Original Article
Effects of C-reactive protein on K+ channel interaction protein 2 in cardiomyocytes

Yong Xie[1,2*], Jing-Ting Mai[1,2*], Fei Wang[3*], Yong-Qing Lin[1,2], Wo-Liang Yuan[1,2], Nian-Sang Luo[1,2], Ming-Cheng Fang[1,2], Jing-Feng Wang[1,2], Yang-Xin Chen[1,2]

[1]Department of Cardiology, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou 510120, China; [2]Guangdong Province Key Laboratory of Arrhythmia and Electrophysiology, Guangzhou 510120, China; [3]Department of Anaesthesiology, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou 510120, China. *Equal contributors.

Abstract: Several studies have found that C-reactive protein (CRP) was associated with QTc interval prolongation and ventricular arrhythmia. However, little is known about the mechanisms involved. K+ channel interaction protein 2 (KChIP2) is a necessary subunit for the formation of transient outward potassium current (Ito.f) which plays a critical role in early repolarization and QTc interval of heart. In this study, we aimed to evaluate the effects of CRP on KChIP2 and Ito.f in cardiomyocytes and to explore the potential mechanism. The neonatal mice ventricular cardiomyocytes were cultured and treated with CRP at different concentrations. The expression of KChIP2 was detected by real time quantitative PCR and Western blot. In addition, Ito.f current density was evaluated by whole cell patch clamp techniques. Our results showed that CRP significantly decreased the mRNA and protein expression of KChIP2 in time and doses dependent manners ($P < 0.05$), and also reduced the current density of Ito.f ($P < 0.05$). In addition, CRP increased the expression of NF-κB and decreased IκBα expression without significant influence on the expression of ERK1/2 and JNK. Meanwhile, the NF-κB inhibitor PDTC significantly attenuated the effects of CRP on KChIP2 and Ito.f current density. In conclusion, CRP could significantly down-regulate KChIP2 expression and reduce current density of Ito.f partly through NF-κB pathway, suggesting that CRP may directly or indirectly influence QTc interval and arrhythmia via influencing KChIP2 expression and Ito.f current density of cardiomyocytes.

Keywords: C-reactive protein, cardiomyocytes, KChIP2, Ito.f current density

Introduction

C-reactive protein (CRP), an acute-phase protein and mainly synthesized in liver, plays important roles in cardiovascular diseases [1-3]. For example, lots of epidemiological studies found that CRP could act as an important risk factor for many cardiovascular diseases, such as acute coronary syndrome, dialated cardiomyopathy, etc [4, 5]. Recently, several reports showed that CRP level was closely associated with QTc (correct QT) interval prolongation and ventricular arrhythmia. In a population-based sample, Kim et al. found a positive correlation between CRP level and the length of heart rate-corrected QTc interval [6]. Also, a study on the hypertensive patients indicated that CRP is a significant predictor for the QTc interval and presence of QT prolongation [7].

Similar to this, a significant correlation between serum CRP level and QTc interval was observed in a study that included a cohort of young healthy men [8]. It has also been demonstrated that higher CRP level was able to increase the ventricular ectopic activity in subjects without cardiovascular diseases [9]. Moreover, a positive correlation between higher serum hs-CRP level (> 3 mg/L) and the occurrence of ventricular arrhythmias was found in a prospective cohort study on the ICD recipient [10]. Beside, Florian Streitner et al found a significantly increased occurrence rate of electronic storm in patients receiving the ICD therapy with a higher CRP level [11]. At present, there is conclusive evidence that CRP is used to predict ventricular arrhythmia in patients with acute coronary syndrome [12]. Hence, all these studies suggest that higher CRP level plays a pro-

arrhythmia role and the QTc interval prolongation may be implicated in the development of the pro-arrhythmia process. However, it remains largely unknown about the mechanisms involved in the QTc interval prolongation induced by higher CRP level.

QTc interval is calculated using Bazett's formula (QT interval divided by square root of RR interval) in clinical setting and is dependent upon the action potential duration of ventricular myocytes. It was mentioned above that a QTc interval prolongation might be induced by higher CRP level in human. In other word, higher CRP level could lengthen the action potential duration of ventricular myocytes. Accordingly, the influencing factors of action potential duration may be involved in the prolongation of QTc interval induced by CRP.

K+ channel interacting proteins 2 (KChIP2), a member of the Ca^{2+}-binding protein, is highly restricted to express in the heart tissue. KChIP2 may interact with Kv4.2 or Kv4.3 to form the transient outward currents (Ito.f) and to participate in regulating the early repolarization of action potential duration and QTc interval of heart [13-15]. The first phase repolarization duration is dependent upon the Ito.f current density which plays an important role in the action potential duration and QTc interval. Hence, the KChIP2 expression is closely correlated with the QTc interval. Meanwhile, it was previously shown that the NF-κB, ERK1/2 and JNK pathways were involved in the regulation of KChIP2 in heart [16, 17]. Recently, functional reduction of Ito.f and KChIP2 has also been reported in many animal models of heart failure, suggesting an important role of KChIP2 in the development of cardiac remodeling [18-20]. It was recently shown that downregulation of KChIP2 causes a prolongation of action potential and QTc interval while upregulation of KChIP2 results in a shortage of action potential and QT interval [21]. Furthermore, Kuo et al found that the defect of KChIP2 gene led to a complete loss of Ito.f and conferred susceptibility to ventricular tachycardia in mice [13]. There is growing evidence that KChIP2 plays important roles in the QTc interval prolongation and the development of ventricular arrhythmia, and the related regulatory pathways NF-κB could be activated by CRP. Accordingly, the primary aim of this study was to explore the effects of CRP on KChIP2 in ventricular myocytes and the potential mechanisms involved.

Materials and methods

Animals

C57BL/6J mice (1-2 day old) were used in this investigation and the study was approved by the Institutional Animal Care and Use Committee of SunYat-sen University. Animal use and care were in accordance with the animal care guidelines, which conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Culture of cardiomyocytes from neonatal mice

Cardiomyocytes were prepared from ventricles of 1-2 day old C57BL/6J mice as described [22]. Briefly, the hearts from neonatal mice were prepared; the atrial and vascular tissues were removed and the ventricles were enzymatically digested in 0.125% trypsin (Gibco) for 5 minutes and then 0.06% collagenase II (MP biomedical, USA) for 2 hours in a thermostat shaker at 37°C and a speed of 62 rpm. Then, we collected the supernatant and resuspended mixed aliquots followed by PBS washing and centrifuge in 0.5 g for 5 minutes. The sediment was collected and resuspended with Dulbecco's modified Eagle medium/Ham's F-12 medium (DMEM/F12) and 10% fetal bovine serum (FBS) together with 100 U/ml penicillin/ streptomycine (Gibco, USA). Then the cells were plated in tissue culture dishes and maintained at 37°C in 5% CO_2 incubator to be differentially plated for 30-35 minutes to remove non-myocytes. Afterward, the supernatant was transferred to new culture dishes and cardiomyocytes were cultured in the DMEM/F12 and 10% FBS and 100 U/ml penicillin /streptomycine and 1% BrdU.

Cell viability assay

The cell viability was detected by MTS assay. The cardiomyocytes (5×10^3 cells/well) seeded in 96-well plates were incubated with CRP (0, 5, 10, 20, 40 and 100 µg/mL) for 24, 48, 72 h respectively and the aliquots (20 µL) of MTS were added into each well and co-cultured 4 h. The absorbance at 490 nm presented the cell viability in microplate reader.
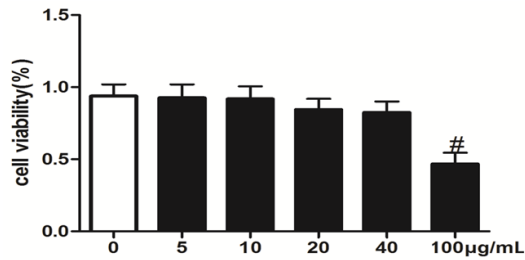
Figure 1. Effects of CRP on cell viability of neonatal mice myocytes. Cells were incubated with different concentrations of CRP for 24, 48, 72 h. Cell viability was determined by the absorbance at 490 nm. Each column represents the mean ± SD from three independent experiments. #$p < 0.05$ vs normal control group.

Cardiomyocytes treated with CRP

After incubation for 24 h in DMEM/F12 with 10% FBS, 100 U/ml penicillin/streptomycine, the cardiomyocytes were maintained in serum-free DMEM/F12 for 24 h and then treated with human recombinant CRP (Merck, USA). Purity of CRP was confirmed by SDS-PAGE showing a single band. We determined the endotoxin level as 0.0005 EU/μg for the CRP preparation by the Limulus amebocyte lysate assay (Associates of Cape Cord, USA). The cardiomyocytes were treated by CRP with different concentrations (0, 5, 10, 20, 30, 40 μg/mL) for different durations (0, 3, 6, 12, 24, 48 h). The NF-κB inhibitor PDTC (10 μM) was pretreated for 1 h and then the cells were stimulated with CRP (10 μg/mL) for 24 h.

Quantitative Real Time PCR analysis

Total RNA was extracted from cardiomyocytes by RNAiso plus (Takara, Japan) and reverse transcribed to cDNA on the specification. Quantization of KChIP2 transcript levels was determined by amplification of cDNA prepared from the isolated RNA with the SYBR Premix Ex Taq (Takara, Japan) and primers specific for KChIP2 (forward, 5'-GGC TTC AAG AAC GAA TGT CC-3'; reverse, 5'-CAG CCA CAA AGT CCT CAA AA-3') and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) as the internal control (forward, 5'-TGT GTC CGT CGT GGA TCT GA-3'; reverse, 5'-TTG CTG TTG AAG TCG CAG GAG-3'). Results were presented as fold difference for each gene against GAPDH by use of $2^{-\Delta\Delta CT}$ method. The melting curve was used to confirm whether only a single product was present.

Western blot analysis

Total proteins and the nuclear proteins were extracted from cultured cardiomyocytes with RIPA buffer (CST, USA) and NE-PER Nuclear Extraction Reagents (Thermo scientific, USA) respectively. 10-40 μg protein samples were separated on 10-13% SDS-PAGE gels and blocked with 5% non-fat milk in tris-buffered saline Tween, the membranes were incubated overnight at 4°C with primary antibodies rabbit original against KChIP2, NF-κBp65, IκBα, GAPDH (Abcam, USA), ERK1/2, p-ERK1/2, JNK, p-JNK (Cell Signaling Technology, USA) and subsequently with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit IgG) for 1 h at room temperature. Western blots membranes were exposed with Gel Documentation and Analysis System (G-box, Syngene, UK) by Western Chemiluminescent HRP Substrate (Millipore Corporation, Billerica, USA).

Measurement of Ito.f current density

Ito.f currents were measured by the whole-cell patch-clamp method with a MultiClamp 700B amplifier (Axon Instruments, Union City, CA). Whole-cell patch-clamp was utilized at 37°C. Micropipettes were pulled from borosilicate glass capillaries (BF150-110-7.5, Sutter Instruments, Novato CA) on a programmable horizontal puller (S-97; Sutter Instruments, Novato CA). The inner tip diameters of pipettes were about 1 to 1.5 μm. The resistance range from 2.5 to 5 MΩ When filled with internal solutions. Data were filtered at 4 KHz with a four-pole low-pass bessel filter and sampled at 10 KHz. The experiments were performed using pCLAMP10.2 software (Axon Instruments, Union City CA). Boltzman's fits were performed as previously described. To record Ito.f currents, the pipette solution contained 130 mM K$^+$ gluconate, 10 mM NaCl, 10 mM EGTA, 1 mM MgCl$_2$, 2 mM Mg-ATP, 2.0 mM CaCl$_2$ and 10 mM HEPES, adjusted to pH 7.2 with KOH. Cells were superfused at room temperature with HEPES-buffered Tyrode's containing 137 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, 5 mM glucose, and 10 mM HEPES (pH was adjusted to 7.4 with NaOH). Voltage clamp correction for a liquid junction potential of -14 mV was made by configuring the recording files in CLAMPEX of pCLAMP10.2. Series resistances were usually less than 1 MΩ after 80% compensation. All
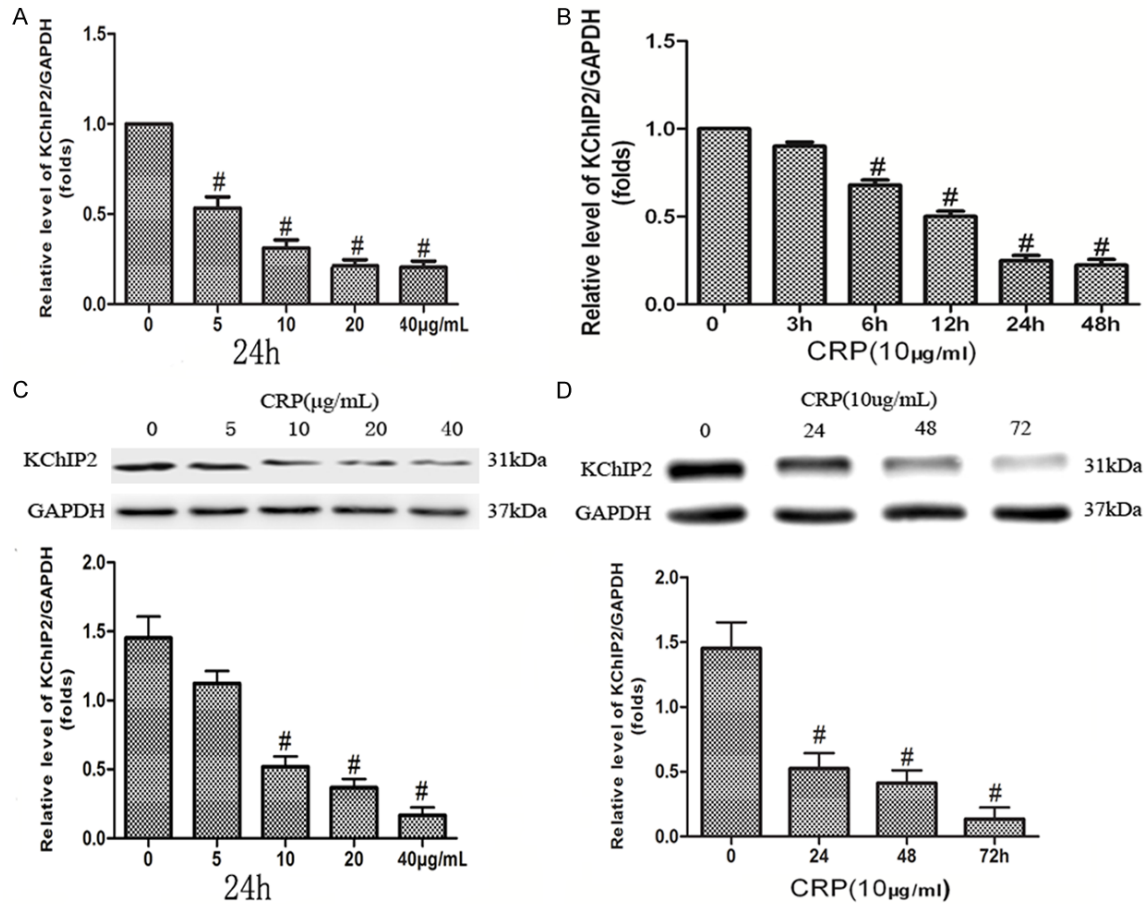
Figure 2. Effects of CRP on the expression of KChIP2 in mRNA and protein level. A, B. In **Figure 2** presented a reducing expression of the KChIP2 mRNA after treated with CRP in different concentration and time respectively. C and D. Showed an identical variance of KChIP2 protein after stimulated with CRP in different concentration and time respectively. Data are presented as mean ± SEM (N = 3). #$P < 0.05$ compared with control.

voltages in current clamp recording were also corrected for the junction potential [23].

The decay phases of the voltage-dependent K⁺ currents in mouse cardiomyocytes may be fit by the sum of 2 exponentials, which denotes the fast transient K current Ito,f, a rapidly activating, very slowly inactivating current, $I_{K,slow}$, and a non-inactivating current, Iss [24, 25]. The decay phases of the outward K⁺ currents evoked during 4.0 s depolarizing voltage steps were fitted by a double exponential function of the form:

$$Y(t) = A_1 exp(-t/T_1) + A_2 exp(-t/T_2) + B.$$

Where t is time, T_1 and T_2 are the decay time constants, A_1 and A_2 are the amplitudes of the inactivating current components (Ito,f and $I_{K,slow}$), and B is the amplitude of the non-inactivating current component, Iss.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) or mean ± SEM. Differences between control and experimental groups were analyzed by *t* test or one-way analysis of variance followed by Dunnett's test, and *p* < 0.05 was considered as statistical significant. All calculations were performed using SPSS 13.0 statistical software (SPSS, Chicago, USA).

Results

*Effects of CRP on the cell viability of cardiomyocytes*

As shown in **Figure 1**, except for the concentration of 100 µg/mL, CRP (5, 10, 20 and 40 µg/mL) had no significant effect on cell viability. Therefore, the concentration of 5, 10, 20, 40
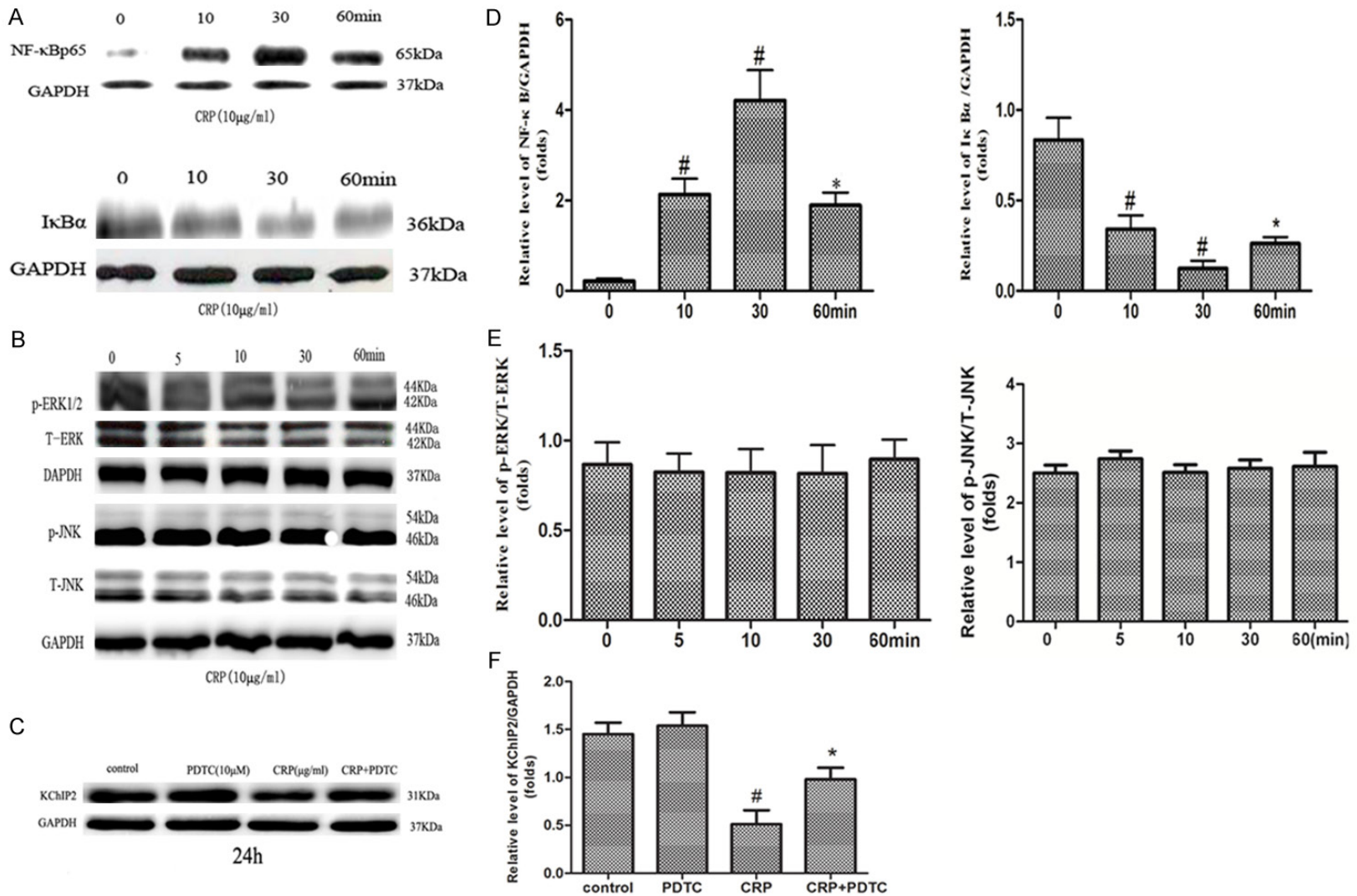
Figure 3. Relevant pathways in the regulation of KChIP2. The results showed an increasing level of NF-κB p65 and a decreasing level of IκBα after CRP treatment (10 μg/ml) (A, D) and the same level of p-ERK and p-JNK between control group and treatment group (B, E) (#$P < 0.05$: Compared with control group, *$P < 0.05$: Compared with previous group in 30 min). Furthermore, the NF-κB inhibitor PDTC (10 μM) could attenuate the effects of CRP on KChIP2 in cardiac myocytes in 24 h (C, F). Data are presented as mean ± SEM (N = 3). #$P < 0.05$: Compared with control group. *$P < 0.05$: Compared with CRP group.
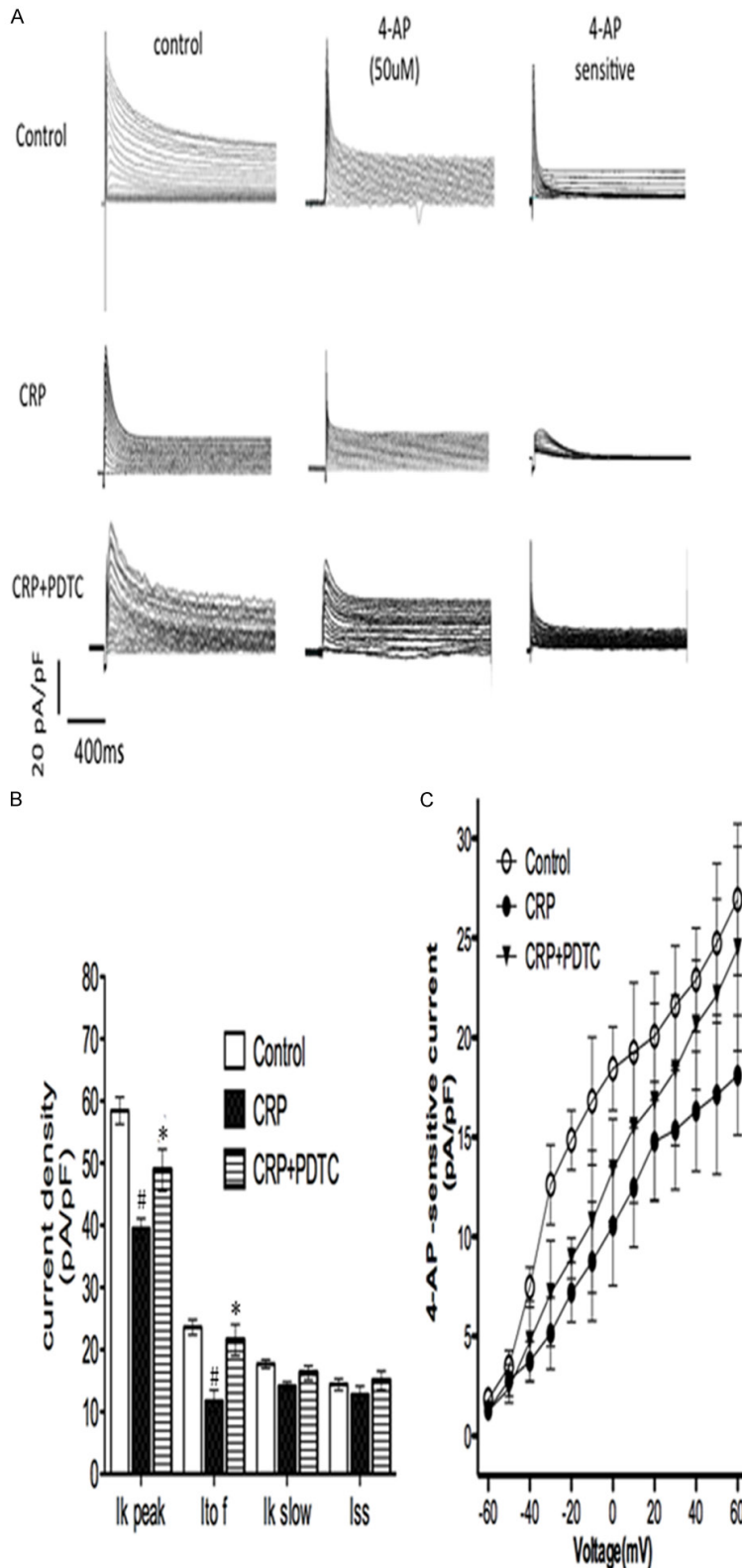
Figure 4. The Ito.f current density decreased in CRP group. Voltage-dependent K⁺current tracings for the control and CRP group. a lower Ito.f current density was presented in CRP group compared with control group. After exposure to 50 μmol/L 4-AP (middle), and the 4-AP sensitive current (A, B). Patch clamp protocols was described in Methods and we obtained 4-AP sensitive current by off-line digital subtraction of records before and after 4-AP application. Graph of current density of 4-AP sensitive current for normal control and CRP group (C). Data were presented as Mean ± SEM, control group (n = 43); CRP group (n = 32) #$P < 0.05$: Compared with control group, *$P < 0.05$: Compared with CRP group. pA/pF indicates picoamps/ picofarads.

μg/mL were applied in the following experiments.

CRP down-regulated the KChIP2 expression in cultured cardiomyocytes

To clarify the effects of CRP on KChIP2 in cardiomyocytes, cells were serum-deprived for 24 h and treated with CRP in different concentrations (0, 5, 10, 20, 40 μg/mL) for 24 h. At every time point, cell lysates were collected for the analysis of KChIP2 mRNA and protein expression. Results in **Figure 2** showed that mRNA and protein expressions of KChIP2 began to decrease after 6 h and reached valley at 48 h in a time and dose-dependent manner ($P < 0.01$).

NF-κB pathways were involved in CRP-induced electrophysiological effects in cardiomyocytes

Results in **Figure 3A**, **3D** showed that CRP stimulation could increase the phosphorylation of NF-κB and decrease IκBα expression in cardiomyocytes. Furthermore, NF-κB inhibitor PDTC could attenuate the effect of CRP on KChIP2 and Ito.f current density (**Figure 3C** and **3F**). But no changes of phosphorylated ERK1/2 and JNK were observed after CRP stimulation (**Figure 3B** and **3E**).

Effects of CRP on Ito.f current density

KChIP2 is a necessary ingredient for the formation of Ito.f. A decreased expression of KChIP2 was found in the cardiomyocytes after exposed to CRP. To well clarify the electrophysiological effects of CRP on the cardiomyocytes, the Ito.f current density was measured by patch clamp. The results showed a reduction of voltage-dependent K$^+$ current after CRP stimulation compared with control group. In mouse cardiomyocytes, at least three distinct voltage dependent K$^+$ currents have been identified. Peak outward Kcurrent (Ik.peak) was reduced in the CRP treatment group (45.9 ± 1.59 picoamps/picofarads [pA/pF]) compared with normal control (58.5 ± 2.19 pA/pF) ($p < 0.05$). The decay phases of the voltage-dependent K$^+$ currents in cardiomyocytes after CRP stimulation might be fit by the sum of 2 exponentials, which included the Ito.f, Ik.slow, Iss current. The analysis of decay phases showed a statistically reduction in the current density of Ito.f (CRP group vs control group: $P < 0.05$) and a non-statistical variation in the current density of Ik.slow and Iss (**Figure 4A**, **4B**). The reduction of Ito.f current density after CRP treatment was confirmed by a pharmacological approach. After exposure to 50 μmol/L 4-AP, a lower Ito.f current density was observed in CRP group than in control group (**Figure 4C**). These data suggested that the Ito.f current density was reduced in cardiomyocytes after the treatment of CRP.

Discussion

The underlying mechanisms about the pro-arrhythmia roles of CRP are poorly understood. Here, we showed that CRP could down-regulate the expression of KChIP2 and reduce the Ito.f current density via NF-κB pathway. As we know, KChIP2 is an important ingredient in the formation of Ito.f and action potential duration in heart. The reduction of KChIP2 and Ito.f current density may affect the cardiac repolarization and cause a prolongation of action potential duration and QTc interval followed by arrhythmias. The results of this study give us a new understanding on the arrhythmogenic roles of CRP in cardiomyocytes.

Previous study had found a close correlation between elevated CRP serum concentrations and the incidence of malignant ventricular arrhythmias in ICD-treated patients [10]. Meanwhile, results from Hidehiro et al study also suggested that a higher serum CRP level was related to the development of VT/VF after reperfused ST-elevation myocardiac infarction [26]. Additional, Epidemic studies shown that even moderate increases of serum CRP levels were also accompanied with a higher risk of future cardiovascular events in both apparently healthy individuals and patients with coronary heart disease [27, 28]. Combined with the results of this experiment, we deduced that higher CRP level might induce the ventricular arrhythmias partly through influencing the KChIP2 expression and Ito.f current density, which could help to explain the potential mechanisms involved in the proarrhythmic effects of CRP in patients with or without known cardiovascular diseases.

CRP is not only a marker but also a mediator of cardiovascular diseases [29]. It was also reported that CRP had a link with vascular and left ventricle remodeling [30]. Toshiyuki Nagai, et al demonstrated that overexpression of human CRP could exacerbate the left ventricular remodeling in transverse aortic constriction mice model [31]. Interestingly, it has been reported that a reduction of KChIP2 and Ito.f current density in ventricle hypertrophy in severe stenosis of the ascending aorta rat modle and a significantly improvement after KChIP2 overexpression by transduced with Ad.KChIP2 [32]. Therefore, a reduction of KChIP2 expression may be involved in adverse effects induced by CRP overexpression on the left ventricular remodeling in transverse aortic constriction mice model. Hence, the results from our study suggest that the cardiac remolding caused by CRP may in part be mediated by affecting the expression and function of KChIP2 and may be as a predictor for the ventricular arrhythmia.

NF-κB, an important transcriptional regulatory factor, is activated and enters the nucleus where it combines with specific DNA motifs and stimulates the expression of various genes after the cells are stimulated by various cytokines such as CRP. As a classic inflammatory pathway, the NF-κB pathway was involved in CRP-induced inflammatory reaction and downstream effects. On the other side, it was found that the NF-κB pathway played a regulatory role in the KChP2 expression and Ito.f current density [33]. As an inflammatory factor, CRP could activate the NF-κB inflammatory pathway and affect the downstream target proteins. Our

results also demonstrated that the NF-κB pathway was involved in regulating KChIP2 expression and Ito.f current density of CRP-treated myocytes. Meanwhile, PDTC, a free radical scavenger, could inhibits NF-κB release from I-κB and seemed as an inhibitor of NF-κB ,could attenuate these effects induced by CRP independent on the actions of PDTC beyond its ability to inhibit NF-κB which further demonstrated the NF-κB pathway was involved in the regulatory process of KChIP2. Interestingly, the ERK1/2 and JNK pathways were not activated in this study. It was reported that the ERK1/2 and JNK were implicated in the reduction of KChIP2 protein and mRNA expression in aortic banded rat ventricles as well as in cultured neonatal rat ventricular myocytes after treatment with the alpha-adrenergic agent phenylephrine and the PKC activator, phorbol 12-myristate 13-acetate (PMA), which suggested that different pathways exerted their regulatory roles for KChIP2 in different case [34]. Therefore, involvement of NF- κB signaling pathway in the regulation of KChIP2 by CRP further demonstrate the closely relation between CRP and KChIP2.

In conclusion, we demonstrated that CRP down-regulated KChIP2 expression and Ito.f current density in cardiomyocytes, and clarified that NF-κB signaling pathways were involved. Our findings suggest that CRP as a predictor for ventricular arrhythmia might be partly associated with its inhibition effect on KChIP2 expression and Ito.f current density.

Limitation

Limitations of our study include a lack of data about the effects of CRP on the expression of other potassium channels as Kv4.2, Kv4.3, Kv1.5 and Kv2.1 and calcium channels and sodium channels which also play important roles in the QTc interval of cardiomyocytes. As a risk factor for ventricular arrhythmia, CRP may also exert an effect on these ion channels, which requires further research to determine. In addition, the data about the resting membrane potential, upstroke velocity and action potential duration in cardiomyocytes treated with CRP were not shown in this paper.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Jing-feng Wang or Yang-xin Chen, Department of Cardiology, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, 107 West Yanjiang Road, Guangzhou 510120, China. E-mail: dr_wjf@hotmail.com (JFW); tjcyx-1995@163.com (YXC)

References

[1] Maseri A. Inflammation, atherosclerosis, and ischemic events: exploring the hidden side of the moon. New Engl J Med 1998; 332: 281-292.

[2] Libby P, Geng YJ, Sukhova GK, Simon DI, Lee RT. Molecular determinants of atherosclerotic plaque vulnerability. Ann N Y Acad Sci 1997; 811: 134-142.

[3] Pasceri V, Yeh ET. A tale of two diseases: atherosclerosis and artificial phosphatidylcholine bilayers and complement. J Immunol rheumatoid arthritis 1999; 100: 2124-2126.

[4] Satoh M, Nakamura M, Akatsu T, Shimoda Y, Segawa I, Hiramori K. C-reactive protein co-expresses with tumor necrosis factor-alpha in the myocardium in human dilated cardiomyopathy. Eur J Heart Fail 2005; 7: 748-754.

[5] Brunetti ND, Troccoli R, Correale M, Pellegrino PL, Di Biase M. C-reactive protein in patients with acute coronary syndrome: correlation with diagnosis, myocardial damage, ejection fraction and angiographic findings. Int J Cardiol 2006; 109: 248-256.

[6] Kim E, Joo S, Kim J, Ahn J, Kim J, Kimm K, Shin C. Association between C-reactive protein and QTc interval in middle-aged men and women. Eur J Epidemiol 2006; 21: 653-659.

[7] Chang KT, Shu HS, Chu CY, Lee WH, Hsu PC, Su HM, Lin TH, Voon WC, Lai WT, Sheu SH. Association between C-reactive protein, corrected QT interval and presence of QT prolongation in hypertensive patients. Kaohsiung J Med Sci 2014; 30: 310-315.

[8] Kazumi T, Kawaguchi A, Hirano T, Yoshino G. C-reactive protein in young, apparently healthy men: associations with serum leptin, QTc interval, and high-density lipoprotein-cholesterol. Metab Clin Exp 2003; 52: 1113-1116.

[9] Theuns DA, Smith T, Szili-Torok T, Muskens-Heemskerk A, Janse P, Jordaens L. Prognostic role of high-sensitivity C-reactive protein and B-type natriuretic peptide in implantable cardioverter-defibrillator patients. Pacing Clin Electrophysiol 2012; 35: 275-282.

[10] Streitner F, Kuschyk J, Veltmann C, Ratay D, Schoene N, Streitner I, Brueckmann M, Schumacher B, Borggrefe M, Wolpert C. Role of proinflammatory markers and NT-proBNP in patients with an implantable cardioverter-defibrillator and an electrical storm. Cytokine 2009; 47: 166-172.

[11] Sajadieh A, Nielsen OW, Rasmussen V, Ole Hein H, Hansen JF. Increased ventricular ectopic activity in relation to C-reactive protein, and NT-pro-brain natriuretic peptide in subjects with no apparent heart disease. Pacing Clin Electrophysiol 2006; 29: 1188-1194.

[12] Nagai T, Anzai T, Kaneko H, Anzai A, Mano Y, Nagatomo Y, Kohsaka S, Maekawa Y, Kawamura A, Yoshikawa T, Ogawa S. Impact of systemic acidosis on the development of malignant ventricular arrhythmias after reperfusion therapy for ST-elevation myocardial infarction. Circ J 2010; 74: 1808-1814.

[13] Kuo HC, Cheng CF, Clark RB, Lin JJ, Lin JL, Hoshijima M, Nguyêñ-Trân VT, Gu Y, Ikeda Y, Chu PH, Ross J, Giles WR, Chien KR. Defect in the Kv Channel-Interacting Protein 2(KChIP2) Gene Leads to a Complete Loss of Ito and Confers Susceptibility to Ventricular Tachycardia. Cell 2001; 107: 801-813.

[14] Guo W, Li H, Aimond F, Johns DC, Rhodes KJ, Trimmer JS, Nerbonne JM. Role of heteromultimers in the generation of myocardial transient outward K$^+$ currents. Circ Res 2002; 90: 586-593.

[15] Bosch RF, Scherer CR, Rüb N, Wöhrl S, Steinmeyer K, Haase H, Busch AE, Seipel L, Kühlkamp V. Molecular mechanisms of early electrical remolding: transcriptional downregulation of ion channel subunits reduce I$_{ca,L}$ and Ito in rapid atrial pacing in rabbit. J Am Coll Cardial 2003; 41: 856-869.

[16] Panama BK, Latour-Villamil D, Farman GP, Zhao D, Bolz SS, Kirshenbaum LA, Backx PH. Nuclear factor kappaB downregulates the transient outward potassium current I(to,f) through control of KChIP2 expression. Circ Res 2011; 108: 537-543.

[17] Barth AS, Kääb S. MAPK = mitogen-activated protein KChIP2? Unraveling signaling pathways controlling cardiac Ito expression. Circ Res 2006; 98: 301-302.

[18] Nass RD, Aiba T, Tomaselli GF and Akar FG. Mechanisms of disease: ion channel remodeling in the failing ventricle. Nat Clin Pract Cardiovasc Med 2008; 5: 196-207.

[19] Radicke S, Cotella D, Graf EM, Banse U, Jost N, Varro A, Tseng GN, Ravens U and Wettwer E. Functional modulation of the transient outward current Ito by KCNE beta-subunits and regional is tribution in human non-failing and failing hearts. Cardiovasc Res 2006; 71: 695-703.

[20] Soltysinska E, Olesen SP, Christ T, Wettwer E, Varró A, Grunnet M, Jespersen T. Transmural expression of ion channels and transporters in human nondiseased and end-stage failing hearts. Pflügers Arch 2009; 459: 11-23.

[21] Fotiadis P, Forger DB. Modeling the effects of the circadian clock on cardiac electrophysiology. J Biol Rhythms 2013; 28: 69-78.

[22] Xu Y, Zhang Z, Timofeyev V, Sharma D, Xu D, Tuteja D, Dong PH, Ahmmed GU, Ji Y, Shull GE, Periasamy M, Chiamvimonvat N. The effects of intracellular Ca^{2+} on cardiac K$^+$ channel expression and activity: novel insights from genetically altered mice. J Physiol 2005; 562: 745-758

[23] Morrow JP, Katchman A, Son NH, Trent CM, Khan R, Shiomi T, Huang H, Amin V, Lader JM, Vasquez C, Morley GE, D'Armiento J, Homma S, Goldberg IJ, Marx SO. Mice with cardiac overexpression of peroxisome proliferator-activated receptor γ have impaired repolarization and spontaneous fatal ventricular arrhythmias. Circulation 2011; 124: 2812-2821.

[24] Xu H, Guo W, Nerbonne JM. Four kinetically distinct depolarization-activated Kcurrents in adult mouse ventricular myocytes. J Gen Physiol 1999; 113: 661-678.

[25] Brunet S, Aimond F, Li H, Guo W, Eldstrom J, Fedida D, Yamada KA, Nerbonne JM. Heterogeneous expression of repolarizing, voltage-gated K currents in adult mouse ventricles. J Physiol 2004; 559: 103-120.

[26] Kaneko H, Anzai T, Naito K, Kohno T, Maekawa Y, Takahashi T, Kawamura A, Yoshikawa T, Ogawa S. Role of Ischemic Preconditioning and Inflammatory Response in the Development of Malignant Ventricular Arrhythmias After Reperfused ST-Elevation Myocardial Infarction. J Card Fail 2009; 15: 775-781.

[27] Danesh J, Wheeler JG, Hirschfield GM, Eda S. C reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004; 350: 1387-1397.

[28] Eisen A, Benderly M, Behar S, Goldbourt U, Haim M. Inflammation and future risk of symptomatic heart failure in patients with stable coronary artery disease. Am Heart J 2014; 167: 707-714.

[29] Cui C, Shi Q, Zhang X, Liu X, Bai Y, Li J, Liu S, Hu S, Wei Y. CRP promotes MMP-10 expressionvia c-Raf/MEK/ERK and JAK1/ERK pathways in cardiomyocytes. Cell Signal 2012; 24: 810-818.

[30] Uehara K, Nomura M, Ozaki Y, Fujinaga H, Ikefuji H, Kimura M, Chikamori K, akaya Y, Ito S.

High sensitivity C reactive protein and left ventricular remodeling in patients with acute myocardial infarction. Heart Vessels 2003; 18: 67-74.

[31] Nagai T, Anzai T, Kaneko H, Mano Y, Anzai A, Maekawa Y, Takahashi T, Meguro T, Yoshikawa T, Fukuda K. C-Reactive Protein Overexpression Exacerbates Pressure Overload-Induced Cardiac Remodeling Through Enhanced Inflammatory Response. Hypertension 2011; 57: 208-215.

[32] Jin H, Hadri L, Palomeque J, Morel C, Karakikes I, Kaprielian R, Hajjar R, Lebeche D. KChIP2 attenuates cardiac hypertrophy through regulation of Ito and intracellular calcium signaling. J Mol Cell Cardiol 2010; 48: 1169-1179.

[33] Panama BK, Latour-Villamil D, Farman GP, Zhao D, Bolz SS, Kirshenbaum LA, Backx PH. Nuclear factor kappaB downregulates the transient outward potassium current I(to,f) through control of KChIP2 expression. Circ Res 2011; 108: 537-543.

[34] Jia Y, Takimoto K. Mitogen-activated protein kinases control cardiac KChIP2 gene expression. Circ Res 2006; 98: 386-393.