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Corticosteroids and Aldose Reductase inhibitor Epalrestat Modulates Cardiac Action Potential via Kv β 1.1 (AKR6A8) subunit of Voltage Gated Potassium Channel

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Abstract

We previously demonstrated the role of Kv β 1.1 subunit of voltage activated potassium channel in heart for its sensory roles in detecting changes in NADH/NAD and modulation of ion channel. However the pharmacological role for the association of Kv β 1 via its binding to ligands such as cortisone and its analogs remains unknown. Therefore, we investigated the significance of Kv β 1.1 binding to cortisone analogs and AR inhibitor epalrestat. In addition the aldose reductase (AR) inhibitor epalrestat was identified as a pharmacological target and modulator of cardiac activity via binding to the Kv β 1 subunit. Using a combination of *ex vivo* cardiac electrophysiology and in silico binding, we identified that Kv β 1 subunit binds and interacts with epalrestat. To identify the specificity of the action potential changes, we studied the sensitivity of the action potential prolongation by probing the electrical changes in the presence of 4-aminopyridine and evaluated the specificity of pharmacological effects in the hearts from Kv β 1.1 knock out mouse. Our results show that pharmacological modulation of cardiac electrical activity by cortisone analogs and epalrestat is mediated by Kv β 1.1.

Keywords

Kv β subunit; heart; aldo-keto reductase; cortisone

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Disclosures:

Authors have no disclosures.

Ethical approval:

All institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee at the University of South Florida (Tampa, FL, USA) at which the studies were conducted.

Introduction

Glucocorticoids are utilized to manage autoimmune diseases and aid in controlling inflammation. However, glucocorticoids present with many cardiac effects including both beneficial as well as deleterious effects. Narayanan *et al.* demonstrated that a low dose treatment with dexamethasone improved contractile performance [1]. Furthermore, pre-treatment with methylprednisolone resulted in significant protection against ischemiareperfusion damage [2]. Chronic use of glucocorticoids however, has demonstrated adverse effects including hypertension and heart failure. In adult rats, dexamethasone given for 15 days resulted in left ventricular hypertrophy and increased systolic pressure [3]. While much of the previous work was focused on understanding the mechanism for glucocorticoid via the activation of glucocorticoid and mineralocorticoid receptors many questions remain in regards to factors that mediate the electrical effects of corticosteroids in the heart.

Previous research has demonstrated that glucocorticoids including cortisone, cortisone-21-acetate, and fluticasone binds Kv β subunit and dissociate the Kv β 1.1 subunits from the Kv channel (Kv1.x) thus removing the Kv β subunit mediated Kv channel inactivation [4, 5]. The Kv β subunits represent a unique class of aldoketo reductases (AKR) which bind and alter many different voltage-gated potassium channels (Kv) [6, 7]. Recently we demonstrated that global knockout of Kv β 1.1 in mouse resulted in significant prolongation of monophasic action potential as well at QTc [8]. Further, we also demonstrated that the binding of pyridine nucleotide to Kv β 1.1 subunits can modulate the gating potential of Kv4.2 a key player in the I_{to} current responsible for the initial phase of the repolarization in cardiac action potentials. The addition of lactate resulted in significant increase in intracellular NADH and led to increased rates of Kv4.2 inactivation cardiac action potential prolongation [7]. Therefore, we hypothesized that Kv β 1.1 can interact with cortisone and other ligands and regulate the cardiac action potential.

While cortisone demonstrated two binding sites for the Kv β subunit, it is the interface site or the β - β interaction and the T1 domain of the Kv channel which induces dissociation and alters Kv β inactivation [4]. Cortisone analogs were shown previously to interact and dissociate the Kv β subunits from Kv channel by binding to same sites [5]. This dissociation leads to abolished inactivation of Kv1.x channels in heterologous system; however the physiological consequences of cortisone and Kv β 1.1 dissociation remain unknown. Therefore, we tested the physiological role of cortisone and fluticasone on *ex vivo* Langendorff perfused hearts from control and Kv β 1.1 knockout hearts.

Kv β subunits belong to the aldo-keto reductase family and share common mechanisms and ancestral origins [9, 10]. Moreover, the Aldose Reductase (AR) inhibitor epalrestat, is utilized for diabetic neuropathy [11]. Previous research has demonstrated that AR inhibitors have profound cardiac effects preserving cardiac contractility after ischemia-reperfusion injury in diabetic and non-diabetic hearts [12, 13]. Clinically, treatment with aldose reductase for over a year in diabetic patients with neuropathy improved contractile function [14]. Therefore in the present study we investigated the role of Kv β 1.1 in modulating the cardiac action potential via its interactions with cortisone analogs and AR inhibitor epalrestat.

Material and Methods

Animals:

Mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA); Kv β 1.1 KO (global knockout) and control or WT mice (C57BL/6NJ)[7]. Mice 14–16 weeks of age (Males) were provided water and food *ad libitum*. All animal work was approved in advance by the Institutional Animal Care and Use Committee at the University of South Florida (Tampa, FL, USA).

Monophasic action potentials:

Monophasic action potentials (MAPs) were recorded from *ex vivo* heart preparations as reported before [7, 15]. Briefly, 1 mg heparin (180 USP, Sigma-Aldrich, St. Louis, MO, USA) was injected into the mice following euthanizing with Somnasol (Pentobarbital Sodium, 50 mg (kg body weight), Henry Schein Animal Health, Dublin, OH, USA) by i.p. injection. A bilateral thoracotomy was performed to remove the hearts which were then retrogradely perfused with Krebs-Hanseleit buffer (mM- NaCl 119, NaHCO₃ 25, KCl 4, KH₂PO₄ 1.2, MgCl₂ 1, CaCl₂ 1.8, D-glucose 10 and Sodium pyruvate 2, pH 7.4) at a constant flow rate of 2.0 ml/min and temperature of 37°C. MAP recordings were obtained from the left ventricular (LV) epicardial surface using a contact electrode (Harvard Apparatus, Holliston, MA, USA). MAP data was obtained after 10 minutes stabilization and acquired using 8 channel PowerLab system (AD Instruments, UK).

WT and KO MAPs were obtained to assess the activity in response to cortisone, fluticasone, eparlestat, and 4-aminopyridine (4-AP). Baseline MAPs from the LV were obtained with Krebs-Hanseleit buffer, subsequently hearts were then perfused for 20 min with a cortisone (500 μ M), fluticasone (100nM), eparlestat (100 μ M), and a combination of cortisone (500 μ M) and 4-AP (2mM) (8). MAP's were recorded using 8 channel PowerLab system (AD Instruments, UK).

Docking study:

In silico analysis for binding of human Kv β 2 (PDB: 1ZSX) to eparlestat ligand (Pubchem-CID 1549120) was performed by using Discovery Studio software. The crystal structure of Kv β 2 co-crystalizes with NADP⁺ at the active site which was utilized for ligand docking study.

Statistical analysis:

Statistical analyses were performed with Sigma Plot (v.11.0). When comparing two groups, an independent Student's t-test was used. Data are expressed as mean $\pm$ SEM; and p 0.05 were considered significant.

Results

Cortisone exposure significantly prolongs cardiac action potentials

Cortisone (500 μ M) exposure to *ex vivo* perfused WT hearts led to a significant increase in action potential durations at 20%, 50%, and 70% repolarization (Figure 1). However similar

exposure of cortisone to Kv β 1.1 KO hearts demonstrated no significant alternations in action potential durations when compared with baseline measurements or those prior to cortisone exposure (Figure 1C). Similar to previously published research, KO hearts demonstrated a significant increase in APD's at 20%, 50%, and 70% when compared with WT hearts at baseline.

Fluticasone exposure significantly alters cardiac action potentials

Acute exposure with fluticasone (100nM), which is a widely used steroid in respiratory illnesses, to WT hearts led to a significant prolongation in action potential durations at 20%, 50%, and 70% repolarization (Figure 2). However, fluticasone resulted in no significant prolongation in KO hearts. Similar baseline values were obtained between WT and KO demonstrating significant prolongation in KO compared with WT hearts.

AR inhibitor Epalrestat exposure prolongs cardiac action potentials

Epalrestat is an aldose reductase inhibitor; however its cardiovascular effects in Kv β 1.1 KO mice remain unknown. Acute exposure to epalrestat in WT hearts led to significant action potential prolongation at 20%, 50%, and 70% repolarization. Interestingly, KO hearts showed no significant increase in action potential durations as compared with baseline measurements (Figure 3).

Cortisone along with 4-aminopyridine significantly prolongs the cardiac action potential

To dissect the 4-AP sensitive portion of the cardiac action potential, we utilized 2 mM 4-amino pyridine to identify the cortisone effects and distinguish the 4-AP mediated effects. 4-AP exposure alone in WT or KO ex vivo hearts demonstrated significant prolongation of action potential durations at 20%, 50%, and 70% repolarization (Figure 4).

WT hearts perfused with cortisone showed significant action potential prolongation at APD 50 and APD 70, however the KO hearts failed to prolong the APD. However a combination of cortisone + 4-AP (500 μ M and 2 mM) demonstrated APD prolongation in both WT and KO hearts suggesting that 4-AP mediates the APD prolongation independent of Kv β 1.1 subunit (Figure 5). [16]

Epalrestat demonstrates binding interaction with the Kv β subunits

As shown in Fig 6A, the comparative alignment of sequences for human Kv β 2, rat Kv β 2, and mouse Kv β 1 shows conserved amino acid residues such as the cysteine (C234 human, 248 rat, and 282 mouse), following this, six amino acids after is noticed by lysine (240 human, 254 rat, 288 mouse) and tyrosine (241 human, 255 rat, and 289 mouse) and ten amino acids after that is an arginine (250 human, 264 rat and 297 mouse)(Fig 6A). The binding pocket created by the key amino acids have been previously demonstrated as an important pyridine nucleotide binding site in which the mutation of rat 264R prevents the NADPH molecule from binding with the Kv β subunit [17, 18]. Novel *in silico* docking in this study identifies that epalrestat interacts with the arginine forming a hydrogen bond thus preventing the necessary interaction between NADPH (shown overlaid in line format) and the Kv β 's arginine residue (Figure 6B). Further amino acid inspection of Kv β subunit and epalrestat demonstrates within this binding pocket the C5 and lysine 240 interact at a

distance of 1.8 and an angle of $\phi=127.074$, $\theta=157.152$ and energy 2.438 (Figure 6C). Epalrestat demonstrates novel *in silico* interactions here while also demonstrating profound physiological alterations within the ex vivo perfused heart in WT and in KO hearts.

Discussion

We identified major pharmacological role for Kv β 1 in the heart based on the modulation of cardiac electrical activity by cortisone, its analogs, and epalrestat (aldose reductase inhibitor). We report *in silico* binding of aldose reductase inhibitor epalrestat to Kv β 1 subunit which allows for action potential prolongation at APD 20, 50 and 70. Therefore, Kv β 1.1 subunit offers a potential target for therapeutic interventions of cardiac arrhythmia.

Previous report by Pan *et al.* show that Kv β subunits demonstrate tight binding based on the X-ray crystallography studies combined with heterologous electrophysiology studies [5]. The regions responsible for cortisone binding are primarily at two locations of the Kv β subunit including one at the active site and the other at the interface between the domains (residues 75 and 76) where Kv β interacts with Kv channel. In the present study, we hypothesized that cortisone binding to Kv β subunits plays a significant pharmacological role in the heart. Our investigation reveals that indeed cortisone allows the electrophysiological modulation at the action potential level by prolonging the action potential duration. We identified that cortisone has the highest propensity for APD prolongation followed by fluticasone. In addition to cortisone, we investigated the aldose reductase inhibitor epalrestat for its pharmacological role in modulating cardiac activity [19]. Based on our data it is clear that epalrestat prolongs the APD in the wild type hearts whereas the Kv β 1 knockout mouse heart failed to prolong the APD, demonstrating that Kv β 1 has a role in mediating the pharmacological activity.

At physiological level, since the cortisone and its analogs bind Kv β 1 subunit, it allows for a direct Kv β 1-Kv channel interaction that can be altered due to the affinity for cortisone. As noted in previous report [4, 5], it is likely that cortisone dissociates the assembly of Kv β 1-Kv interaction leading to a decreased Kv channel prowess causing prolongation of APD. Since the Kv β 1 subunit is knocked out in the Kv β 1.1 KO mouse heart, it is plausible that the effects of cortisone were abolished and the APD remained unaltered due to appearance of cortisone or fluticasone. To identify the specificity of cortisone mediated action potential changes, we utilized 4-amino pyridine which selectively alters Kv channel mediated responses. Based on the combined effects demonstrated by cortisone and 4-aminopyridine, we identified that cortisone mediates the action potential prolongation via its ability to bind Kv β 1.1 subunit, whereas 4-aminopyridine mediated changes are due to its direct effects on the Kv channel. Therefore, our results suggest that the cortisone mediated alteration in action potential is different than the 4-aminopyridine sensitive action potential.

Cortisone is a stress hormone which increases under physiological stress [20]. However the precise effect of cortisone on heart and electrical modulation remain unclear. To identify the effects of starvation on heart function and its relation to cortisone levels, another study identified the effects and revealed that prolonged starvation leads to stress related cortisone levels and impacts cardiac output and morphology in rats [21]. The present study clearly

demonstrates that cortisone and its analogs can directly bind to Kv β 1 and modulate the cardiac action potential; revealing the potential of such interactions for their utility as a pharmacological targets under metabolic stress.

Aldose reductase inhibitor epalrestat has been previously studied for assessing diabetic neuropathy in patients [19]. The use of epalrestat delays the progression of diabetic neuropathy by reducing sorbitol accumulation in different tissues such as sciatic nerves, ocular nerves and erythrocytes. The anti-ischemic activity of epalrestat was reported by Calderone *et al.* identifying the role for AR inhibitors in diabetic ischemia-reperfusion injury [12]. However, the electrical modulation caused by the pharmacological inhibitor remains unknown. In the present study, we investigated the role of aldose reductase inhibitor epalrestat and its interaction with Kv β 1 in the heart. Based on our findings, we identified that epalrestat an aldose reductase inhibitor prolongs the cardiac action potential. However deletion of Kv β 1.1 from the heart fails to invoke further prolongation observed in the wild type hearts suggesting that binding of epalrestat to Kv β 1 is likely causing the electrical modulation in the heart. Further, the sequence alignments and *in silico* binding demonstrate that epalrestat can interact with Kv β subunits arginine, a key amino acid, in binding NADPH to the active site as well as lysine which form the pyridine nucleotide binding pocket conserved in all Kv β proteins. This novel interaction likely interferes with the ability of NADPH to bind and interact with the Kv β subunit and modulate Kv β mediated Kv channel gating.

Conclusions

This is the first study demonstrating the physiological role of Kv β 1.1 and its interactions with corticosteroids to modulate cardiac action potential in the heart. The AR inhibitor epalrestat also shows *in silico* binding to Kv β subunit and modulates cardiac action potential *ex vivo*. Therefore, the present study provides new pharmacological information in terms of action potential modulation by Kv β subunit by corticosteroids or epalrestat.

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Figure 1: Cortisone exposure leads to prolongation in wildtype monophasic action potentials

A. Representative traces of monophasic action potentials (MAPs) from wildtype and Kv β 1.1 KO mouse heart without cortisone (black) and with cortisone (blue). Note significant prolongation in the wildtype. **B.** Action potential duration (APD) at 20% repolarization showing significant increase after cortisone treatment in WT while no such increase in KO hearts. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05 **C.** Action potential duration (APD) at 50% repolarization showing significant increases after cortisone treatment in WT but no increase in KO hearts. The data represents mean $\pm$ SEM (n= 5 hearts) * <0.05 **D.** Action potential duration (APD) at 70% repolarization Again, significant increases after cortisone treatment in WT while no increase in the Kv β 1.1 KO hearts. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05.

Figure 2: Fluticasone exposure prolong monophasic action potentials in hearts from wildtype mice.

A. Representative traces of wildtype and Kv β 1.1 KO MAPs without fluticasone (black) and with fluticasone (red) showing significant prolongation in wildtype. **B.** APD at 20% repolarization showing significant increases after fluticasone treatment in WT while having no effect in Kv β 1.1 KO hearts. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05 **C.** APD at 50% repolarization showing significant increase after fluticasone treatment in WT but no effect in KO hearts. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05 **D.** APD at 70% repolarization showing increases after fluticasone treatment in WT but no effect in Kv β 1.1 KO hearts. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05.

Figure 3: Treatment with Epalrestat prolongs monophasic action potentials in hearts of wildtype mice

A. Representative traces of wildtype and Kv β 1.1 KO knockout MAPs without epalrestat (blue) and with epalrestat (red) showing prolongation in wildtype. **B.** APD at 20% repolarization showing increases after epalrestat treatment in WT while no increase in KO Kv β 1.1 hearts. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05 **C.** APD at 50% repolarization showing increases after epalrestat treatment while having no effect in Kv β 1.1 KO hearts. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05 **D.** APD at 70% repolarization showing increases after epalrestat treatment in WT but no in Kv β 1.1 KO hearts. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05.

Figure 4: Action potential prolongs in wildtype and KO hearts exposed to 4-aminopyridine. Representative traces of MAPs in wildtype and Kv β 1.1 knockout hearts in nontreated controls (black), 4-aminopyridine; 4-AP-treated (brown). **B.** APD at 20% repolarization showing increases after 4-AP in WT and Kv β 1.1 KO hearts. **C.** APD at 50% repolarization showing increases after 4-AP treatment in WT and Kv β 1.1 KO hearts. **D.** APD at 70% repolarization showing increases after 4-AP treatment in WT and Kv β 1.1 KO hearts. Although 4-AP prolonged the APD's at 20, 50 and 70% repolarization, the magnitude of response is lower in KO group compared with WT after 4-AP treatment. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05.

Figure 5: Treatment with Cortisone and 4-aminopyridine prolongs monophasic action potentials in hearts from wildtype mice.

A. APD at 50% repolarization showing increases after cortisone in WT but no effect in Kv β 1.1 KO hearts. Treatment with Cortisone in combination with 4-AP further prolongs APD as compared with cortisone alone in WT. Combination of Cortisone and 4-AP in Kv β 1.1 KO hearts prolongs APD solely due to the effect of 4-AP. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05 **B.** APD at 70% repolarization showing increases after cortisone treatment in WT while exerting no effect in Kv β 1.1 KO hearts. Combination treatment with Cortisone and 4-AP further prolongs the APD compared with cortisone alone in WT. Also, the combination treatment with Cortisone and 4-AP in Kv β 1.1 KO hearts prolongs APD solely due to the effect of 4-AP. The data represent mean $\pm$ SEM (n= 5 hearts) * p<0.05.

Figure 6: Epalrestat modeling of the Kv β AKR domain

A. Sequence alignment of human Kv β 2, rat Kv β 2, and mouse Kv β 1 demonstrate a conserved active site and NADPH binding pocket. A cysteine residue followed exactly by 5 amino acids then a lysine and tyrosine followed exactly by 10 amino acids then an arginine make up the binding pocket for pyridine nucleotide entry. **B.** Docking of epalrestat (labeled grey arrow shown in balloon model) with Kv β 2 binding pocket depicting key residue arginine (Human 250), overlaid is the NADPH (labeled grey arrow shown in stick model) molecule which is inhibited from interacting with arginine (grey arrow) due to epalrestat interactions. **C.** Stick modeling of the different amino acids within the Kv β binding pocket demonstrating epalrestat to additionally interact with the 240 Lysine shown by the grey arrow.