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RESEARCH PAPER

Bepridil up-regulates cardiac Na^+ channels as a long-term effect by blunting proteasome signals through inhibition of calmodulin activity

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Background and purpose: Bepridil is an anti-arrhythmic agent with anti-electrical remodelling effects that target many cardiac ion channels, including the voltage-gated Na^+ channel. However, long-term effects of bepridil on the Na^+ channel remain unclear. We explored the long-term effect of bepridil on the Na^+ channel in isolated neonatal rat cardiomyocytes and in a heterologous expression system of human $Na_v1.5$ channel.
Experimental approach: Na^+ currents were recorded by whole-cell voltage-clamp technique. Na^+ channel message and protein were evaluated by real-time RT-PCR and Western blot analysis.
Key results: Treatment of cardiomyocytes with 10 $\mu mol \cdot L^{-1}$ bepridil for 24 h augmented Na^+ channel current (I_{Na}) in a dose- and time-dependent manner. This long-term effect of bepridil was mimicked or masked by application of W-7, a calmodulin inhibitor, but not KN93 [2-[N-(2-hydroxyethyl)-N-(4-methoxy benzenesulphonyl)]-amino-N-(4-chlorocinnamyl)-N-methylbenzylamine], a Ca^{2+}/calmodulin-dependent kinase inhibitor. During inhibition of protein synthesis by cycloheximide, the I_{Na} increase due to bepridil was larger than the increase without cycloheximide. Bepridil and W-7 significantly slowed the time course of $Na_v1.5$ protein degradation in neonatal cardiomyocytes, although the mRNA levels of $Na_v1.5$ were not modified. Bepridil and W-7 did not increase I_{Na} any further in the presence of the proteasome inhibitor MG132 [N-[(phenylmethoxy)carbonyl]-L-leucyl-N-[(1S)-1-formyl-3-methylbutyl]-L-leucinamide]. Bepridil, W-7 and MG132 but not KN93 significantly decreased 20S proteasome activity in a concentration-dependent manner.
Conclusions and implications: We conclude that long-term exposure of cardiomyocytes to bepridil at therapeutic concentrations inhibits calmodulin action, which decreased degradation of the $Na_v1.5$ α-subunit, which in turn increased Na^+ current.
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Keywords: bepridil; Na^+ channel; anti-electrical remodelling; calmodulin; post-transcriptional; proteasome

Abbreviations: AF, atrial fibrillation; CaM, calmodulin; CaM-K, Ca^{2+}/CaM-dependent kinase; HEK, human embryonic kidney; *I–V*, current–voltage; I_{Na}, Na^+ channel current; KN93, 2-[N-(2-hydroxyethyl)-N-(4-methoxy benzenesulphonyl)]-amino-N-(4-chlorocinnamyl)-N-methylbenzylamine; MG132, N-[(phenylmethoxy) carbonyl]-L-leucyl-N-[(1S)-1-formyl-3-methylbutyl]-L-leucinamide; SDS, sodium dodecyl sulphate; SR, sinus rhythm

Introduction

Bepridil is known as a powerful anti-arrhythmic agent with anti-anginal properties (Hollingshead *et al.*, 1992; Prystowsky, 1992). Although bepridil is primarily classified as a Ca^{2+} channel antagonist, it is reported to block many cardiac ion channels including the slow (I_{Ks}), rapid (I_{Kr}), and ultrarapid (I_{Kur}) delayed rectifier K^+ channels (Wang *et al.*, 1999; Kobayashi *et al.*, 2001; Kamiya *et al.*, 2006), the ATP-sensitive K^+ (I_{KATP}) channel (Sato *et al.*, 2006), the Na^+-activated K^+ (I_{KNa}) channel (Li *et al.*, 1999; Sato *et al.*, 2006), the L- and T-type Ca^{2+} channels (Yatani *et al.*, 1986; Uchino *et al.*, 2005) and the Na^+ channel (Nawada *et al.*, 1995; Sato *et al.*, 1996). Probably because of its multi-channel blocking properties, bepridil is effective for the treatment of intractable cardiac arrhythmias including ventricular tachycardia (Levy *et al.*, 1984; Brembilla-Perrot *et al.*, 1992; Izumi *et al.*, 2007) and persistent atrial fibrillation (AF) (Nakazato *et al.*, 2005;Miyaji *et al.*, 2007). Furthermore, several recent reports have demonstrated that bepridil exhibits anti-electrical remodelling effects in the heart (Fujiki *et al.*, 2003; Nishida *et al.*, 2007), similar to those of amiodarone in clinical and experimental models of AF

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