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Original article

Kir2.x inward rectifier potassium channels are differentially regulated by adrenergic α_{1A} receptors

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Abstract

Inhibition of I_{K1} currents by adrenergic α_1 receptors has been observed in cardiomyocytes and has been linked to arrhythmogenesis in an animal model. Both PKC-dependent and PKC-independent pathways have been implied in this regulation. The underlying molecular mechanisms, however, have not been elucidated to date. The molecular basis of native I_{K1} current is mainly formed by Kir2.1 (KCNJ2), Kir2.2 (KCNJ12) and Kir2.3 (KCNJ4) channels that are differentially regulated by protein kinases. We therefore sought to investigate the role of those different Kir2.x channel subunits in this regulation and to identify the major signalling pathways involved.Adrenergic α_{1A} receptors (the predominant cardiac isoform) were co-expressed with cloned Kir2.1, Kir2.2 and Kir2.3 channels in *Xenopus* oocytes and electrophysiological experiments were performed using two-microelectrode voltage clamp. Native I_{K1} currents were measured with the whole-cell patch clamp technique in isolated rat ventricular cardiomyocytes.Activation of co-expressed adrenergic α_{1A} receptors by phenylephrine induced differential effects in Kir2.x channels. No effect was noticed in Kir2.1 channels. However, a marked inhibitory effect was observed in Kir2.2 channels. This regulation was not attenuated by inhibitors of PKC, CamKII and PKA (chelerythrine, KN-93, KT-5720), and mutated Kir2.2 channels lacking functional phosphorylation sites for PKC and PKA exhibited the same effect as Kir2.2 wild-type channels. By contrast, the regulation could be suppressed by the general tyrosine kinase inhibitor genistein and by the src tyrosine kinase inhibitor PP2 indicating an essential role of src kinases. This finding was validated in rat ventricular cardiomyocytes where co-application of PP2 strongly attenuated the inhibitory regulation of I_{K1} current by adrenergic α_1 receptors. The inactive analogue PP3 was tested as negative control for PP2 and did not reproduce the effects of PP2. In Kir2.3 channels, a marked inhibitory effect of α_{1A} receptor activation was observed. This regulation could be attenuated by inhibition of PKC with chelerythrine or with Ro-32-0432, but not by tyrosine kinase inhibition with genistein.In summary, on the molecular level the inhibitory regulation of I_{K1} currents by adrenergic α_{1A} receptors is probably based on effects on Kir2.2 and Kir2.3 channels. Kir2.2 is regulated via src tyrosine kinase pathways independent of protein kinase C, whereas Kir2.3 is inhibited by protein kinase C-dependent pathways. Src tyrosine kinase pathways are essential for the inhibition of native I_{K1} current by adrenergic α_1 receptors. This regulation may contribute to arrhythmogenesis under adrenergic stimulation.

Keywords: Cardiac electrophysiology; Potassium current; Adrenergic receptors; Signal transduction; Protein kinases; *Xenopus* oocytes; Arrhythmias; Patch clamp

1. Introduction

Cardiac inwardly rectifying potassium current I_{K1} is of major importance in the terminal phase of repolarisation and it is essential to maintain the resting membrane potential (RMP) of cardiomyocytes [1]. As a consequence, inhibition of I_{K1} prolongs cellular action potentials and disturbs the stability of RMP favouring irregular afterdepolarisations. In animal models, suppression of I_{K1} by gene transfer induces ectopic focal activity and may even be suitable to generate a "biological pacemaker" [2,3].

A reduction of I_{K1} current density is observed in patients suffering from Andersen's syndrome, a channelopathy caused by mutations in Kir2.1 inwardly rectifying potassium channels [4]. Because of the broad spectrum of physiological processes

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affected by Kir2.1 dysfunction, Andersen's syndrome is a multi-system disorder that may be associated with periodic paralysis, electrolyte disturbances, skeletal dysmorphisms, mild cognitive impairment and arrhythmias [4]. Notably, the cardiac manifestation has been classified as Long QT Syndrome Type 7 (LQT-7) characterised by QT prolongation, complex ventricular ectopy and polymorphic ventricular tachycardia [5]. Interestingly, I_{K1} has also been found to be downregulated in electrical remodelling in chronic heart failure [6].

The exact molecular composition of I_{K1} has not been fully elucidated yet. However, there is an increasing body of evidence that a major part of the current is formed by three closely related potassium channels: Kir2.1 (KCNJ2), Kir2.2 (KCNJ12) and Kir2.3 (KCNJ4) [7–11]. Kir2.1-knockout mice do not have measurable I_{K1} currents whereas knockout of Kir2.2 leads to a reduction of I_{K1} by 50% [7]. Kir2.2 is the main component of I_{K1} current in guinea pig ventricle [8]. Kir2.1 and Kir2.2 have also been reported to be predominant in human and rabbit ventricle [9–11]. Kir2.3 probably has a less prominent role in the mammalian heart, but there are reports of a higher expression in human atrium and in sheep heart [10,12].

Adrenergic α_1 receptors are expressed in human heart and are involved in the process of cardiac hypertrophy [13]. Among the three subtypes (α_{1A}, α_{1B} and α_{1D}), α_{1A} receptors (formerly classified as α_{1c}) show the most abundant cardiac expression and are likely to be functionally predominant [13]. It has been demonstrated in native cardiomyocytes from human atria that activation of adrenergic α_1 receptors induces inhibition of inwardly rectifying I_{K1} current [14]. The same regulation was also observed in mammalian ventricular myocytes [15–18]. The underlying signal transduction pathways appear to be complex as both PKC-dependent and PKC-independent effects have been reported by different research groups [14–18]. Recently, Sosunov and co-workers [18] demonstrated that phenylephrine potentiates the occurrence of ventricular arrhythmias in an established animal model due to an inhibition of I_{K1} currents in susceptible animals.

Thus, inhibition of native cardiac I_{K1} currents by adrenergic α_1 receptors is well documented and has been linked to arrhythmogenesis in an animal model. In this study, we sought to investigate the contribution of different Kir2.x channel subunits to this regulation and the underlying signal transduction. Our results indicate that Kir2.x channels are differentially regulated by adrenergic α_{1A} receptors *via* both protein kinase C-dependent and src tyrosine kinase-dependent pathways.

2. Materials and methods

2.1. Solutions and drug administration

Two-microelectrode voltage clamp measurements of *Xenopus* oocytes were performed in a low K^+ solution containing (in mmol/l) 5 KCl, 100 NaCl, 1.5 $CaCl_2$, 2 $MgCl_2$ and 10 HEPES (pH 7.4). Current and voltage electrodes were filled with 3 mol/l KCl solution. All measurements were carried out at room temperature (20 °C) as described previously [19,20]. Patch clamp measurements of rat ventricular cardiomyocytes were performed as described previously [19,21,22] in a bath solution containing (in mmol/l) 3.5 KCl, 140 NaCl, 1.5 $CaCl_2$, 1.4 $MgSO_4$ and 10 HEPES (pH 7.4), as well as 10 μmol/l nisoldipine to block calcium currents, 10 μmol/l chromanol 293B to inhibit the slow component I_{Ks} of the delayed rectifier potassium current, 1 μmol/l dofetilide to block the rapid component I_{Kr} of the delayed rectifier potassium current and 1 μmol/l glibenclamide to inhibit ATP-dependent $I_{K(ATP)}$. The pipette solution contained (in mmol/l) 140 KCl, 0.5 $CaCl_2$, 1.5 $MgCl_2$, 5 KATP, 1 EGTA and 10 HEPES (pH 7.4). The high concentration of ATP served to contribute to full inhibition of $I_{K(ATP)}$.

Phenylephrine (PE; Sigma, Germany) was dissolved in DMSO to stock solutions of 10 mmol/l and stored at −20 °C. Protein kinase inhibitors staurosporine, chelerythrine, KT-5720, KN-93, PP2, genistein, PP3, genistin and Ro-32-0432 (all purchased from Sigma or Calbiochem) were also dissolved in DMSO to stock solutions of 10 mmol/l and stored at −20 °C. Nisoldipine (Sigma), glibenclamide (Sigma) and chromanol 293B (Aventis, Germany) were dissolved in DMSO to stock solutions of 10 mmol/l and stored at −20 °C. Dofetilide (Pfizer Central Research, Sandwich, Kent, England) was dissolved in water to a stock solution of 1 mmol/l and stored at −20 °C. On the day of experiments, aliquots of the stock solution were diluted to the desired concentrations with the bath solution. The maximum concentration of DMSO in the bath had no effect on the measured currents in cardiomyocytes or *Xenopus* oocytes.

2.2. Electrophysiology and data analysis

Cardiomyocytes were isolated from rat ventricles by enzymatic dispersion with the use of collagenase and papain and were measured with the whole-cell patch clamp technique as described previously [19,21,22]. The two-microelectrode voltage-clamp configuration was used to record currents from *Xenopus laevis* oocytes as described previously [19–21]. Only recordings with <10% leak current were considered for data analysis. Statistical data are presented as mean ± standard error (SEM). Statistical significance was evaluated using Student's *t* test for pairwise comparison and ANOVA for comparison of several groups of data. Differences were considered to be significant if the *p*-value was <0.05 and highly significant if the *p*-value was <0.01.

2.3. Mutagenesis and heterologous expression in Xenopus oocytes

Kir2.1 and Kir2.3 clones (GenBank accession no. U12507 and U07364) were a kind gift of Dr. C.A. Vandenberg (Santa Barbara, USA). Adrenergic α_{1A} receptors (GenBank accession no. NM001957) were a kind gift of Prof. R.W. Graham (Darlinghurst, Australia) and the Kir2.2 clone (GenBank accession no. L36069) of Dr. B.A. Wible (Cleveland, USA). Complementary RNA was prepared as described previously [19,20]. Generation of mutant Kir2.2 channels used in this study has already been reported [19,20]. Injection of cRNA (50 to 500 ng/μl) into stage V and VI defolliculated oocytes was performed using a Nanoject automatic injector (Drummond,