Biochemical Pharmacology 150 (2018) 120–130

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Regulation of Kv4.3 and hERG potassium channels by KChIP2 isoforms and DPP6 and response to the dual K^+ channel activator NS3623

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ARTICLE INFO

Keywords:
Heart
Action potential
Potassium ion channels
Transient outward current
hERG
KChIP2
DPP6
NS3623

ABSTRACT

Transient outward potassium current (I_{to}) contributes to early repolarization of many mammalian cardiac action potentials, including human, whilst the rapid delayed rectifier K^+ current (I_{Kr}) contributes to later repolarization. Fast I_{to} channels can be produced from the *Shal* family *KCNDE* gene product Kv4.3s, although accessory subunits including KChIP2.x and DPP6 are also needed to produce a near physiological I_{to}. In this study, the effect of KChIP2.1 & KChIP2.2 (also known as KChIP2b and KChIP2c respectively), alone or in conjunction with the accessory subunit DPP6, on both Kv4.3 and hERG were evaluated. A dual I_{to} and I_{Kr} activator, NS3623, has been recently proposed to be beneficial in heart failure and the action of NS3623 on the two channels was also investigated. Whole-cell patch-clamp experiments were performed at 33 ± 1 °C on HEK293 cells expressing Kv4.3 or hERG in the absence or presence of these accessory subunits. Kv4.3 current magnitude was augmented by co-expression with either KChIP2.2 or KChIP2.1 and KChIP2/DPP6 with KChIP2.1 producing a greater effect than KChIP2.2. Adding DPP6 removed the difference in Kv4.3 augmentation between KChIP2.1 and KChIP2.2. The inactivation rate and recovery from inactivation were also altered by KChIP2 isoform co-expression. In contrast, hERG (Kv11.1) current was not altered by co-expression with KChIP2.1, KChIP2.2 or DPP6. NS3623 increased Kv4.3 amplitude to a similar extent with and without accessory subunit co-expression, however KChIP2 isoforms modulated the compound's effect on inactivation time course. The agonist effect of NS3623 on hERG channels was not affected by KChIP2.1, KChIP2.2 or DPP6 co-expression.

1. Introduction

About 10 distinct potassium (K^+) channels participate in the repolarization of cardiac action potentials (APs) [1]. However, how they map into the net AP repolarizing current is complicated; in the ventricles, the rapid and slow delayed rectifier K^+ currents (I_{Kr} and I_{Ks}) influence AP repolarization over plateau voltages, whilst the inward rectifier K^+ current (I_{K1}) is involved in both setting the resting potential and mediating the final repolarization phase of the AP [1,2]. The transient outward K^+ current, I_{to}, contributes to phase 1 repolarization but will also affect later repolarization phases of the AP by modifying the time- and voltage-dependent recruitment of other K^+ currents (such as I_{Kr}, I_{Ks}) as well as L-type Ca^{2+} current ($I_{Ca,L}$) [2]. In addition, I_{to} will affect NCX current via effects on $I_{Ca,L}$ dependent Ca^{2+} release as well as via Ca-dependent inactivation of $I_{Ca,L}$ [3–5]. Native I_{to} has components with fast and slow recovery kinetics ($I_{to,f}$ and $I_{to,s}$ respectively) and *KCND2* (Kv4.2) and *KCND3* (Kv4.3) underlie $I_{to,f}$, while *KCNA4* (Kv1.4) is responsible for $I_{to,s}$ [2,6].

The normal physiological behaviour of many cardiac K^+ channels appears to require both pore-forming (α) and accessory (β) subunits to be co-expressed and associated [7]. Native $I_{to,f}$ channels require interactions between α-subunits and K^+ Channel interacting Protein 2 (KChIP2) β-subunits, but other proteins such as DPP6 and members of the *KCNE* family may also modulate the current [6,8–11]. Two splice variants of KChIP2 (called KChIP2L and KChIP2S) were discovered by RT-PCR cloning [12] and the shorter form KChIP2S (also called KChIP2 isoform-1 or KChIP2.1) was identified as the predominant isoform in human heart [13]. Additional expression cloning from human revealed another splice variant of *KChIP2*, KChIP 2.2 which was 32 amino acids shorter than KChIP2.1 and which, like KChIP 2.1, also increased Kv4.2 channel cell-surface expression and slowed inactivation [14] (for a review of KChIP isoforms and nomenclature see Table 1 in [15]). KChIP2.1 and KChIP2.2 are produced by alternative splicing from the *KChIP2* gene removing exons 3 and 2 + 3 to produce isoforms coding of 252 and 220 amino acids respectively [16].

The AP depolarization also activates I_{Kr} which plays a key role in determining action potential duration [17]. Recordings at physiological temperature from recombinant hERG channels (Kv11.1) expressed in

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https://doi.org/10.1016/j.bcp.2018.01.036
Received 25 October 2017; Accepted 22 January 2018
Available online 31 January 2018

Table 1
Comparison of biophysical parameters for HEK 293 cells expressing recombinant Kv4.3 alone or in the presence of KChIP2 isoforms and/or DPP6.

	Kv4.3	KChIP2.2	KChIP2.1	KChIP2.2/DPP6	KChIP2.1/DPP6
$I_{pA/pF}$ @ +40 mV[#]	453 ± 62 (n = 33)	780 ± 87 (n = 16)[§]	1044 ± 88 (n = 13)[§†]	873 ± 79 (n = 14)[§]	828 ± 107 (n = 15)[§]
Recovery τ_{rec} (ms)[#]	46.10 ± 6.19 (n = 18)	5.88 ± 0.55 (n = 12)[§]	11.99 ± 1.8 (n = 13)[§]	5.30 ± 0.47 (n = 13)[§]	8.21 ± 1.03 (n = 14)[§]
Decay τ_f (ms)[#]	11.16 ± 0.89 (n = 23)[‡]	13.82 ± 0.74 (n = 12)[‡]	21.24 ± 2.88 (n = 12)	9.17 ± 0.59 (n = 12)[‡]	10.52 ± 1.10 (n = 13)[‡]
Decay τ_s (ms)[#]	84.7 ± 4.9 (n = 23)	132.8 ± 17.3 (n = 12)[§]	146.4 ± 23.9 (n = 12)[§]	82.6 ± 4.1 (n = 12)[†]	71.2 ± 6.9 (n = 13)[†‡]
$I_{pA/pF}$ +NS3623 @ +40 mV[#]	567 ± 87 (n = 16)	796 ± 145 (n = 9)	1142 ± 124 (n = 10)[§]	948 ± 90 (n = 12)[§]	917 ± 95 (n = 10)[§]
Recovery τ_{rec} (ms) +NS3623[#]	146.2 ± 18.1 (n = 14)	21.3 ± 3.8 (n = 9)[§]	30.9 ± 3.4 (n = 10)[§]	19.4 ± 5.1 (n = 8)[§]	31.4 ± 5.4 (n = 9)[§]
Decay τ_f (ms) +NS3623[#]	9.85 ± 0.69 (n = 14)	19.37 ± 1.03 (n = 9)[§,‡]	26.8 ± 3.42 (n = 10)[§]	14.54 ± 0.97 (n = 12)[‡]	14.83 ± 1.13 (n = 11)[‡]
Decay τ_s (ms) +NS3623	65.1 ± 2.53 (n = 14)	71.3 ± 7.1 (n = 9)	86.8 ± 9.6 (n = 10)[§]	60.3 ± 4.2 (n = 12)[‡]	67.4 ± 4.7 (n = 11)
% Change ($I_{pA/pF}$)	144 ± 5% P = .0001	131 ± 2% P = .0078	132 ± 2% P = .002	141 ± 3% P = .0002	129 ± 3% P = .002
Fold change (τ_{rec})[*]	3.83 ± 0.57 P = .0001	4.68 ± 0.39 P = .0039	3.84 ± 0.47 P = .0002	3.04 ± 0.65 P = .0078	3.72 ± 0.40 P = .0039
% Change (τ_f)[*#]	92 ± 5% P = .11	135 ± 6%[§] P = .0078	132 ± 8%[§] P = .0098	159 ± 6%[§] P < .0005	156 ± 15%[§] P = .001
% Change (τ_s)[*]	77 ± 3% P = .0001	51 ± 8% P = .0039	69 ± 12% P = .054	73 ± 4% P = .001	98 ± 9%[†‡] P = .557
Fold change (AUC)[#¥]	1.27 ± 0.06	1.50 ± 0.07 P = 0.769	1.45 ± 0.11 P = 0.865	2.09 ± 0.22 P = 0.0005	2.26 ± 0.18 P < 0.0001

[#] One-way ANOVA.
[*] Wilcoxon matched-paired signed rank test.
[§] Compared to Kv4.3.
[†] Compared to KChIP2.2.
[‡] Compared to +KChIP2.1.
[¥] P values shown are against the extent of increase in current integral (AUC) due to NS3623 compared to Kv4.3 alone.

mammalian cells closely approximate native I_{Kr} [18]. The accessory subunits of native I_{Kr} channels have been a matter of some debate as hERG can co-assemble with both KCNE1 and KCNE2 and clinically observed mutations in these subunits can influence hERG current and the channel's pharmacological sensitivity [19–21]. The potassium channel regulatory protein KCR1 has also been shown experimentally to influence drug sensitivity of hERG channels [22] and the possible interaction with other K^+ channel regulatory units is uncertain. Recent data suggest that hERG channel current magnitude is influenced by Kv4.3 co-expression [23], but no such information exists for I_{to} beta subunits. KChIP2 has recently been identified to act as a core transcriptional regulator of cardiac excitability [24]. That KChIP may also effect other K channels (such as hERG/Kv11.1) is suggested by the observation that KChIP knockdown in myocytes (from guinea pig) that do not express I_{to} increases action potential duration [25]. While this did not seem to be associated with a detectable change in I_{Kr}, the natural low level of expression of KChIP2 in guinea pig leaves open the possibility of interactions at higher levels of expression. The promiscuous nature of KChIP2 interactions is further underscored by data demonstrating altered L-type Ca current magnitude in murine myocytes lacking KChiP2 and a direct interaction between KChiP2 and Cav1.2 [26]. Such interactions could have important ramifications for drug design in the future and need evaluation.

Small molecule activators of cardiac K^+ channels that both increase repolarizing current and prolong post-repolarisation refractoriness have the potential to offer novel antiarrhythmic actions [27]. Furthermore, activators of I_{to} may restore early repolarization and inhibit dyssynchronous Ca^{2+} release that occurs consequent to loss of the early repolarization notch of the AP in heart failure [4,5]. A prototypical I_{to} activator, NS5806, has been shown to enhance native ventricular I_{to} in dog and rabbit and it also increases recombinant Kv4.2 and 4.3 currents [28–31]. The agonist effect of NS5806 on Kv4.x channels requires the presence of KChIP2.1 [32]. Canine atrial I_{to} is augmented by NS5806 to a much smaller extent than ventricle, whilst rabbit atrial I_{to} is paradoxically inhibited by the compound [31,33]. NS5806 also has an off-target effect of atrial-selective Na channel inhibition [31,33], and a related compound, NS3623, has been reported to be a dual activator of I_{Kr} and I_{to} and to increase repolarization reserve in cellular and multicellular canine preparations[29]. NS3623 was originally described as a chloride channel inhibitor [34] but it also activates hERG/I_{Kr} [35]. Paradoxically, NS3623 had no significant effect on Kv4.3 channels expressed in *Xenopus* oocytes at 30 μM [35], despite effects on epicardial AP notch, J wave amplitude and increasing I_{to} (and I_{Kr}) in canine preparations (at 1–5 μM) [29]. These conflicting results led us to hypothesize that the lack of sensitivity of Kv4.3 to NS3623 could be due to the absence of KChIP2/DPP6 in the aforementioned oocyte experiments [35] or that the augmentation of I_{to} [29] might be due to some other mechanism that affects Kv4.3 current density rather than an effect on the channel (and/or Kv4.3 β-subunits) *per se*.

The present study had three aims: first, to compare the gating properties of Kv4.3 co-expressed with either KChIP2.1 or KChIP2.2 with and without DPP6. Second, to examine the effects of NS3623 on Kv4.3 and hERG, in both the presence and absence of KChIP2 and DPP6 in a mammalian cell expression system. Finally, to examine the possibility that KChIP2.1, KChIP2.2 and DPP6 expression may affect Kv11.1 (hERG) by transfecting each β-subunit (along with GFP protein as a reporter) in a stably transfected mammalian (HEK 293) cell line which expresses hERG channels.

2. Methods

2.1. DNA constructs

The cDNA constructs coding for human short Kv4.3 isoform 1 precursor (*KCND3* gene cloned in pcDNA3, NCBI reference sequence NM_172198.2) [36] and KChIP2 variant 2 (*KCNIP2* gene cloned in pcDNA3, NCBI reference sequence NM_173195.2) [14], herein designated as KChIP2.2 (220 amino acid long) were kindly provided by