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Inhibition by a novel anti-arrhythmic agent, NIP-142, of cloned human cardiac K^+ channel Kv1.5 current

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Abstract

NIP-142 was shown to prolong atrial effective refractory period and to terminate atrial fibrillation and flutter in in vivo canine models. To obtain information on its antiarrhythmic action, we examined the effect of NIP-142 on cloned human cardiac K^+ channel Kv1.5 (hKv1.5) currents stably expressed in a human cell line using whole-cell voltage clamp methods. NIP-142 inhibited the hKv1.5 current in a concentration-dependent and voltage-independent manner. The inhibition was larger at the end of depolarizing pulse than at the outward current peak. The IC_{50} for inhibition of the steady-state phase was 4.75 μM. A cross-over phenomenon was observed when current traces in the absence and presence of NIP-142 were superimposed. Inhibition of hKv1.5 current by NIP-142 was frequency-independent; changing the depolarizing pulse frequencies (0.1, 0.2, 1 Hz) and little effect on the degree of inhibition. NIP-142 decreased the maximal peak amplitude of kHv1.5 current at the first command pulse after 3 min rest in the presence of the drug. These results suggest that NIP-142 has inhibitory effects on the hKv1.5 current through interaction with both open and closed states of the channel, which may underlie its antiarrhythmic activity in the atria. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: NIP-142; Kv1.5; Atria; Atrial fibrillation

Introduction

Atrial fibrillation is one of the most frequent types of arrhythmia and is the major risk factor for thromboembolism, especially cerebral embolism. It is also reported to double the risk of deaths due to cardiovascular diseases (1). At present, atrial fibrillation is treated with class I and class III antiarrhythmic agents (2–4), such as flecainide, propafenone and dofetilide. However, the major problem with these agents are that they also affect ventricular repolariza-

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tions, which may sometimes cause increase in QT interval and life-threating arrhythmia such as *torsades de pointes.* Thus, drugs with atrial selectivity are desired for the treatment of atrial fibrillation.

NIP-142 is a novel benzopyran derivative shown to terminate arrhythmias both with canine vagal stimulation-induced atrial fibrillation model (5) and with Y-shaped incision-induced atrial flutter model (6). These effects are attributed to prolongation of atrial effective refractory period (ERP). Interestingly, NIP-142 was shown to prolong ERP in atrium, but not in ventricle (5, 7). Thus, NIP-142 appears to be an ideal leading compound for the development of therapeutic agents against atrial fibrillation, but the precise mechanisms of its antiarrhythmic activity with atrium specific nature has not yet been clarified.

The ultra-rapidly activating delayed rectifier potassium current (I_{kur}) has been shown to be present in atrial myocytes of many species including human, dog and rat (8–11). I_{kur} is considered to play an important role in atrial repolarization and to be a determinant of the refractory period (10, 11). The I_{kur} in human atrial myocyte is electrophysiologically and pharmacologically similar to the current carried by Kv1.5 channels cloned from the human heart (hKv1.5) (10). The presence of the hKv1.5 channel protein in human myocardium was confirmed immunohistologically (12). Accordingly, the hKv1.5 channel is considered to be the molecular identity of I_{kur} in human myocardium. Antiarrhythmic agents used in the treatment of atrial fibrillation, e.g. quinidine and propafenone, inhibit I_{kur} in human atrial myocytes (13, 14) and the hKv1.5 current (15, 16) at clinically relevant concentrations. Since NIP-142 was shown to inhibit I_{kur} in human atrial myocytes (17), the hKv1.5 channel protein could be considered as a target for NIP-142 action.

In the present study, we examined the effect of NIP-142 on hKv1.5 current stably expressed in HEK293 cells. We found that NIP-142 concentration-dependently inhibits the current and further studied the mode of its action. A preliminary account of this work was presented at the 73th General Meeting of the Japanese Pharmacological Society in Yokohama, Japan in 2000 (18).

Methods

Cloning of hKv1.5 cDNA and stable expression in HEK293 cells

Human Kv1.5 cDNA fragments were PCR-amplified from a human heart cDNA library (Takara Shuzo Co., Ltd.) with oligonucleotide primers designed based on the published hKv1.5 cDNA sequence (19, GenBank accession number M60451). The cDNA fragments obtained were assembled with standard ligation techniques and the full length hKv1.5 cDNA was inserted into the pIRES2-EGFP expression vector (Clontech). The expression vector with hKv1.5 cDNA was introduced into cultured HEK293 cells with Lipofectamine (Gibco BRL) and stable transformants were obtained by clone culture in the presence of 500 μg/ml G418 (Geneticin; Gibco BRL). Transformed HEK293 cell clones were observed under excitation at 488 nm and hKv1.5 currents were measured as described below. The background current level in non-transfected HEK293 cell was $<$50 pA. Among the EGFP-fluorescence-positive clones expressing the hKv1.5 current, a clone with a current amplitude of about 1 to 5 nA was chosen for analysis.

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