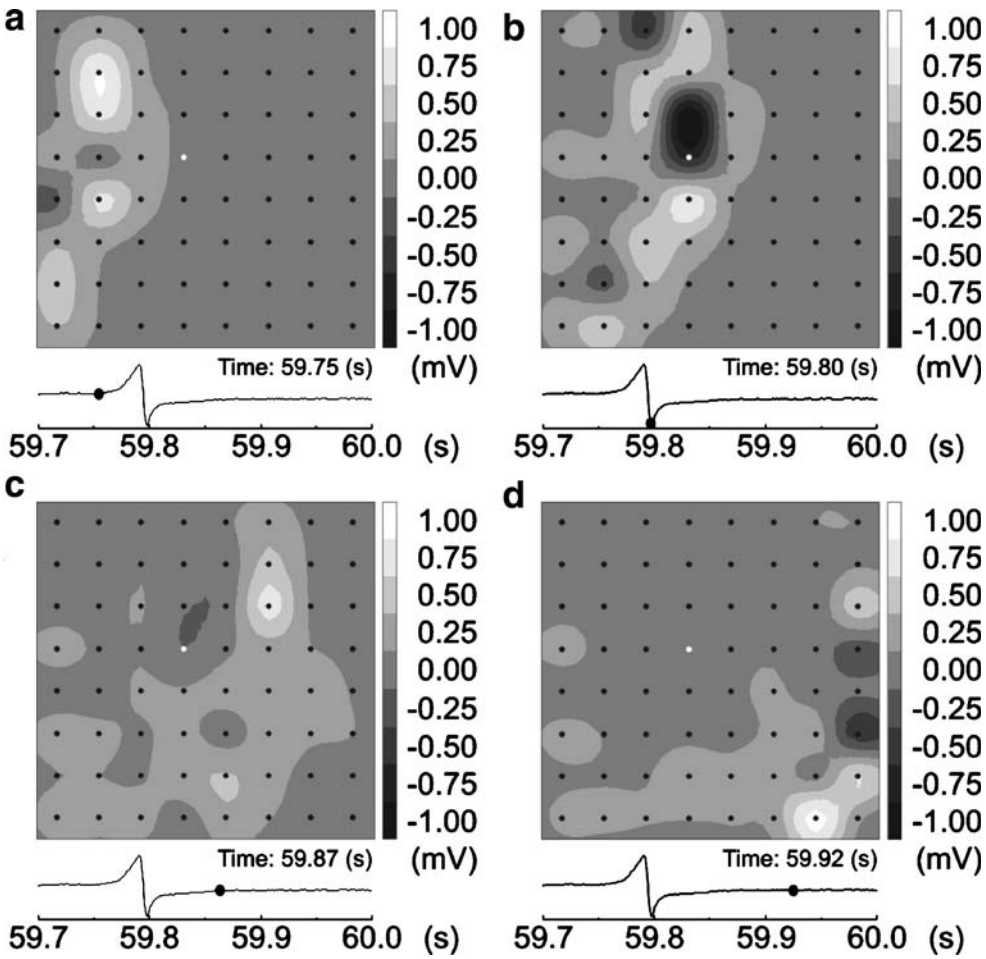

Fig. 2 Visualisation of an action potential wave propagation of a cardiac myocyte monolayer across the 64 (8×8) MEA. The propagation can be seen travelling from left to right (**a**–**d**). The action potential was recorded from electrode no. 28 (*white dot*). Recordings from each electrode have been converted into greyscale maps. Four screenshots extracted from the complete movie are shown. We developed a software routine in Matlab® to visualise the signal propagation and to extract the signal velocity for the recordings (see supplementary material)

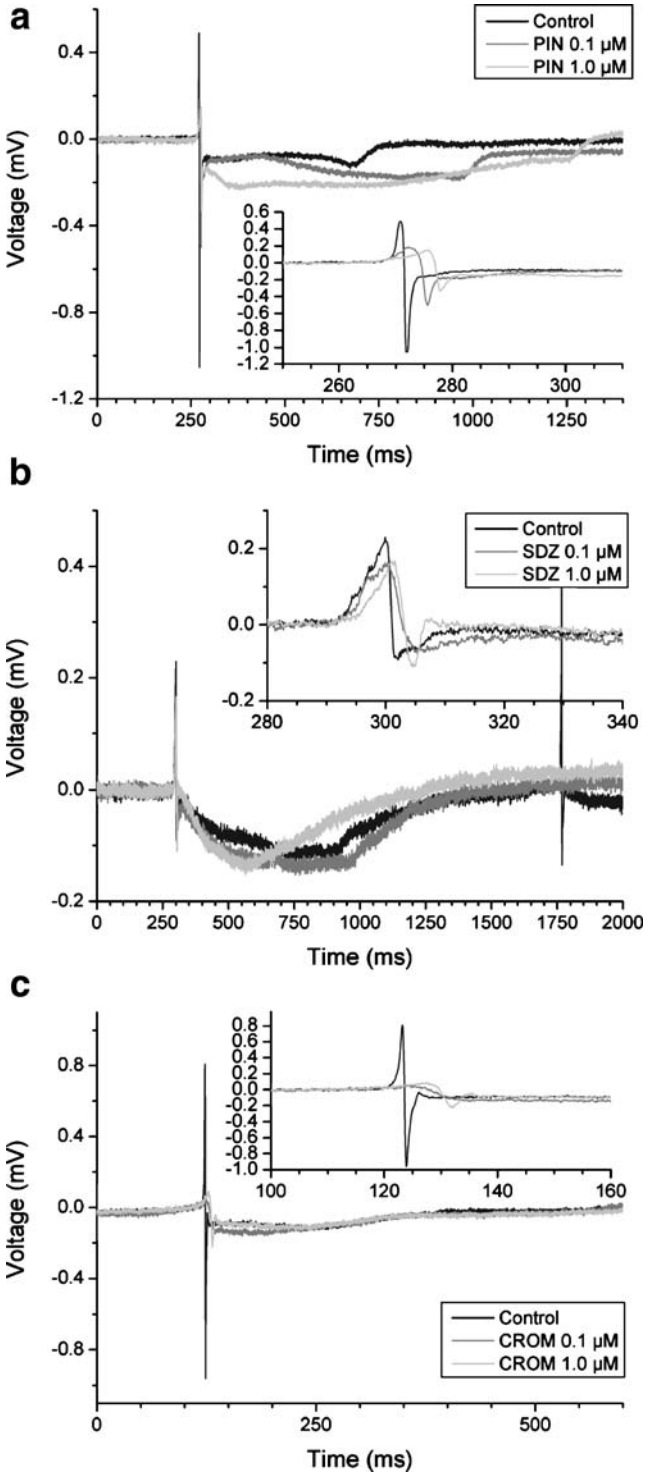

Fig. 3 The signal shapes (action potentials) of cardiac myocytes in the presence of (**a**) pinacidil (*PIN*), (**b**) SDZ PCO400 (*SDZ*), and (**c**) cromakalim (*CROM*) (0.1 and 1.0 μM vs. controls). All three drugs caused a concentration-dependent reduction in the rate of change of voltage over time. The extent of depolarisation (the magnitude of voltage change) also decreased concentration-dependently in the presence of these potassium channel openers (KCOs). In addition, the presence of CROM affected the sodium component of the recording more significantly than the presence of PIN or SDZ (**c**) voltage change from the baseline. In addition, the presence of CROM (0.1 and 1.0 μM) caused a more pronounced reduction in sodium influx than the presence of PIN or SDZ.

Effects of KCOs on rate of beating and propagation speed

As it was unlikely that identical numbers of cardiac myocytes would be cultured on each chip, variable rates of beating and speeds of propagation were observed with means (95% CL) of 52.2±8.6 bpm (34.2–70.2, n=20 channels) and 62.6±14.4 mm/s (21.7–93.5, n=15 channels), respectively. The control rate of beating was stable throughout the entire experiment.

PIN, CROM, and SDZ caused concentration-dependent reductions in the rate of beating (Fig. 4a) and propagation speed (Fig. 4b). The pD_2 values of these KCOs are shown in Table 1. From these values, it can be deduced that SDZ was the most potent in reducing the rate of beating and propagation speed followed by CROM and then PIN. In

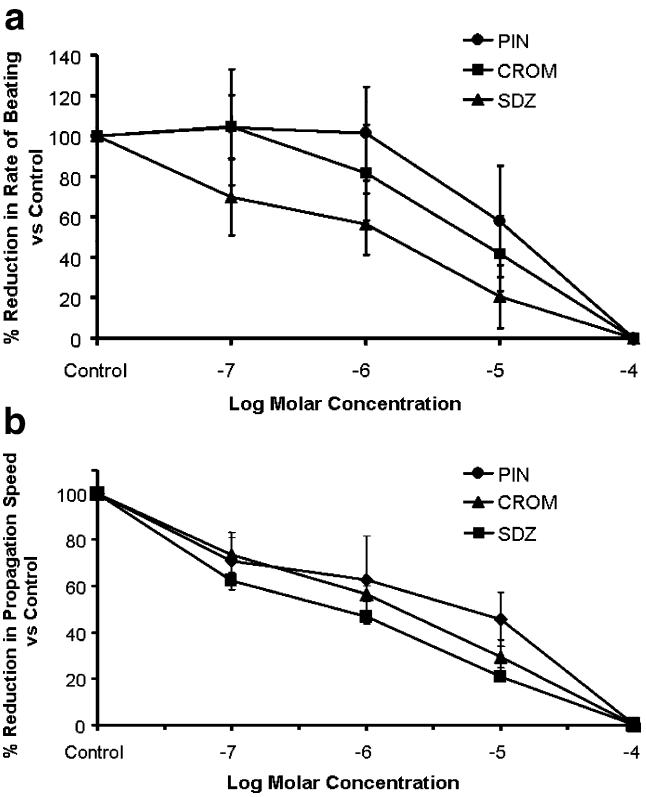

Fig. 4 Effects of PIN, CROM, and SDZ on the rate of beating (**a**) and on the propagation speed (**b**) of cardiac myocytes cultured on the MEAs. The effect of each KCO is concentration-dependent, with SDZ being the most potent. The results are expressed as the percentage reductions in the rate of beating (± standard error of the mean, SEM) and the percentage reductions in propagation speed (± SEM) in the presence of different concentrations of KCOs (log molar concentration) compared with their respective control, which was taken as 100%

Table 1 Effects of potassium channel openers (KCOs) on rate of beating and propagation speed

	Rate of beating			Propagation speed		
	SDZ	CROM	PIN	SDZ	CROM	PIN
Mean pD_2	6.12± 0.36	5.93± 0.35	5.27± 0.38	6.03± 0.43	5.93± 0.24	5.67± 0.57
Equivalent concentration (μM)	0.75	1.17	5.37	0.93	1.17	2.14
n	5	4	6	5	3	4

The results are expressed as pD_2 ± SEM, with SDZ PCO400 (*SDZ*) having the most potent effects on these parameters. *CROM* cromakalim, *PIN* pinacidil, *n* the number of microelectrode array chips used in each experiment

terms of the effect on the rate of beating, SDZ was approximately 1.6 and 7.2 times more potent than CROM and PIN, respectively. In terms of the effect on the propagation speed, SDZ was approximately 1.3 and 2.3 times more potent than CROM and PIN, respectively. In addition, the relative influence of all the KCOs on these two parameters showed a close correlation, with values being close to 1 (Table 2). The close correlation suggests that similar concentrations of these KCOs are needed in order to elicit reductions in the rate of beating and propagation speed to the same extent and that both effects are strongly linked to each other.

The present cardiac myocyte–MEA hybrid system detects the electrophysiology of cardiac myocytes as a single cell (rate of beating, signal shape) and as a colony of cells (propagation speed). Each microelectrode plate is essentially a network of detection electrodes (64 channels in this case). Cardiac myocytes cultured on these plates form a network of interconnecting cells—the basis for an in vitro model of the heart. Electrophysiological recordings obtained can, therefore, be treated as a reflection of physiological responses of the 'whole organ'. Cell communication in this context has been interpreted by those working in this area as highly relevant to functional and developmental morphogenesis (i.e. segmentation, synaptogenesis, etc.) and signal processing in cells that are often described as myogenic (myocardium, vascular, and alimentary canal). The MEA is able to provide separate data on the rate of beating, which is related to the electrophysiological characteristics of an individual cell due to ion fluxes, and the propagation speed, which is related to the connectivity of cells within the cultured monolayer. This connectivity, which affects the propagation of electrical excitation of cultured cardiac myocytes, is related to the gap junction proteins such as connexin45 [37]. Indeed, the MEA system has been used to study the involvement of gap junction proteins and signal propagation in cultured ventricular myocytes [38]. As demonstrated in the present study, the concentrations of these to produce a particular level of effect on the rate of beating and propagation speed were closely correlated.

Table 2 Correlation of the KCO concentrations used in reducing the rate of beating and propagation speed based on their corresponding pD_2 values

Substance	pD_2 of KCO on rate of beating	pD_2 of KCO on propagation speed	Correlation
SDZ	6.12	6.03	1.01
CROM	5.93	5.93	1.00
PIN	5.27	5.67	0.93

A correlation value of 1 means that the concentrations of a given KCO that are required to reduce the rate of beating and propagation speed of cardiac myocytes cultured on the microelectrode arrays were closely correlated

In addition to evaluating the effects of KCOs on the reductions in the rate of beating and conductivity, their effects on the extracellular action potential in terms of ionic changes can also be determined by signal shape analysis of the same data. This is useful information in that these shapes may support the notion of using the signal shapes obtained by the MEA system as 'physiological signatures'. Further studies are currently under way to validate this possibility.

There are also other studies that have used the MEA to study effects of drugs on the heart [7, 39]. Other than cultured cells, ventricular slice preparation is also possible [40]. Furthermore, other methods, such as immunostaining and microdialysis, can be used in conjunction with the MEA system [40, 41]. It seems the MEA may be able to make a substantial contribution to biomedical research. Before it can be used routinely, however, further work must be performed to cross-compare results obtained using different MEA systems that are presently available. Issues such as the number of cells per MEA, the distance between cells and electrodes, and how the chips are made should not be overlooked. In spite of these issues, the present study demonstrated that this 64-channel MEA system can be used to screen a number of drugs quickly and effectively. It also provides a means of preliminary drug profiling before further in-depth studies are performed.

Conclusion

The results of the present study suggest the use of MEAs in pharmacological bioassay is possible. This MEA system is

sensitive and is able to detect minute differences between different ionic components of an action potential. In addition to the analysis of the rate of beating and conductivity of the cellular syncytium, the MEA may be able to provide a further means of classification of drug actions based on signal shapes. It may be possible to identify these characteristic signal shapes as 'signatures' of drugs in the future.

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