Physiol. Meas. **30** (2009) 155–167 doi:10.1088/0967-3334/30/2/004

The use of microelectrode array (MEA) to study the protective effects of potassium channel openers on metabolically compromised HL-1 cardiomyocytes

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Received 7 August 2008, accepted for publication 5 December 2008
Published 9 January 2009
Online at stacks.iop.org/PM/30/155

Abstract

The microelectrode array (MEA) was used to evaluate the cardioprotective effects of adenosine triphosphate sensitive potassium (K_{ATP}) channel activation using potassium channel openers (KCOs) on HL-1 cardiomyocytes subjected to acute chemically induced metabolic inhibition. Beat frequency and extracellular action potential (exAP) amplitude were measured in the presence of metabolic inhibitors (sodium azide (NaN_3) or 2-deoxyglucose (2-DG)) or KCOs (pinacidil (PIN, a cyanoguanidine derivative, activates sarcolemmal K_{ATP} channels) or SDZ PCO400 (SDZ, a benzopyran derivative, activates mitochondrial K_{ATP} channels)). The protective effects of these KCOs on metabolically inhibited HL-1 cells were subsequently investigated. Signal shapes indicated that NaN_3 and 2-DG reduced the rate of the sodium (Na^+) influx signal as reflected by a reduction in beat frequency. PIN and SDZ appeared to reduce both rate of depolarization and extent of the Na^+ influx signals. Pre-treating cardiomyocytes with PIN (0.1 mM), but not SDZ, prevented the reduction of beat frequency associated with NaN_3- or 2-DG-induced metabolic inhibition. The exAP amplitude was not affected by either KCO. The cardioprotective effect of PIN relative to SDZ may be due to the opening of different K_{ATP} channels. This metabolic inhibition model on the

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MEA may provide a stable platform for the study of cardiac pathophysiology in the future.

Keywords: chemical hypoxia, extracellular electrophysiology, HL-1 cell-line, microelectrode array, mouse cardiomyocytes, potassium channel openers

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Hypoxia is a pathophysiological condition whereby cells or tissues are deprived of oxygen. Hypoxia could be the result of cardiac arrest, carbon dioxide poisoning, suffocation or high altitudes where atmospheric O_2 concentrations are low (Banchero 1987, Ping and Jenkins 1978). During acute hypoxia, an insufficient oxygen supply could lead to lactic acid production and accumulation, and prolonged hypoxia could lead to cell death (Kubasiak *et al* 2002). In the case of cardiac hypoxia, the heart rate decreases gradually and then apoptosis occurs (McCully *et al* 2004). In order to seek means to protect the heart from the lethal consequences of short- or long-term hypoxia, studies of the possible protective effects of certain agents against metabolically compromised cardiac cells would be useful. A group of agents collectively known as potassium channel openers (KCOs) may have such properties (Rodrigo and Standen 2005).

KCOs are an expanding category of cardiovascular medicines with actions at different sites within the cardiovascular system. Besides working as peripheral and coronary vasodilators (Rodrigo and Standen 2005), they have been shown to protect the myocardium from damage during hypoxic and ischaemic insults (Nielsen-Kudsk *et al* 1996). Cardiac potassium channels (K^+ channels) are membrane-spanning proteins that allow the passive movement of K^+ across the cell membrane along its electrochemical gradient (Tamargo *et al* 2004). K^+ channels play a major role in controlling the resting membrane potential, the shape and duration of the action potential and consequently the heart rate (Tamargo *et al* 2004). Amongst different types of cardiac K^+ channels, the ligand-gated adenosine triphosphate sensitive (K_{ATP}) channel is a target for studying the importance of K^+ channels in hypoxia/ischaemia. The opening of K_{ATP} channels favours the efflux of K^+, accelerates the repolarization process and thereby shortens the cardiac action potential (Nielsen-Kudsk *et al* 1996). The shortening of the plateau phase of the action potential reduces the time available for calcium (Ca^{2+}) influx, which in turn causes a decline in contractile function, decreases energy demand and perhaps delays lethal injury (Nielsen-Kudsk *et al* 1996). This action seems to represent a possible protective mechanism against metabolic stress.

The patch-clamp technique has been employed to study electrophysiological responses of cells for many decades, and it has provided plenty of valuable information on cardiac pathophysiology (Roden and George 1997). Recently, the advent of microelectronic devices, such as microelectrode arrays (MEAs), has offered scientists a useful alternative (Stett *et al* 2003). The present custom-made MEA system involves the detection of extracellular physiological signals with 64 planar metal microelectrodes. Due to rapid improvement in microelectronic fabrication techniques and in low noise amplifier design over the years, the present MEA system has much higher signal-to-noise (S/N) ratios than past systems (Wrobel *et al* 2007, Zhang *et al* 2005). The data obtained from the MEA were comparable to those from

the patch-clamp system, and a number of pharmacological studies using the MEA to study cardiac (Ingebrandt *et al* 2001, Reppel *et al* 2007, Ecken *et al* 2003), neuronal (Nisch *et al* 1994, Kelly *et al* 2007), gastrointestinal (Nakayama *et al* 2006) and immune functions (Abassi *et al* 2004, Yeung *et al* 2008) have been documented. The MEA has numerous advantages over the traditional patch-clamp technique. For example, the MEA system is non-invasive; therefore, cells can be used for a number of experiments as long as they remain uncontaminated and the drug treatment to the cells is reversible. Furthermore, the logistics of experimentation is less time-consuming, and there is a potential to record signals from all 64 microelectrodes simultaneously. We have previously used the MEA system to detect changes in extracellular signal shapes of cultured primary rat heart cells in the presence of KCOs (Yeung *et al* 2007).

In the present study, chemically induced metabolic inhibition (i.e. an inhibition of the essential energy conversion processes) of cultured HL-1 cells was established with metabolic inhibitors, as opposed to creating a hypoxic state using 95% N_2/5% CO_2 mixture, in order to better control the extent of metabolic stress. Two classes of K_{ATP} channel openers were used to characterize their protective roles in this metabolically compromised condition. The understanding of how K_{ATP} channel openers modify damaged caused by metabolic inhibition could be useful for the treatment of hypoxia and ischaemia in men. Furthermore, this study aimed to show the potential of using this metabolically inhibited model on the MEA as a stable platform for the study of drug treatment in cardiac pathophysiology in the future.

2. Methods

2.1. Solutions and reagents

The standard culture medium was Claycomb Medium (SAFC Biosciences, Lenoxa, Kansas) supplemented with 10% foetal bovine serum (FBS) (v/v) (JRH Biosciences), penicillin (100 U/ml) (Life Technologies), norepinephrine (0.1 mM) (Sigma) and l-glutamine (2 mM, Life Technologies).

The stocks of metabolic inhibitors were made using distilled water, while the stocks of KCOs (10 mM) were made using 40% (v/v) ethanol in distilled water. The required concentrations of all the metabolic inhibitors (0.1 μM–30 mM) and KCOs (0.1 μM–1 mM) were prepared using the standard culture medium. Two KCOs, pinacidil [PIN; (±)-*N*-cyano-4-pyridyl-*N*-1,2,2-trimethylpropylguanidine monohydrate] and SDZ PCO400 [SDZ; (−)-(3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(3-oxo-cyclopent-enyloxy)-2H-1-benzopyran-6-carbonitrile], were used in the present study to activate K_{ATP} channels. Sodium azide (NaN_3) or 2-deoxyglucose (2-DG) was used to elicit two different acute metabolically inhibited conditions. NaN_3 is an inhibitor of mitochondrial oxidative phosphorylation (Inomata and Tanaka 2003), whereas 2-DG is an inhibitor of glycolysis (Orloff and McCarl 1973).

PIN and SDZ were generous gifts from Leo Pharmaceuticals (Princes Risborough, Buckinghamshire, UK) and Sandoz Pharmaceuticals, respectively. Metabolic inhibitors were purchased from Sigma.

2.2. Cell culture

The HL-1 cell line (Louisiana State University Health Science Center, New Orleans, LA, USA), which maintains the phenotype and contractile activity of differentiated cardiomyocyte *in vitro*, was derived from AT-1 cells (mouse cardiomyocyte tumour). The HL-1 cell line has an organized but less differentiated ultrastructure that is similar to the embryonic myocyte. Since these cells do not contain atrial granules, as can be observed morphologically in adult

myocytes, they represent a hybrid between embryonic and adult myocytes rather than at an intermediate stage of myocyte maturation (Claycomb *et al* 1998).

2.3. Microelectrode arrays

MEA chips were fabricated in a previous project (Krause *et al* 2000). The MEAs were built on borofloat glass wafers (Borofloat 33, SCHOTT GLAS, Mainz, Germany) using standard optical lithography. The planar 64-channel gold MEAs (8 × 8) were designed with a diameter of 10 or 20 μm and an electrode-to-electrode pitch of 200 μm. The chip surface was passivated by an oxide–nitride–oxide layer consisting of 500 nm SiO_2, 500 nm Si_3N_4 and 100 nm SiO_2. For measurements, the MEA chips were cleaned and coated with gelatin/fibronectin. Fifty microlitres of cell suspension was initially added, giving rise to a cell density of about 2000 cells mm^{-2}. After 4 h of initial adherence, the chips were filled with 500 μl of culture medium. The HL-1 cells would beat spontaneously once a confluence state had been reached (usually 2–4 days *in vitro*), and measurements could begin at this point. The medium was changed every second day.

2.4. Recording setup

The present amplifier system contained 64 microelectrodes that were coupled directly to the inputs of high-impedance operational amplifiers (Wrobel *et al* 2007, Zhang *et al* 2005), and it was then coupled to a main amplifier similar to what was used in a previous study (Ecken *et al* 2003). As a result of this ultimate high-impedance input, the bandwidth (3 dB cut-off) of this system was enhanced to 1.7 Hz–3.7 kHz for planar gold microelectrodes with a diameter of only 10 μm (Wrobel *et al* 2007, Yeung *et al* 2007, Zhang *et al* 2005). Because of the high impedance input of the recording system and the small size of the microelectrodes relative to the size of individual cardiomyocytes, the recorded extracellular signals from individual cells are different from the usually recorded field potentials obtained using other larger, lower impedance microelectrode systems. As such, these signals are termed 'extracellular action potentials' (exAPs) and signal shape changes of exAPs can be directly correlated with drug action on 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 Co. Ltd, Japan). A Ag/AgCl wire was used as a quasi-reference electrode to define a stable reference potential for the electrolyte solution during all MEA measurements.

2.5. Pharmacology

The medium on each chip was changed and incubated for an hour prior to recording. The concentration–response curves to metabolic inhibitors (NaN_3 or 2-DG, 1 μM–30 mM) or KCOs (PIN or SDZ, 0.1 μM–1 mM) or their respective vehicle controls were performed by taking 30 s recordings of the HL-1 cells before and after the addition of the required agent. To study the effect of KCOs on metabolic inhibition, the cells were pre-incubated with 0.1 mM of PIN or SDZ for 15 min. The medium was then subsequently changed to one that contained both KCO (PIN or SDZ) and metabolic inhibitor (NaN_3 at 1 mM or 2-DG at 10 mM). These concentrations, obtained from the concentration–response curves performed above, were chosen as these metabolic inhibitors elicited an approximately 60–70% maximal reduction in cellular activity.

2.6. Measurements taken

The beat frequency (beats per minute, bpm) and the exAP amplitude (from the positive peak of depolarization to the negative peak of the sodium (Na^+) signal, mV) were recorded using the MED64 software. The results were subsequently analysed using a programme implemented in MATLAB® (Version 7.0, The MathWorks, USA). The means of these two parameters from all available channels of each MEA chip were calculated (i.e. $n = 1$) before obtaining the final means of all the chips from the same treatment group. Since the cells on different MEA chips would have different beat frequencies and amplitudes, the data were normalized against their respective control and expressed as percentage change of these parameters ±SEM. The actions of KCOs and metabolic inhibitors were expressed as IC_{50} values (the concentration required to produce half of the full inhibitory response) ±SEM. The extracellular signal shapes are composed of several signal components, and the interpretations of signal shapes of embryonic cardiomyocyte have been described (Yeung *et al* 2007). Similar interpretations of the extracellular signal shape components of the HL-1 cardiomyocyte are outlined as follows.

(1) The fast up-spike is related to the depolarization 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 Na^+ currents through the small cleft between the membrane and the sensor surface.
(3) The slow negative signal component is mainly the result of Ca^{2+} influx.
(4) The slow positive signal is the result of the repolarizing K^+ efflux.

The sum of components (1) and (2) is equal to the exAP amplitude measured in the present study.

The differences between the effects of metabolic inhibitors and KCOs were analysed using an unpaired *t*-test. The effects of a metabolic inhibitor in the absence or presence of KCOs were compared using ANOVA, with *post hoc* Bonferroni *t*-tests, as appropriate. $P < 0.05$ indicates a significant difference between values.

3. Results

Physiological concentration–responses of the HL-1 cells to metabolic inhibitors (NaN_3, 2-DG) and KCOs (PIN, SDZ) were first characterized, and the possible protective effects of these KCOs on NaN_3- and 2-DG-induced metabolic inhibition were subsequently investigated. Beat frequency and exAP amplitude were used to show the differences in electrophysiogical responses of the HL-1 cells between normoxic and metabolically compromised conditions.

3.1. Effects of metabolic inhibitors on HL-1 cells

The control beat frequency and exAP amplitude were 65.1 ± 7.2 bpm and 0.8 ± 0.1 mV ($n = 18$), respectively. NaN_3 and 2-DG reduced the beat frequency and exAP amplitude of HL-cells in a concentration-dependent manner (figures 1(a) and (b)). NaN_3 was more potent than 2-DG by 17.4-fold and 7.7-fold ($P < 0.001$, $n = 3$–4) in reducing the beat frequency and exAP amplitude, respectively (table 1). The traces of propagating exAP of HL-1 cells in the presence of NaN_3 (1 mM) and 2-DG (10 mM) relative to their respective controls are shown in figures 2(a) and (c), respectively. While the general signal shapes indicated that the rates of depolarization (the fast up-spikes) remained at similar levels to that of the controls in the presence of either metabolic inhibitor, the extent of Na^+ influx signals, especially with

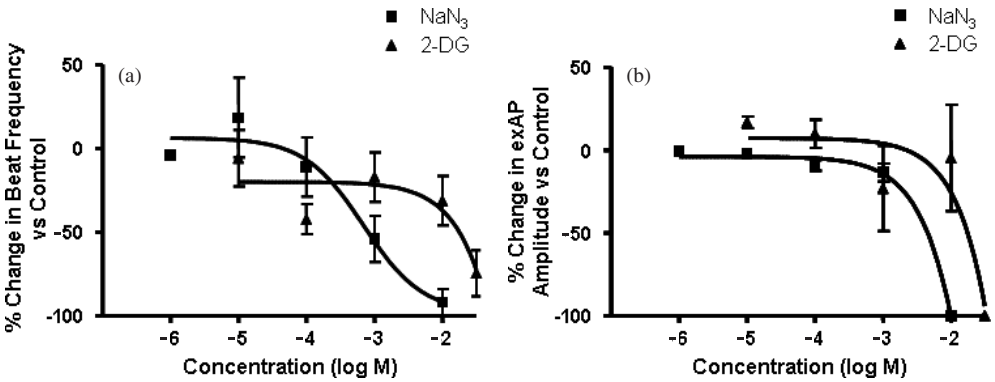

Figure 1. Effects of metabolic inhibitors, NaN_3 and 2-DG (1 μM–30 mM), on HL-1 cells (n = 3–4). Both metabolic inhibitors caused concentration-dependent reductions in beat frequency (a) and extracellular action potential (exAP) amplitude (b). The results are expressed as % change ±SEM.

Table 1. Effects of metabolic inhibitors, sodium azide (NaN_3) or 2-deoxyglucose (2-DG), and potassium channel openers, pinacidil (PIN) or SDZ PCO400 (SDZ), on beat frequency and extracellular action potential (exAP) amplitude (n = 3–7). The IC_{50}s (the concentration that elicits a 50% reduction in response ± SEM) showed that NaN_3 was more potent than 2-DG, whereas SDZ was more potent than the PIN in reducing the beat frequency and exAP amplitude.

Beat frequency		exAP amplitude	
	IC_{50} (mM)		IC_{50} (mM)
NaN_3	1.07 ± 0.33	NaN_3	2.60 ± 0.21
2-DG	18.64 ± 2.98	2-DG	19.94 ± 0.58
PIN	0.20 ± 0.03	PIN	0.23 ± 0.01
SDZ	0.06 ± 0.03	SDZ	0.18 ± 0.04

2-DG, reduced as indicated by smaller fast down-spikes than their corresponding controls (figures 2(b) and (d)).

3.2. Effects of KCOs on HL-1 cells

The beat frequency (figure 3(a)) and exAP amplitude (figure 3(b)) of HL-1 cells decreased as the concentrations of PIN and SDZ increased. These changes were reflected by reductions in the rate of depolarization (the fast up-spikes) and the extent of Na^+ influx (the fast down-spikes) (figure 4). SDZ was approximately 3.3 times more potent than PIN at decreasing beat frequency of the HL-1 cells ($P < 0.05$, n = 4–5). While both KCOs affected the exAP amplitude to comparable extents with similar IC_{50} values (table 1), SDZ prolonged the time it took the HL-1 cells to complete Na^+ influx (figure 4(d)). The vehicle (⩽4% alcohol) had no effect.

As the beat frequency decreased, the exAP amplitude also proportionately decreased. Based on the respective pD_2 values (the negative log of IC_{50}s) of all four agents, the concentration ratios were very close to 1, indicating that these two parameters are closely correlated (table 2).

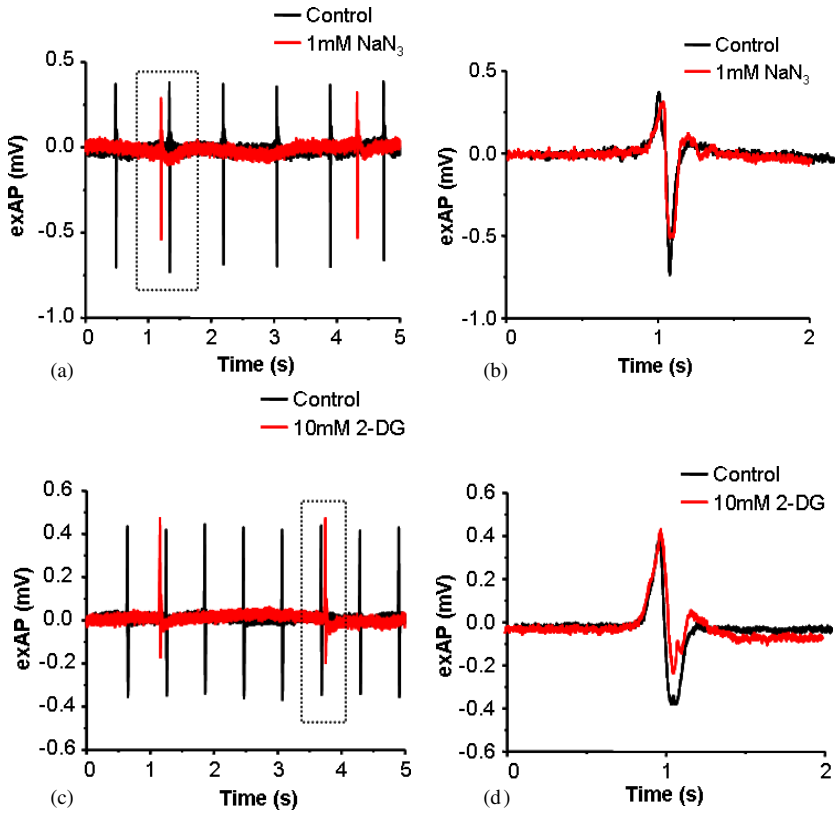

Figure 2. Representative traces of multiple and individual extracellular action potentials (exAP) of HL-1 cells following the addition of NaN_3 (1 mM) or 2-DG (10 mM). Both metabolic inhibitors reduced the beat frequency (a, c). While the rate of depolarization remained comparable to their respective controls, the extent of the Na^+ influx signal reduced in both cases, especially with 2-DG (b, d).

Table 2. Correlations (i.e. concentration ratios ± SEM) between the respective pD_2 values (the negative log of IC_{50}s) of KCOs and metabolic inhibitors in reducing the beat frequency and extracellular action potential (exAP) amplitude ($n = 3$–7). A ratio close to 1 suggests that similar concentrations of drugs are needed in order to reduce the beat frequency and exAP amplitude to the same extent.

	pD_2 of drugs on beat frequency	pD_2 of drugs on exAP amplitude	Concentration ratios
NaN_3	3.07 ± 0.19	2.59 ± 0.03	1.19 ± 0.08
2-DG	1.74 ± 0.07	1.70 ± 0.01	1.03 ± 0.04
PIN	3.73 ± 0.07	3.65 ± 0.03	1.02 ± 0.02
SDZ	4.51 ± 0.30	3.83 ± 0.17	1.18 ± 0.11

3.3. Effects of KCOs on metabolically inhibited HL-1 cells

The protective effect of the KCOs (0.1 mM) was evaluated against a single concentration of NaN_3 at 1 mM or 2-DG at 10 mM, which elicited a 60–70% maximal reduction in cellular activity. PIN pre-treated HL-1 cells were able to overcome the metabolic inhibitor-induced

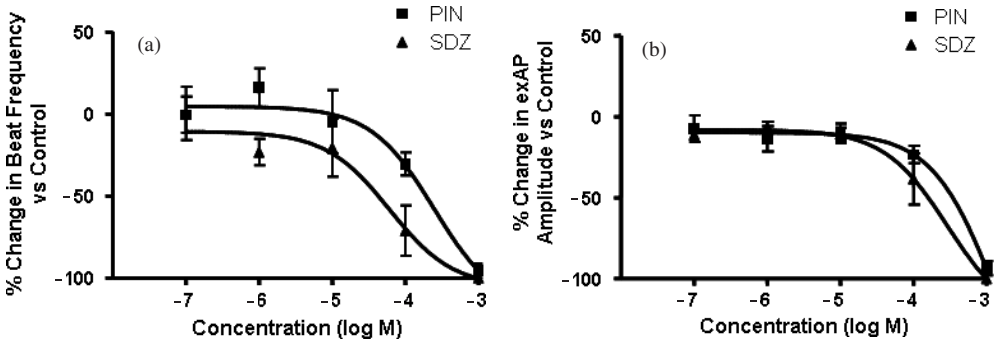

Figure 3. Effects of KCOs, PIN and SDZ (0.1 μM–1 mM), on HL-1 cells (n = 3–7). Both KCOs caused concentration-dependent reductions in beat frequency (a) and extracellular action potential (exAP) amplitude (b), with SDZ being more potent than PIN. The results are expressed as % change ± SEM.

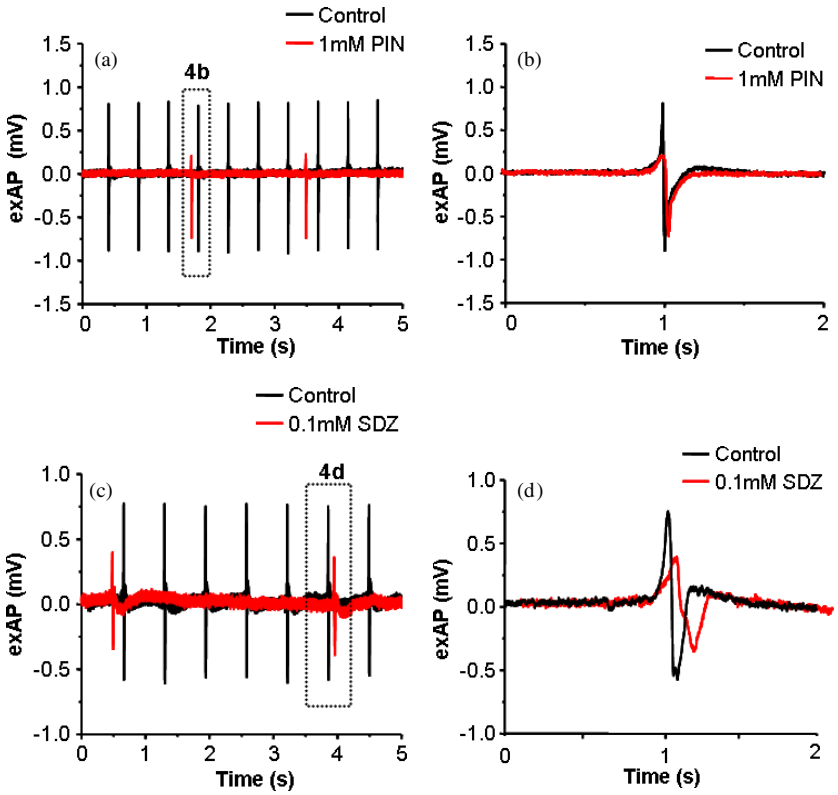

Figure 4. Representative traces of multiple and individual extracellular action potentials (exAP) of HL-1 cells following the addition of PIN (1 mM; (a, b)) or SDZ (0.1 mM; (c, d)). Both rates of depolarization and extents of the Na^+ influx signal reduced in the presence of KCOs, with SDZ appeared more potent than PIN. SDZ also prolonged the duration of the Na^+ influx signal.

reduction in the beat frequency. With PIN pre-treated cells, the beat frequency not only returned to its basal level but also actually increased upon the addition of either NaN_3 or

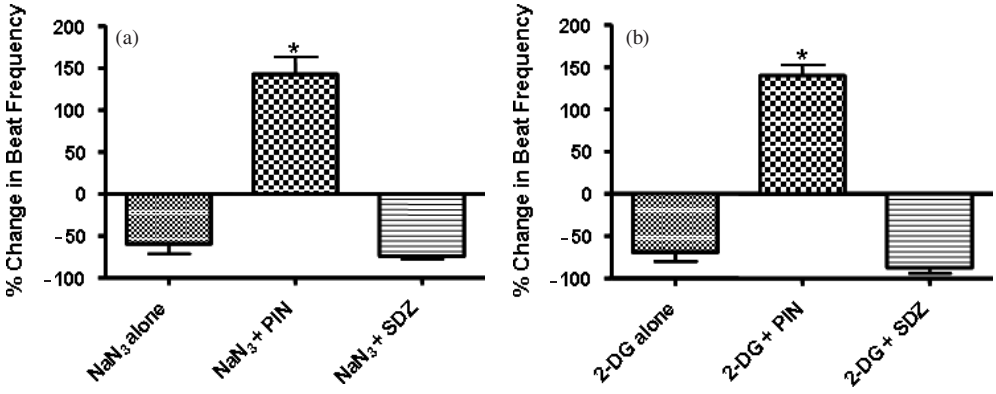

Figure 5. Effects of PIN and SDZ, both at 0.1 mM, on the beat frequency of metabolically compromised HL-1 cells induced by 1 mM of NaN_3 (a) or 10 mM of 2-DG (b) ($n = 3$–7). These concentrations of metabolic inhibitors elicited an approximately 60–70% reduction in cellular activity. Significant recovery in beat frequency was observed between the control (metabolic inhibitors only) and PIN pre-treated cells (% change ± SEM, $*P < 0.01$). SDZ had no effect.

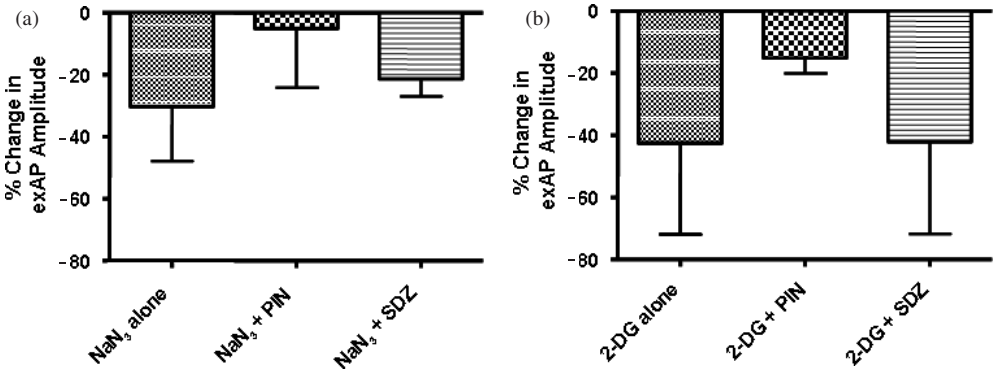

Figure 6. Effects of PIN and SDZ, both at 0.1 mM, on the extracellular action potential (exAP) amplitude of metabolically compromised HL-1 cells induced by 1 mM of NaN_3 (a) or 10 mM of 2-DG (b) ($n = 3$–7). No significant differences in exAP amplitude (% change ± SEM) were found between KCOs pre-treated cells and their respective controls.

2-DG (figures 5(a) and (b)). This, however, was not observed with SDZ pre-treated HL-1 cells. Neither PIN nor SDZ could prevent the exAP amplitude reduction of the metabolically inhibited cells in the presence of either inhibitor (figures 6(a) and (b)), although a slightly smaller reduction in amplitude was observed with PIN-treated cells.

4. Discussion

The present study demonstrated, for the first time, that the MEA system could be used to study metabolic inhibition using cardiomyocytes derived from the HL-1 cell line, and the results revealed that PIN may have advantages over SDZ under acute metabolically compromised conditions.

In previous studies, metabolic inhibitors and KCOs have already been shown to reduce the beat frequency of cardiomyocytes (Hasin and Barry 1984, Orloff and McCarl 1973,

Yeung *et al* 2007). The close correlation between the beat frequency and exAP amplitude suggests that similar concentrations of drugs are needed in order to reduce these parameters to the same extent, and that these observed physiological changes are intricately linked. In this study, the inhibitors of mitochondrial oxidative phosphorylation (NaN_3) and glycolysis (2-DG) mimicked hypoxic conditions as reflected by reductions in the beat frequency and exAP amplitude. The present results show that PIN, but not SDZ, has a cardioprotective role under these metabolically compromised conditions.

The relative potency of PIN (pD_2: 5.27) and SDZ (pD_2: 6.12), with regard to their effects on the beat frequency, on cultured rat neonatal primary cardiomyocytes has been evaluated previously (Yeung *et al* 2007). While the order of potency of PIN and SDZ on HL-1 cells is the same (i.e. SDZ (pD_2: 4.51) > PIN (pD_2: 3.73)), SDZ was found to be more potent than PIN by 7.2- and 3.3-fold on primary cultured cardiomyocytes and HL-1 cells, respectively. The difference in efficacies of these KCOs may simply be a tissue-dependent effect, since the HL-1 cells were derived from the mouse cardiomyocyte tumour, whereas, in the previous study, the embryonic cardiomyocytes were obtained from the rat. The neonatal cardiomyocytes, which lack many of the adult cardiomyocyte characteristics, are different from the HL-1 cells (Claycomb *et al* 1998). Since the HL-1 cell line maintains the phenotype and contractile activity of differentiated cardiomyocyte, they have been used in various pathophysiological studies including hypoxia, hyperglycaemia and apoptosis (White *et al* 2004). It is possible that the HL-1 cells may be better suited for the cellular study of cardiac physiology.

K^+ channels contribute to both electrical and mechanical properties of the heart, and it is believed that activations of certain K^+ channels play a significant role in cardioprotection. KCOs are able to manifest cardioprotection by shortening the cardiac action potential and thus reserving energy consumption in response to hypoxia and ischaemia, thereby maintaining cardiac contractile functions (Crestanello *et al* 2000). The effect of KCOs on metabolically compromised cells is probably due to the opening of K_{ATP} channels since the protective effect is known to diminish in the presence of a K_{ATP} channel blocker glibenclamide (Crestanello *et al* 2000, Critz *et al* 1997, Matar *et al* 2000). The K_{ATP} channels can be further divided into two subtypes: one that is expressed in mitochondria, the mitochondrial K_{ATP} ($mitoK_{ATP}$) channel, and one that is located on the cell membrane, the sarcolemmal K_{ATP} ($sarK_{ATP}$) channel. The opening of $mitoK_{ATP}$ channels conserves energy and preserves mitochondrial function (Takeo and Nasa 1999), and this is believed to be one of the mechanisms in cardioprotection (Crestanello *et al* 2000, O'Rourke 2000). Similarly, the opening of $sarK_{ATP}$ channels also reduces the energy requirement of cells (Lin *et al* 2004, Matar *et al* 2000, Saltman *et al* 2000).

In this study, PIN pre-treated myocytes were able to overcome the challenge by both metabolic inhibitors, thus demonstrating the relationship between K^+ channel activation and cardioprotection. The ineffectiveness of SDZ pre-treated cells under the present metabolically compromised conditions was surprising, and this discrepancy may be due to different sites of action for PIN and SDZ or duration of inhibition or both. One important distinction that could account for this discrepancy is that the $sarK_{ATP}$ channel is responsible for the shortening of action potential duration during *acute* hypoxia, whereas the $mitoK_{ATP}$ channel is related to *chronic* hypoxia (Tamargo *et al* 2004). It is believed that PIN, a cyanoguanidine derivative, exerts its cardioprotective effect primarily via the opening of $sarK_{ATP}$ channels (Lin *et al* 2004, Matar *et al* 2000, Saltman *et al* 2000). In contrast, SDZ, a benzopyran derivative, and related compounds such as KR-31378 (Moon *et al* 2004), KR 466 (Jung *et al* 2003), BMS-191095 (Fischbach *et al* 2004, Neckar *et al* 2002) and bimakalim (Eells *et al* 2000) have all been shown to act specifically via the opening of $mitoK_{ATP}$ channels, which are more relevant to preconditioning and sustained hypoxia and ischaemia of the heart. It is very possible that while the potent hyperpolarization effect of SDZ alone could be observed, its protective effect on

metabolically compromised cardiomyocytes could not be seen because the present study only dealt with acute metabolic inhibition. Further studies are now underway in order to confirm the involvement of the sarK$_{ATP}$ channel in acute hypoxia by using selective K$_{ATP}$ channel antagonist (e.g. glibenclamide), mitoK$_{ATP}$ channel antagonist (e.g. 5-hydroxydecanoate) and sarK$_{ATP}$ channel antagonist (e.g. HMR-1098) (Eells *et al* 2000, Weyermann *et al* 2004). The understanding of this aspect could be very useful for the treatment of different types of hypoxia in the future.

The successful adoption of the present MEA system for detecting electrophysiological changes during a physiologically compromised state has many advantages over the conventional patch-clamp technique. The MEA system is easy to operate, and multiple electrophysiological recordings can be performed within a short period of time. In contrast, the patch-clamp is very time consuming to perform and can only provide electrophysiological information of up to a few cells at any one time. By considering the entire syncytium on an MEA, a more global change of cellular behaviour can be seen. Furthermore, controls (e.g. before hypoxic insult or any treatments) and repetitive long-term recordings (e.g. hypoxia-induced changes over time, effects of preconditioning, recovery or reperfusion injury) of the same cell(s) can be carried out, which is simply impossible with patch-clamp experiments. The success in mimicking a metabolically inhibited condition enables a high-throughput study of effects of drugs on cardiomyocytes with a controlled and known level of stress to be carried out. The ability of the present system to distinguish the effects of the two distinct groups of KCOs could have significant implication for the treatment of different types of hypoxia in the future. The application of the MEA can be enhanced and substantiated by incorporating biochemical and molecular studies of stress proteins that are involved in hypoxia and ischaemia in order to correlate the extent of electrophysiological change with the level of stress protein expression.

5. Conclusion

The present MEA system may be useful for the study of both long- and short-term effects of metabolic inhibition on cultured cardiomyocytes. The present metabolic inhibition model of the heart on the MEA may provide an easily adoptable and reliable platform for the discovery and study of cardioprotective drugs. Furthermore, the relative potency and the effect of different drugs of the same group could also be compared and elucidated using the present system easily.

Acknowledgments

This work is supported by an Earmarked Grant from the Research Grant Council of Hong Kong under the contract number 611205 and Helmholtz Association of National Research Centres, Germany. The authors would like to thank Norbert Wolters (IBN-2, Electronic Workshop) and Yi Zhang (IBN-2) for their technical expertise in designing the current MEA system. In addition, the authors would like to thank Frank Sommerhage for implementing the signal analysis software in MATLAB®. The measurements presented in this study were performed by Jessica K Y Law during a research stay at the Research Centre Jülich, Germany.

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