RESEARCH PAPER

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

L Kang, MQ Zheng, M Morishima, Y Wang, T Kaku and K Ono

Department of Pathophysiology, Oita University School of Medicine, Oita, Japan

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\textsuperscript{+} channel. However, long-term effects of bepridil on the Na\textsuperscript{+} channel remain unclear. We explored the long-term effect of bepridil on the Na\textsuperscript{+} channel in isolated neonatal rat cardiomyocytes and in a heterologous expression system of human Na\textsubscript{1.5} channel.

Experimental approach: Na\textsuperscript{+} currents were recorded by whole-cell voltage-clamp technique. Na\textsuperscript{+} channel message and protein were evaluated by real-time RT-PCR and Western blot analysis.

Key results: Treatment of cardiomyocytes with 10 \textmu m\textsuperscript{-1} bepridil for 24 h augmented Na\textsuperscript{+} channel current (\textit{i}_{\text{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\textsuperscript{2+}/calmodulin-dependent kinase inhibitor. During inhibition of protein synthesis by cycloheximide, the \textit{i}_{\text{Na}} increase due to bepridil was larger than the increase without cycloheximide. Bepridil and W-7 significantly slowed the time course of Na\textsubscript{1.5} protein degradation in neonatal cardiomyocytes, although the mRNA levels of Na\textsubscript{1.5} were not modified. Bepridil and W-7 did not increase \textit{i}_{\text{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\textsubscript{1.5} \alpha-subunit, which in turn increased Na\textsuperscript{+} current. British Journal of Pharmacology (2009) 157, 404–414; doi:10.1111/j.1476-5381.2009.00174.x; published online 9 April 2009

Keywords: bepridil; Na\textsuperscript{+} channel; anti-electrical remodelling; calmodulin; post-transcriptional; proteasome

Abbreviations: AF, atrial fibrillation; CaM, calmodulin; CaM-K, Ca\textsuperscript{2+}/CaM-dependent kinase; HEK, human embryonic kidney; \textit{l}–\textit{v}, current–voltage; \textit{i}_{\text{Na}} Na\textsuperscript{+} 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 \textit{et al.}, 1992; Prystowsky, 1992). Although bepridil is primarily classified as a \textit{Ca}\textsuperscript{2+} channel antagonist, it is reported to block many cardiac ion channels including the slow (\textit{i}_{\text{Ks}}), rapid (\textit{i}_{\text{Kr}}), and ultrarapid (\textit{i}_{\text{Kur}}) delayed rectifier K\textsuperscript{+} channels (Wang \textit{et al.}, 1999; Koba- yashi \textit{et al.}, 2001; Kamiya \textit{et al.}, 2006), the ATP-sensitive K\textsuperscript{+} (\textit{i}_{\text{KATP}}) channel (Sato \textit{et al.}, 2006), the Na\textsuperscript{+}-activated K\textsuperscript{+} (\textit{i}_{\text{Na}}) channel (Li \textit{et al.}, 1999; Sato \textit{et al.}, 2006), the L- and T-type Ca\textsuperscript{2+} channels (Yatani \textit{et al.}, 1986; Uchino \textit{et al.}, 2005) and the Na\textsuperscript{+} channel (Nawada \textit{et al.}, 1995; Sato \textit{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 \textit{et al.}, 1984; Brembilla-Perrot \textit{et al.}, 1992; Izumi \textit{et al.}, 2007) and persistent atrial fibrillation (AF) (Nakazato \textit{et al.}, 2005; Miyaji \textit{et al.}, 2007). Furthermore, several recent reports have demonstrated that bepridil exhibits anti-electrical remodelling effects in the heart (Fujiki \textit{et al.}, 2003; Nishida \textit{et al.}, 2007), similar to those of amiodarone in clinical and experimental models of AF...