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Characterization and molecular basis for the block of Kv1.3 channels induced by carvedilol in HEK293 cells

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ABSTRACT

Carvedilol is a non-selective β-adrenoreceptor antagonist and exhibits a wide range of biological activities. The voltage-gated K^+ (Kv) channel is one of the target ion channels of this compound. The rapidly activating Kv1.3 channel is expressed in several different tissues and plays an important role in the regulation of physiological functions, including cell proliferation and apoptosis. However, little is known about the possible action of carvedilol on Kv1.3 currents. Using the whole-cell configuration of the patch-clamp technique, we have revealed that exposure to carvedilol produced a concentration-dependent blocking of Kv1.3 channels heterologously expressed in HEK293 cells, with an IC_{50} value of 9.7 μM. This chemical decelerated the deactivation tail current of Kv1.3 currents, resulting in a tail crossover phenomenon. In addition, carvedilol generated a markedly hyperpolarizing shift (20 mV) of the inactivation curve, but failed to affect the activation curve. Mutagenesis experiments of Kv1.3 channels identified G427 and H451, two related sites of TEA block, as important residues for carvedilol-mediated blocking. The present results suggest that carvedilol acts directly on Kv1.3 currents by inducing closed- and open-channel block and helps to elucidate the mechanisms of action of this compound on Kv channels.

1. Introduction

Functional Kv1.3 is composed of a homotetramer of pore forming α subunits, each of which comprises six transmembrane helices, arranged around a central K^+-selective pore (Wulff et al., 2009). Expression of Kv1.3 channels is detected in distinct cells, including T and B lymphocytes (Wulff et al., 2004), osteoclasts (Arkett et al., 1994), neurons (Tubert et al., 2016), and smooth muscle cells (Perez-Garcia et al., 2018). Accumulated evidence indicates that Kv1.3 channels exhibit multiple regulatory actions in setting the resting membrane potential, apoptosis, cell volume and proliferation (Cahalan and Chandy, 2009; Deutsch and Chen, 1993; Gulbins et al., 2010; Liu et al., 2002). As a predominant Kv channel in T lymphocyte, Kv1.3 channels could be potently blocked by immunosuppressants, and accordingly its inhibition leads to a reduction of the proliferation in effector memory T (T_{EM}) cells (Wulff et al., 2001). Thus, Kv1.3 channels are taken as a substantial therapeutic target for the treatment of autoimmune diseases, such as multiple sclerosis (Rus et al., 2005) and rheumatoid arthritis (Beeton et al., 2006). This channel is also detected in insulin-sensitive tissues such as adipose tissue, liver and skeletal muscle and therefore is considered as a potential drug target in diabetic therapy (Choi and Hahn, 2010). In addition, Kv1.3 channels have been considered a potentially new molecular target in both the diagnostics and therapy of some cancer diseases (Teisseyre et al., 2015). The inhibition of Kv1.3 channel activity has a beneficial action on above diseases. Hence, extensive works have been conducted to explore inhibitors of Kv1.3 channels as a treatment of related diseases. Concurrently, it is gradually revealed that Kv1.3 channels are also suppressed by chemicals that are originally assumed with other specific effects, such as verapamil (Kuras and Grissmer, 2009) and clofazimine (Faouzi et al., 2015). Nevertheless, only limited data are available for the molecular explanation of the interaction between drugs and Kv1.3 channels. To avoid unwanted side actions and reveal new pharmacological effects, it is worth exploring more about the mechanism of effects of drugs on Kv1.3 channels.

Carvedilol is an α- and β-adrenoceptor antagonist that has been in clinical practice in patients with hypertension (McTavish et al., 1993) or myocardial infarction (Ruffolo and Feuerstein, 1997). Besides blocking adrenergic receptors, this drug also has multiple biological actions, such as scavenging free radicals (Yue et al., 1992), as well as protecting against apoptosis, inflammation and mitochondrial damage (Abreu et al., 2000; Savitz et al., 2000). A growing body of evidence

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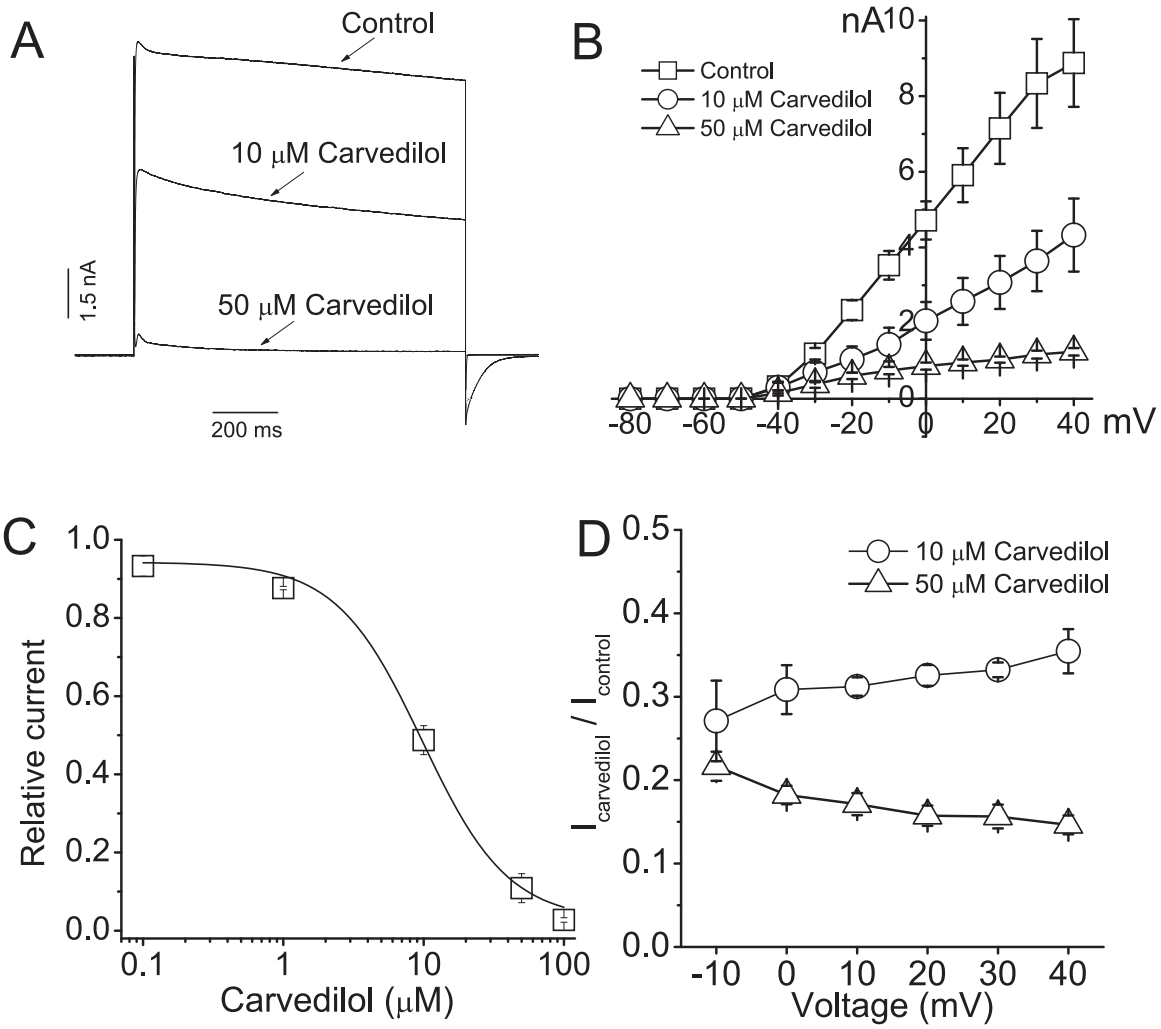

Fig. 1. Blocking of Kv1.3 currents in HEK293 cells by carvedilol. (A) Representative Kv1.3 current traces were shown under control conditions and treatment with 10 and 50 μM carvedilol. Currents in HEK293 cells were routinely evoked in response to 1 s voltage pulses from a holding potential of − 80 mV, to test potentials of + 60 mV. (B) Plot of current-voltage (*I - V*) relationships of Kv1.3 currents before and after exposure to 10 μM and 50 μM carvedilol ($n = 10$). (C) The relative currents in the presence of different concentrations of carvedilol have been obtained, and the solid line was the concentration-response relations of carvedilol action on Kv1.3 currents, which was generated by fitting data points of relative currents using Hill equation ($n = 6$). (D) Normalized block shown as relative current ($I_{carvedilol}/I_{control}$) with 10 μM and 50 μM carvedilol was plotted against the varying voltages ($n = 6$).

suggests that Kv channel is one of its targets in distinct tissues. For example, several components of cardiac outward K^+ currents, including the slowly and rapidly activating delayed rectifier currents (IKr and IKs) as well as the ultrarapid activating delayed currents (IKur) (Deng et al., 2007), are suppressed by carvedilol. Treatment with carvedilol also inhibits the transient outward K^+ currents (Ito), which play important roles in the phase 1 repolarization of cardiac action potential (Liu et al., 1993). Nevertheless, there is not any report for the interaction between carvedilol and Kv1.3 channels. In the current study, we examine the inhibitory effect of carvedilol on Kv1.3 channels and explore the molecular basis of this action.

2. Materials and methods

2.1. Cell culture

The human embryonic kidney cell line, HEK293, was maintained in modified Dulbecco's medium (MEM) with high glucose containing 10% fetal bovine serum in a humidified, 5% CO_2 incubator at 37 °C. The cultures were passed every 2–3 days after brief trypsin treatment. Cells were plated onto glass coverslips 1 day prior to use for patch-clamp experiments.

2.2. Mutagenesis and transfection

The vectors of pIRES2-EGFP containing the sequence of the human Kv1.3 channels were gifts from Dr. Zhi-Jian Cao (Wuhan University, Wuhan). Mutations to Kv1.3 channels were conducted with the QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). Predicted mutations were clarified by sequence analysis. HEK293 cells transiently expressing Kv1.3 channels were produced as described previously (Chen et al., 2012). Briefly, plasmids with Kv1.3 channels were transfected into HEK293 cells using Lipofectamine 2000 (Life Technologies, Bethesda, MD). At 4 h after treatment, the transfection reagent was replaced with regular culture medium. The next day, cells were allowed to settle on the bottom of recording chamber mounted on an inverted microscope (IX-73, Olympus, Osaka, Japan) for electrical measurements.

2.3. Patch-clamp recording

The whole-cell recording mode of the patch-clamp technique was