Biochemical and Biophysical Research Communications 418 (2012) 74–80

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Properties and functions of K_{ATP} during mouse perinatal development

Li Nie [a], Ming Tang [a], Ying Zeng [c], Huaping Jiang [a], Hangchuan Shi [a], Hongyan Luo [a], Xinwu Hu [a], Linlin Gao [a], Jiaoya Xi [a], Aifen Liu [a], Michael Reppel [b], Jürgen Hescheler [b], Huamin Liang [a,*]

[a] Department of Physiology, Tongji Medical College, Huazhong University of Science and Technology, Hangkong Road 13, Wuhan 430030, China
[b] Institute of Physiology, University of Cologne, Cologne, Germany
[c] Wuhan Union Hospital, Huazhong University of Science and Technology, China

ARTICLE INFO

Article history:
Received 21 December 2011
Available online 9 January 2012

Keywords:
Cardiogenesis
ATP-sensitive potassium channel
Mouse embryo
Acute hypoxia–ischemia
APD_{90}

ABSTRACT

Background: Prevailing data suggest that ATP-sensitive potassium channels (K_{ATP}) contribute to a surprising resistance to hypoxia in mammalian embryos, thus we aimed to characterize the developmental changes of K_{ATP} channels in murine fetal ventricular cardiomyocytes.
Methods: Patch clamp was applied to investigate the functions of K_{ATP}. RT-PCR, Western blot were used to further characterize the molecular properties of K_{ATP} channels.
Results: Similar K_{ATP} current density was detected in ventricular cardiomyocytes of late development stage (LDS) and early development stage (EDS). Molecular–biological study revealed the upregulation of Kir6.1/SUR2A in membrane and Kir6.2 remained constant during development. Kir6.1, Kir6.2, and SUR1 were detectable in the mitochondria without marked difference between EDS and LDS. Acute hypoxia–ischemia led to cessation of APs in 62.5% of tested EDS cells and no APs cessation was observed in LDS cells. $SarcK_{ATP}$ blocker glibenclamide rescued 47% of EDS cells but converted 42.8% of LDS cells to APs cessations under hypoxia-ischemic condition. $MitoK_{ATP}$ blocker 5-HD did not significantly influence the response to acute hypoxia–ischemia at either EDS or LDS. In summary, $sarcK_{ATP}$ played distinct functional roles under acute hypoxia-ischemic condition in EDS and LDS fetal ventricular cardiomyocytes, with developmental changes in $sarcK_{ATP}$ subunits. $MitoK_{ATP}$ were not significantly involved in the response of fetal cardiomyocytes to acute hypoxia–ischemia and no developmental changes of K_{ATP} subunits were found in mitochondria.

1. Introduction

K_{ATP} is a complex of inwardly-rectifying potassium channel subunits (Kir6.1 and Kir6.2) which form the channel pore and the sulfonylurea receptor subunits (SUR1 and SUR2) which confer a regulatory role [1]. Previous studies have shown that tissue-specific expressions of different K_{ATP} channel subunits contribute to their pharmacological properties [2–4]. Both $sarcK_{ATP}$ and $mitoK_{ATP}$ exist in the heart [5–7], and take important roles in cardioprotection [6,8–10]. At the molecular level, cardiac $sarcK_{ATP}$ channels are primarily composed of Kir6.2 and SUR2A subunits [11,12]. However, the constitutions of $mitoK_{ATP}$ are still unclear [9]. Both Kir6.1 and Kir6.2 are suspected to localize in mitochondria [13,14].

$SarcK_{ATP}$ channels gamer attention in the field of cardioprotection in ischemia, their opening leads to the cardiac action potential duration (APD) shortage, the calcium entry reduction thus preserves the energy stores of myocytes [15]. $MitoK_{ATP}$ channels are postulated to play an important role in hypoxia–ischemia [10] by triggering an increase in mitochondrial reactive oxygen species (ROS) production leading to gene transcription and cell growth [9]. Cardiac protection of ischemia is abolished by either $mitoK_{ATP}$ channel inhibitor [10,16,17] or $sarcK_{ATP}$ channel blocker [18,19].

Interestingly, mammalian embryos display a surprising resistance to hypoxia, their sensitivity to hypoxia during development is still controversial [20–22]. K_{ATP} is suspected to be involved in such developmental changes in the heart [23]. However, distinct developmental changes in K_{ATP} are reported by different research groups [10,24]. Therefore we aimed to study the properties and functions of K_{ATP} during cardiac development, and found the developmental changes in $sarcK_{ATP}$ subunits (Kir6.1, SUR2) except Kir6.2, the consistent expression of each $mitoK_{ATP}$ subunit (Kir6.1, Kir6.2 and SUR1). $SarcK_{ATP}$ played distinct functional roles under acute hypoxia-ischemic condition in EDS and LDS fetal ventricular cardiomyocytes. $MitoK_{ATP}$ were not significantly involved in the response of fetal cardiomyocytes to acute hypoxia–ischemia.

* Corresponding author. Fax: +86 27 83691903.
E-mail address: lianghuamin@hotmail.com (H. Liang).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.12.133

2. Methods and materials

2.1. Harvesting and isolation of embryonic ventricular myocytes

Mice were superovulated and the hearts were harvested at the early embryonic stage (EDS, E9.5–E10.5) and late embryonic stage (LDS, E16.5–E17.5) [25,26]. Ventricles were enzymatically dissociated to get single cells as previously described [27,28]. The isolated cells were plated on sterile gelatin-coated glass cover slips, cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) containing 20% fetal bovine serum (FBS, Gibco) and kept in the incubator for 24 h. Spontaneously beating cardiomyocytes were used for functional studies.

2.2. RT-PCR

The ventricles of mouse embryos were rapidly removed and flash-frozen in −80 °C to avoid RNA degradation. Total RNA was prepared using Trizol method [13]. The final RNA pellet was resuspended in diethylpyrocarbonate-treated water or in RNA storage solution. Reverse transcription was carried out with oligo (dT) primers, using superscript first-strand cDNA synthesis kit (Invitrogen, USA). PCR was performed using 1 μl of the RT reaction as the template. The cycling conditions consisted of an initial denaturing time of 5 min (at 95 °C) followed by 29 cycles of denaturing at 95 °C for 30 s, annealing at (Tm-3) of each gene for 30 s, and extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. PCR products (10 μl) were separated by agarose gel (2%) electrophoresis and visualized by staining with gold view. Primers used for genes of interest in RT-PCR were listed in Table 1.

2.3. Western blotting

Total and sarcolemmal protein extracts of EDS or LDS ventricular samples were prepared as described previously [13]. Mitochondria were collected by discontinuous percoll gradient purification using a mitochondria subtract kit (Solabia Co., China). Sarcolemmal or mitochondrial proteins were fractioned on SDS–polyacrylamide gels and transferred to PVDF membranes. After incubation for 1 h in TBS-Tween blocking solution with 5% nonfat milk, proteins were incubated overnight at 4 °C with primary antibody against Kir6.1 (1:500, rabbit anti-mouse, ab80972), Kir6.2 (1:500, rabbit anti-mouse, ab79171), SUR1 (rabbit anti-mouse, 1:500, ab32844), or ABCC9 (1:500, rabbit anti-mouse, ab84299) in TBS-Tween containing 2% BSA. Membranes were washed and incubated for 1 h at room temperature with secondary antibodies (goat anti-rabbit IgG 1:2000, Proteintech Group, USA). Bound antibodies were detected using enhanced luminal and oxidizing reagents as specified by the manufacturer (ECL, Amersham Biosciences).

Table 1
Primer sequences used for PCR to detect mRNA levels of each K_{ATP} subunit.

Species	Gene bank	Pairs	Primer sequences	Product size (bp)
Kir 6.1	NM_008428	F	5′-ACCAGAATTCTCTGCGG-3′	297
		R	5′-GCCCTGAACTGGTGAGT-3′	
Kir 6.2	NM_001204411	F	5′-TTGGAAGGCGTGGTAGAAAC-3′	312
		R	5′-GGACAAGGAATCTGGAGAGA-3′	
SUR 1	NM_001044720	F	5′-GCCTTCGTGAGAAAGACCAG-3′	217
		R	5′-GAAGCTTCTCCGGTTTGTCA-3′	
SUR 2	NM_011511	F	5′-CCATCATCAGTGTTCAAAAGC-3′	148
		R	5′-GGCTGCTTCCTGTTTATTGG-3′	

2.4. Electrophysiological recordings

Action potentials (APs) and membrane currents were recorded with conventional whole-cell patch-clamp technique using an Axopatch 200A amplifier and a Digital 1200 interface controlled by PCLAMP 9.0 software. Currents were filtered at 1 kHz and sampled at 2 kHz. Data were analyzed using Clampfit software (Axon Instruments, USA). Patch pipettes (2–3 MΩ tip resistance) were fabricated from borosilicate capillaries using an electrode puller (700C, Japan). All patch-clamp experiments were performed at 35 °C. Cardiomyocytes were superfused with the normal Tyrode's solution containing (mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10, D-glucose 10 (pH 7.4 with NaOH). Patch pipettes were filled with the internal solution (mM): KCl 50, K-asparate 80, EGTA 10, HEPES 10, Na_2ATP 3.0, $MgCl_2$ 1.0 (pH 7.4 with KOH). Hypoxia–ischemia solution was established by replacing D-glucose with 2-deoxyglucose and bubbled with 100% nitrogen [29]. Since increased extracellular K^+ concentration increases potassium conductance [30], the normal Tyrode's solution was modified as following to record K_{ATP} current (mM): KCl 30, NaCl 115, $CaCl_2$ 1.8, $MgCl_2$ 1.0, HEPES 10, D-glucose 10, EGTA 1.0, $CdCl_2$ 0.5 (pH 7.4 with NaOH). A selective K_{ATP} opener, pinacidil (20 μM) (Sigma Chemical Co., USA) was applied to activate K_{ATP} channel under normal condition. K_{ATP} was further identified as the 10 μM glibenclamide (Sigma Chemical Co., USA) sensitive current. Currents were recorded at a holding potential of −40 mV to inactivate the voltage-gated Na^+ channels, followed by a testing potential at −70 mV for 1 s.

2.5. Data analysis and statistical analysis

For the analysis of individual electrophysiological experiments the control data were obtained by averaging APs recorded in the last five seconds before the respective treatment using AP analysis software programmed by Dr. Philipp Sasse (University of Bonn, Germany). Data of the experimental group were obtained from the last five seconds of treatment. If halt of APs occurred, action potential duration (APD) in the experimental group was obtained from the last five seconds before AP halt. Data were presented as mean ± SEM, with N represented the number of experiments or cells for analysis. Student's paired or unpaired t test was used where applicable. A value of $^{*}p < 0.05$ or $^{\#}p < 0.05$ was considered statistically significant.

3. Results

3.1. Functions of sarcK_{ATP} under physiological condition

K_{ATP} specific opener pinacidil (20 μM) and sarcK_{ATP} specific blocker glibenclamide (10 μM) were applied to investigate the functional role of K_{ATP} under physiological condition. Glibenclamide had no effects on APs recorded in both EDS and LDS cardiomyocytes (Fig. 1A), indicating the inactivation status of sarcK_{ATP} under physiological condition. Meanwhile, K_{ATP} of similar current density was elicited by pinacidil in EDS and LDS cells (EDS: −15.0 ± 3.0 pA/pF, $N = 15$; LDS: −16.0 ± 3.8 pA/pF, $N = 18$, $p > 0.05$ EDS vs. LDS) (Fig. 1B).

3.2. Different reactions to acute hypoxia-ischemia during development

Exposure to acute hypoxic-ischemic condition led to a halt of APs in 62.5% of tested EDS cardiomyocytes ($p < 0.05$, $N = 10$) while cells persisted in beating in all tested LDS cells ($N = 10$). Upon reoxygenation, the effect was largely restored to pre-anoxia (Fig. 2A). The hypoxia induced APD shortening, similarly at EDS

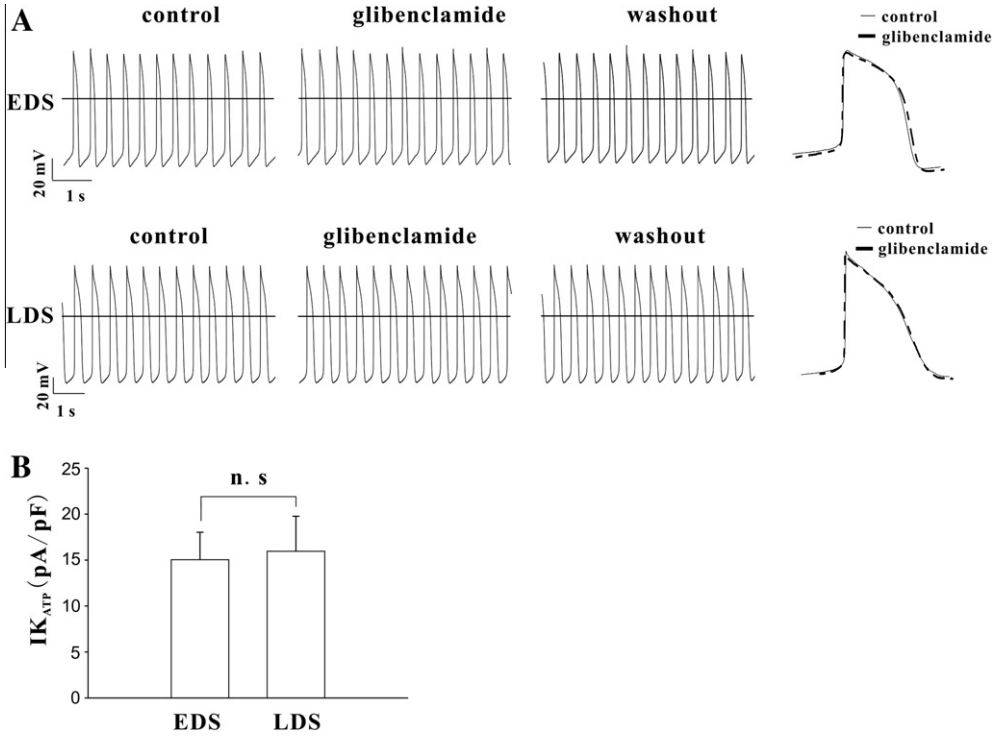

Fig. 1. K_{ATP} current and its role under normal condition in fetal ventricular cardiomyocytes. (A) Effects of 10 μM glibenclamide on APs recorded in EDS cells (upper panel) and LDS cells (lower panel). (B) 10 μM pinacidil elicited K_{ATP} current density in EDS cells and LDS cells. n.s insignificantly changed.

(by 21.25 ± 3.6%, $N = 7$) and LDS (by 25.64 ± 4.8%, N = 7) ($p > 0.05$ EDS vs. LDS), obvious hyperpolarization of MDP was observed at LDS (3.95 ± 0.6 mV, $N = 14$, $p < 0.05$) while slightly at EDS (1.1 ± 0.2 mV, $N = 10$, $p > 0.05$) (Fig. 2A, Table 2). Present study indicated that EDS cardiomyocytes were more sensitive to acute hypoxia–ischemia than LDS cells.

3.3. Functions of sarcK_{ATP} under acute hypoxia-ischemic condition

Generally accepted sarcK_{ATP} blocker glibenclamide (10 μM) was applied in the hypoxia–ischemia solution to investigate functional contribution of sarcK_{ATP} to the above responses to acute hypoxia–ischemia. Contrary effects of glibenclamide were revealed in EDS and LDS ventricular cells. The additional application of glibenclamide led to APs cessation in less EDS cells (33.3% of tested EDS, $p < 0.05$) whereas elevated the incidence of APs cessation to 42.8% at LDS ($N = 10$, $p < 0.05$). In parallel, glibenclamide restored the APD shortening ($N = 12$, $p < 0.05$) at EDS while not at LDS. Moreover, glibenclamide reversed acute hypoxia–ischemia induced MDP hyperpolarization at LDS ($p < 0.05$) and did not significantly changed MDP at EDS (Fig. 2B, Table 2). These data suggested the distinct functional roles of sarcK_{ATP} in acute hypoxia–ischemia during development.

3.4. Functions of mitoK_{ATP} under acute hypoxia-ischemic condition

MitoK_{ATP} blocker 5-HD (100 μM) was applied in the hypoxia–ischemia solution to investigate functional contribution of mito-K_{ATP} to the above responses to acute hypoxia–ischemia. Additional application of 5-HD led to APs cessation in 60% of EDS cells whereas no AP cessation occurred at LDS, which were similar to the direct responses to acute hypoxia–ischemia. Furthermore, 5-HD slightly attenuated the response of APD shortening and hyperpolarization of MDP to acute hypoxia–ischemia at EDS and LDS (Fig. 2C, Table 2), however, without significant differences ($p > 0.05$). These suggested the minor contribution of mitoK_{ATP} to the response of EDS or LDS cell to acute hypoxia–ischemia.

3.5. Developmental changes in K_{ATP} channel subunits

The electrophysiological data strongly suggested the distinct contribution of K_{ATP} channels in the above response of EDS/LDS cells under physiological or acute hypoxia–ischemic condition Thus expression of each K_{ATP} channel subunit in EDS and LDS cardiomyocytes were studied. As demonstrated by RT-PCR data, expressions of Kir6.1, SUR1 and SUR2 mRNA were low at EDS and up-regulated at LDS ($N = 4$, $p < 0.05$ EDS vs. LDS). No significant changes in Kir6.2 from EDS to LDS were detected ($N = 4$, $p > 0.05$ EDS vs. LDS) (Fig. 3A). Total protein extracts from EDS and LDS ventricles displayed similar developmental changes of each subunit to that revealed at mRNA level (Fig. 3B). At EDS Kir6.2 was expressed slightly higher than Kir6.1, whereas at LDS Kir6.1 was predominantly expressed. SUR2A seemed to take a major part in regulatory subunits.

Special analysis on the sarcolemmal protein extract from EDS and LDS ventricles displayed developmental changes of sarcK_{ATP} subunits. In each case, the expression level of Kir6.1, SUR2A was lower in the EDS membrane than that in the LDS membrane ($N = 3$, $p < 0.05$ EDS vs. LDS). Kir6.2 expression was not significantly up-regulated ($N = 3$, $p > 0.05$ EDS vs. LDS). Moreover, SUR1 was not found at EDS or LDS (Fig. 3D).

Western blotting data gave an additional explanation of each K_{ATP} channel subunit in mitochondria. The expressions of Kir6.1, Kir6.2, and SUR1 in the mitochondria were detected, while SUR2A was undetectable. No marked difference was found in the expression of Kir6.1, Kir6.2 and SUR1 between EDS and LDS ($N = 3$, $p > 0.05$ EDS vs. LDS) (Fig. 3D).

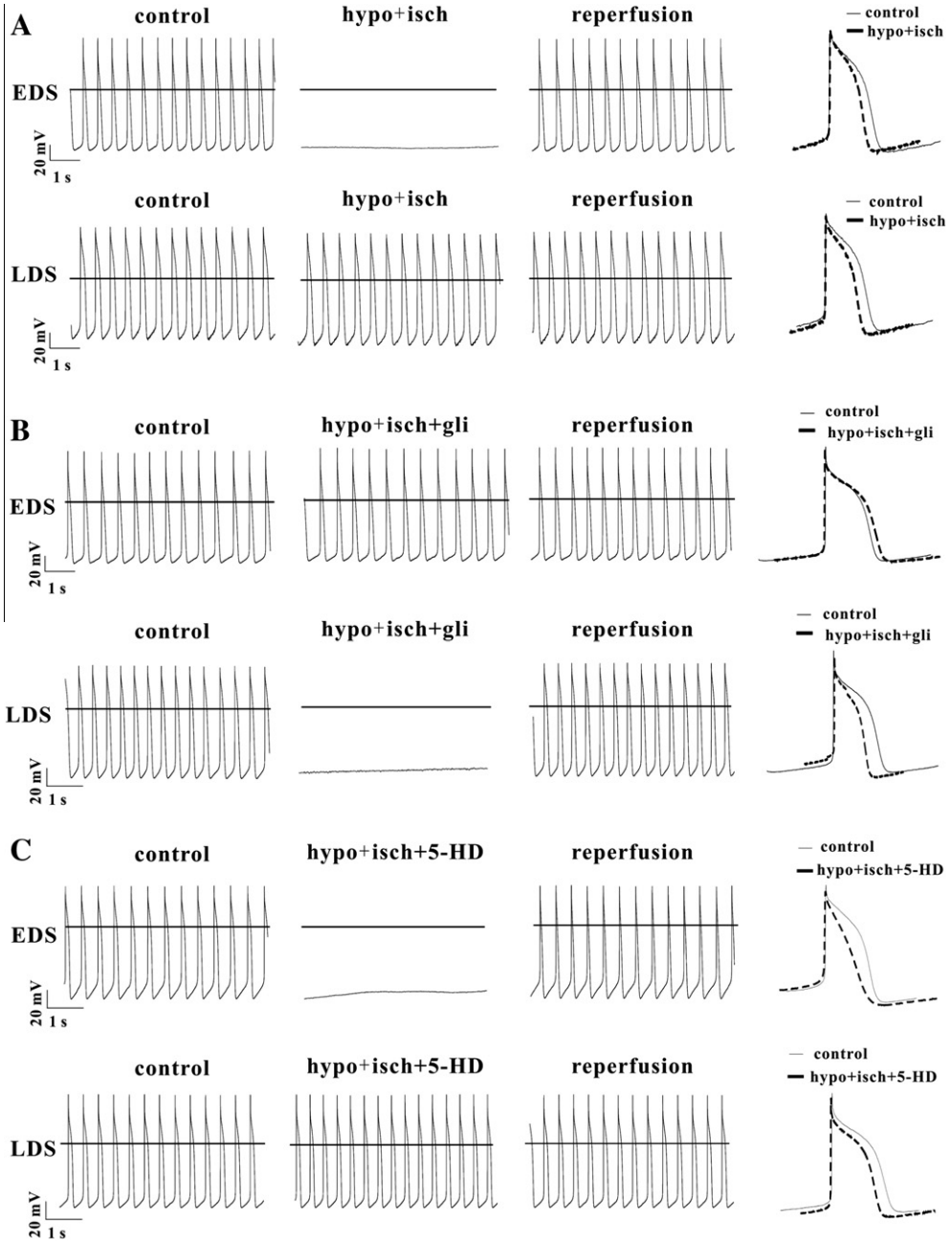

Fig. 2. Responses to acute hypoxia–ischemia and functional roles of K_{ATP} channel in such responses. (A) Distinct sensitivity of EDS and LDS cells to acute hypoxia–ischemia. (B) Effects of sarcK_{ATP} blocker glibenclamide on response of fetal cardiomyocytes to acute hypoxia–ischemia. (C) Effects of mitoK_{ATP} blocker 5-HD on response of fetal cardiomyocytes to acute hypoxia–ischemia.

Table 2
Changes in action potential parameters during hypoxia–ischemia (H) in the absence and presence of glibenclamide (H + gli) or 5-HD (H + 5-HD).

	EDS			LDS		
	H	H + gli	H + 5-HD	H	H + gli	H + 5-HD
AP cessation (%)	62.5*	33.3#	60	0	42.8#	0
MDP (by mV)	1.1 ± 0.2	0.6 ± 0.1	0.8 ± 0.3	3.95 ± 0.6*	−1.54 ± 0.3#	2.92 ± 0.4
APD_{90} (%)	21.25 ± 3.6*	−7.9 ± 1.9#	17.66 ± 4.3	25.64 ± 4.8*	23.69 ± 3.2	20.02 ± 4.3

H: acute hypoxia–ischemia, gli: glibenclamide.
* $p < 0.05$ vs. control.
# $p < 0.05$ vs. H.

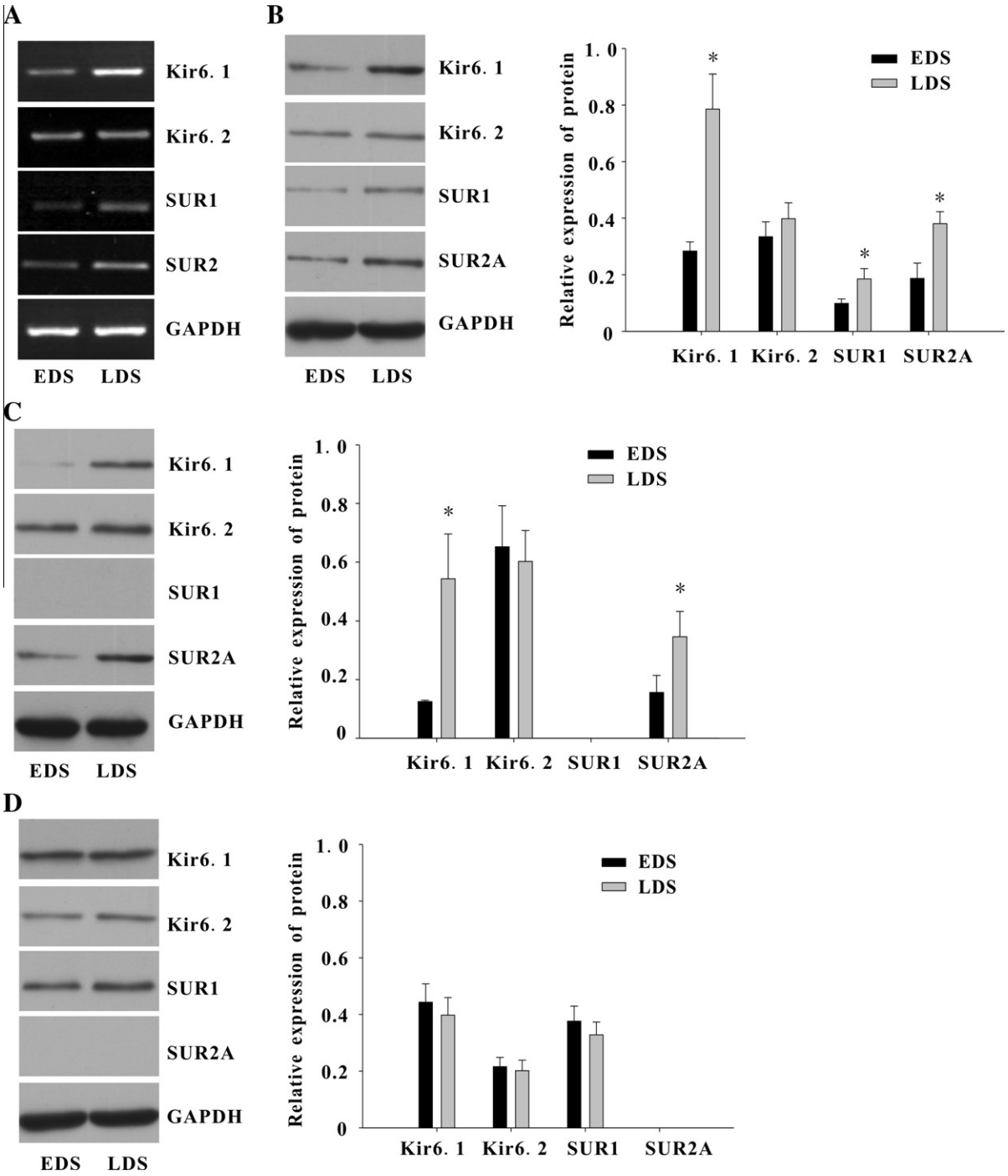

Fig. 3. Molecular analysis of K_{ATP} channel subunits in fetal ventricles. (A) Expressions of Kir6.1, Kir6.2, SUR1, and SUR2 at mRNA level in EDS and LDS ventricular tissues. (B) Expressions of Kir6.1, Kir6.2, SUR1, and SUR2A at protein level in EDS and LDS ventricles. (C) Expressions of Kir6.1, Kir6.2, SUR1, and SUR2A at protein level in EDS and LDS ventricular membrane. (D) Expressions of Kir6.1, Kir6.2, SUR1, and SUR2A at protein level in EDS and LDS ventricular mitochondria. $^{*}p < 0.05$ EDS vs. LDS.

4. Discussion

The present study revealed that (1) $SarcK_{ATP}$ current density remained constant during development and kept inactivation status under physiological condition. (2) EDS cardiomyocytes were more sensitive to acute hypoxia–ischemia than LDS cells. $SarcK_{ATP}$ blocker glibenclamide rescued most EDS cells under hypoxia-ischemic condition by reversing the shortage of APD. At LDS, glibenclamide increased the sensitivity of cells to acute hypoxia–ischemia by reversing the MDP hyperpolarization. $MitoK_{ATP}$ blocker 5-HD had little effect on the sensitivity of fetal cardiomyocytes to acute hypoxia–ischemia. (3) Developmental changes were observed in $sarcK_{ATP}$ subunits while not in $mitoK_{ATP}$ subunits. SUR1 was observed on mitochondria and undetectable on cell membrane, SUR2A was localizing on cell membrane and absent on mitochondria whereas.

In consistent with previous investigation, K_{ATP} opener pinacidil elicited similar K_{ATP} current density in EDS and LDS murine ventricular cardiomyocytes [24]. Moreover, analysis on sarcolemmal protein extracts revealed that the $sarcK_{ATP}$ subunits Kir6.1 and

SUR2A up-regulated during fetal development, however, Kir6.2 remained constant. The expression of Kir6.2 was predominantly higher than Kir6.1 at EDS, and SUR2A seemed to be the only regulatory subunit in sarcK_{ATP}. Although the well accepted model of sarcK_{ATP} was Kir6.2/SUR2A with stoichiometry of 4:4 [11,31], the impaired expression level of Kir6.2 and SUR2A strongly suggested involvement of Kir6.1 in fetal sarcK_{ATP}, as postulated previously that heteromultimeric interaction between Kir6.1 and Kir6.2 subunits [32–34] may occur to form the immature heart K_{ATP} channels [35]. Overexpression of SUR2A gene resulted in increase in level of sarcK_{ATP} channels [13], thus upregulation of SUR2A in fetal development might favor the consistent whole cell K_{ATP} current density.

Opening of K_{ATP} during myocardial ischemia caused membrane hyperpolarization, shortened the APD and decreased Ca^{2+} influx through L-type channels, thus prevented cardiac Ca^{2+} overload and increased cell survival [36]. This could well explain our observation that sarcK_{ATP} blocker glibenclamide converted the beating LDS cells to APs cessation under acute hypoxic–ischemic condition. However, the present study demonstrated a contrary effect of sarcK_{ATP} opening on ventricular cardiomyocytes. Glibenclamide rescued most EDS cells under hypoxia-ischemic condition by reversing the shortage of APD. This might due to the less expression of L-type calcium channel in EDS cells [37,38] and Ca^{2+} influx were essential for EDS cell excitability [37,39,40]. APD shortening decreased the Ca^{2+} influx therefore the Ca^{2+} influx were not sufficient to initiate the AP generation.

Some reports revealed the upregulation of each K_{ATP} subunit during fetal cardiac development [35] and the biophysical and pharmacological properties of that differed from that in the adult [41,42]. All these reports provided neither the molecular biochemical evidence of both sarcK_{ATP} and mitoK_{ATP}, nor the K_{ATP} current density during fetal ventricle development as detailed in the present study. The western blot data showed the absence of SUR1 in sarcK_{ATP} subunits in fetal ventricles, with the expression of SUR1 elevated in ventricles during development but remained constant in mitochondria, suggesting some unknown localization of SUR1. Furthermore, our data revealed that Kir6.1/Kir6.2 and SUR1 could be the constitutions of mitoK_{ATP}. 5-HD specially blocked channels formed by SUR1/Kir6.1 or Kir6.2 [43]. Thus data in this study excluded the essential role of mitoK_{ATP} in the response of fetal cardiomyocytes to acute hypoxia–ischemia.

Several studies reported that mammalian embryos showed a surprising degree of resistance to hypoxia, embryos became more sensitive to hypoxia during the middle of gestation in rat [20,22,44]. Other study showed that EDS cardiomyocytes were more sensitive to acute hypoxia–ischemia, as demonstrated in an early chicken embryo model [21]. Sensitivity to hypoxia–ischemia during development was still disputed. The previous findings were under hypoxic condition *in vivo*, our work firstly applied patch clamp to investigate the electrophysiology properties under acute hypoxia at cell level, and provided a proof that EDS cardiomyocytes were more sensitive to acute hypoxia–ischemia than LDS cells.

It is the first study that reveals the functional roles of K_{ATP} channels in fetal cardiomyocytes under acute hypoxia-ischemic condition. This gives a new insight into cardiogenesis as well as the new knowledge on K_{ATP} channels.

Acknowledgments

This work is supported by grants from National Nature Science Foundation of China (No.: 30700262, No.: 81100818 and No.: 31100828), a Doctoral Fund of Ministry of Education of China (No.: 20070487012), and a Grant assisted by the Fundamental Research Funds for the Central Universities (HUST: 2010MS052) for Huamin Liang.

References

[1] N. Inagaki, T. Gonoi, J.P.T. Clement, N. Namba, J. Inazawa, G. Gonzalez, L. Aguilar-Bryan, S. Seino, J. Bryan, Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor, Science 270 (1995) 1166–1170.
[2] J.P. Arena, R.S. Kass, Activation of ATP-sensitive K channels in heart cells by pinacidil: dependence on ATP, Am. J Physiol. 257 (1989) H2092–2096.
[3] N. Inagaki, Y. Tsuura, N. Namba, K. Masuda, T. Gonoi, M. Horie, Y. Seino, M. Mizuta, S. Seino, Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart, J. Biol. Chem. 270 (1995) 5691–5694.
[4] W.A. Chutkow, M.C. Simon, M.M. Le Beau, C.F. Burant, Cloning, tissue expression, and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle, and vascular KATP channels, Diabetes 45 (1996) 1439–1445.
[5] H. Hu, T. Sato, J. Seharaseyon, Y. Liu, D.C. Johns, B. O'Rourke, E. Marban, Pharmacological and histochemical distinctions between molecularly defined sarcolemmal KATP channels and native cardiac mitochondrial KATP channels, Mol. Pharmacol. 55 (1999) 1000–1005.
[6] B. O'Rourke, Evidence for mitochondrial K^+ channels and their role in cardioprotection, Circ. Res. 94 (2004) 420–432.
[7] G.C. Kane, X.K. Liu, S. Yamada, T.M. Olson, A. Terzic, Cardiac KATP channels in health and disease, J. Mol. Cell Cardiol. 38 (2005) 937–943.
[8] S. Sanada, M. Kitakaze, H. Asanuma, K. Harada, H. Ogita, K. Node, S. Takashima, Y. Sakata, M. Asakura, Y. Shinozaki, H. Mori, T. Kuzuya, M. Hori, Role of mitochondrial and sarcolemmal K(ATP) channels in ischemic preconditioning of the canine heart, Am. J. Physiol. Heart Circ. Physiol. 280 (2001) H256–263.
[9] D.B. Foster, J.J. Rucker, E. Marban, Is Kir6.1 a subunit of mitoK(ATP)?, Biochem Biophys. Res. Commun. 366 (2008) 649–656.
[10] J.D. McCully, S. Levitsky, Mitochondrial ATP-sensitive potassium channels in surgical cardioprotection, Arch. Biochem. Biophys. 420 (2003) 237–245.
[11] N. Inagaki, T. Gonoi, J.P. Clement, C.Z. Wang, L. Aguilar-Bryan, J. Bryan, S. Seino, A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels, Neuron 16 (1996) 1011–1017.
[12] A.P. Babenko, G. Gonzalez, L. Aguilar-Bryan, J. Bryan, Reconstituted human cardiac KATP channels: functional identity with the native channels from the sarcolemma of human ventricular cells, Circ. Res. 83 (1998) 1132–1143.
[13] Q. Du, S. Jovanovic, A. Clelland, A. Sukhodub, G. Budas, K. Phelan, V. Murray-Tait, L. Malone, A. Jovanovic, Overexpression of SUR2A generates a cardiac phenotype resistant to ischemia, FASEB J. 20 (2006) 1131–1141.
[14] H. Singh, D. Hudman, C.L. Lawrence, R.D. Rainbow, D. Lodwick, R.I. Norman, Distribution of Kir6.0 and SUR2 ATP-sensitive potassium channel subunits in isolated ventricular myocytes, J. Mol. Cell Cardiol. 35 (2003) 445–459.
[15] M. Suzuki, N. Sasaki, T. Miki, N. Sakamoto, Y. Ohmoto-Sekine, M. Tamagawa, S. Seino, E. Marban, H. Nakaya, Role of sarcolemmal K(ATP) channels in cardioprotection against ischemia/reperfusion injury in mice, J. Clin. Invest. 109 (2002) 509–516.
[16] K.D. Garlid, P. Dos Santos, Z.J. Xie, A.D. Costa, P. Paucek, Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K(+) channel in cardiac function and cardioprotection, Biochim. Biophys. Acta 1606 (2003) 1–21.
[17] Y. Liu, T. Sato, B. O'Rourke, E. Marban, Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection?, Circulation 97 (1998) 2463–2469.
[18] G.R. Budas, S. Jovanovic, R.M. Crawford, A. Jovanovic, Hypoxia-induced preconditioning in adult stimulated cardiomyocytes is mediated by the opening and trafficking of sarcolemmal KATP channels, FASEB J. 18 (2004) 1046–1048.
[19] T.P. Flagg, C.G. Nichols, Sarcolemmal K(ATP) channels: what do we really know?, J Mol. Cell Cardiol. 39 (2005) 61–70.
[20] J. Hoerter, Changes in the sensitivity to hypoxia and glucose deprivation in the isolated perfused rabbit heart during perinatal development, Pflugers Arch. 363 (1976) 1–6.
[21] J. Altimiras, L. Phu, Lack of physiological plasticity in the early chicken embryo exposed to acute hypoxia, J. Exp. Zool. 286 (2000) 450–456.
[22] W.S. Webster, D. Abela, The effect of hypoxia in development, Birth Defects Res. C Embryo Today 81 (2007) 215–228.
[23] H.J. Ranki, R.M. Crawford, G.R. Budas, A. Jovanovic, Ageing is associated with a decrease in the number of sarcolemmal ATP-sensitive K+ channels in a gender-dependent manner, Mech. Ageing Dev. 123 (2002) 695–705.
[24] O. Gryshchenko, I.R. Fischer, M. Dittrich, S. Viatchenko-Karpinski, J. Soest, M.M. Bohm-Pinger, P. Igelmund, B.K. Fleischmann, J. Hescheler, Role of ATP-dependent K(+) channels in the electrical excitability of early embryonic stem cell-derived cardiomyocytes, J. Cell Sci. 112 (Pt 17) (1999) 2903–2912.
[25] H.M. Liang, M. Tang, C.J. Liu, H.Y. Luo, Y.L. Song, X.W. Hu, J.Y. Xi, L.L. Gao, B. Nie, S.Y. Li, L.L. Lai, J. Hescheler, Muscarinic cholinergic regulation of L-type calcium channel in heart of embryonic mice at different developmental stages, Acta Pharmacol. Sin. 25 (2004) 1450–1457.
[26] G.L. Song, M. Tang, C.J. Liu, H.Y. Luo, H.M. Liang, X.W. Hu, J.Y. Xi, L.L. Gao, B. Fleischmann, J. Hescheler, Developmental changes in functional expression and beta-adrenergic regulation of I(f) in the heart of mouse embryo, Cell. Res. 12 (2002) 385–394.
[27] B.K. Fleischmann, Y. Duan, Y. Fan, T. Schoneberg, A. Ehlich, N. Lenka, S. Viatchenko-Karpinski, L. Pott, J. Hescheler, B. Fakler, Differential subunit

composition of the G protein-activated inward-rectifier potassium channel during cardiac development, J. Clin. Invest. 114 (2004) 994–1001.
[28] A. Liu, M. Tang, J. Xi, L. Gao, Y. Zheng, H. Luo, X. Hu, F. Zhao, M. Reppel, J. Hescheler, H. Liang, Functional characterization of inward rectifier potassium ion channel in murine fetal ventricular cardiomyocytes, Cell Physiol. Biochem. 26 (2010) 413–420.
[29] S. Shigematsu, M. Arita, Anoxia-induced activation of ATP-sensitive K+ channels in guinea pig ventricular cells and its modulation by glycolysis, Cardiovasc. Res. 35 (1997) 273–282.
[30] Z. Fan, K. Nakayama, M. Hiraoka, Pinacidil activates the ATP-sensitive K+ channel in inside-out and cell-attached patch membranes of guinea-pig ventricular myocytes, Pflugers Arch. 415 (1990) 387–394.
[31] B. Schwappach, N. Zerangue, Y.N. Jan, L.Y. Jan, Molecular basis for K(ATP) assembly: transmembrane interactions mediate association of a K+ channel with an ABC transporter, Neuron 26 (2000) 155–167.
[32] Y. Cui, J.P. Giblin, L.H. Clapp, A. Tinker, A mechanism for ATP-sensitive potassium channel diversity: Functional coassembly of two pore-forming subunits, Proc. Natl. Acad. Sci. USA 98 (2001) 729–734.
[33] D.J. Pountney, Z.Q. Sun, L.M. Porter, M.N. Nitabach, T.Y. Nakamura, D. Holmes, E. Rosner, M. Kaneko, T. Manaris, T.C. Holmes, W.A. Coetzee, Is the molecular composition of K(ATP) channels more complex than originally thought?, J Mol. Cell. Cardiol. 33 (2001) 1541–1546.
[34] Y. Kono, M. Horie, M. Takano, H. Otani, L.H. Xie, M. Akao, K. Tsuji, S. Sasayama, The properties of the Kir6.1-6.2 tandem channel co-expressed with SUR2A, Pflugers Arch. 440 (2000) 692–698.
[35] A. Morrissey, L. Parachuru, M. Leung, G. Lopez, T.Y. Nakamura, X. Tong, H. Yoshida, S. Srivastiva, P.D. Chowdhury, M. Artman, W.A. Coetzee, Expression of ATP-sensitive K+ channel subunits during perinatal maturation in the mouse heart, Pediatr. Res. 58 (2005) 185–192.
[36] H. Gogelein, Inhibition of cardiac ATP-dependent potassium channels by sulfonylurea drugs, Curr. Opin. Investig. Drugs 2 (2001) 72–80.
[37] S. Kawano, R.L. DeHaan, Developmental changes in the calcium currents in embryonic chick ventricular myocytes, J. Membr. Biol. 120 (1991) 17–28.
[38] X. Yan, S. Gao, M. Tang, J. Xi, L. Gao, M. Zhu, H. Luo, X. Hu, Y. Zheng, J. Hescheler, H. Liang, Adenylyl cyclase/cAMP-PKA-mediated phosphorylation of basal L-type Ca(2+) channels in mouse embryonic ventricular myocytes, Cell Calcium 50 (2011) 433–443.
[39] H. Liang, M. Halbach, T. Hannes, B.K. Fleischmann, M. Tang, H. Schunkert, J. Hescheler, M. Reppel, Electrophysiological basis of the first heart beats, Cell Physiol. Biochem. 25 (2010) 561–570.
[40] S. Viatchenko-Karpinski, B.K. Fleischmann, Q. Liu, H. Sauer, O. Gryshchenko, G.J. Ji, J. Hescheler, Intracellular Ca2+ oscillations drive spontaneous contractions in cardiomyocytes during early development, Proc. Natl. Acad. Sci. USA 96 (1999) 8259–8264.
[41] F. Chen, G.T. Wetzel, W.F. Friedman, T.S. Klitzner, ATP-sensitive potassium channels in neonatal and adult rabbit ventricular myocytes, Pediatr. Res. 32 (1992) 230–235.
[42] S. Jovanovic, A. Jovanovic, Sarcolemmal K(ATP) channels in ageing, Ageing Res. Rev. 3 (2004) 199–214.
[43] Y. Liu, G. Ren, B. O'Rourke, E. Marban, J. Seharaseyon, Pharmacological comparison of native mitochondrial K(ATP) channels with molecularly defined surface K(ATP) channels, Mol. Pharmacol. 59 (2001) 225–230.
[44] A. Vleugels, E. Carmeliet, S. Bosteels, M. Zaman, Differential effects of hypoxia with age on the chick embryonic heart. Changes in membrane potential, intracellular K and Na, K efflux and glycogen, Pflugers Arch. 365 (1976) 159–166.