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THE NOVEL CLASS III ANTIARRHYTHMICS NE-10064 AND NE-10133 INHIBIT I_{SK} CHANNELS EXPRESSED IN *XENOPUS* OOCYTES AND I_{KS} IN GUINEA PIG CARDIAC MYOCYTES

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Slowly activating, voltage-dependent I_{sK} channels were expressed in *Xenopus* oocytes after injection of rat I_{sK} protein cRNA and recorded with the two-microelectrode voltage-clamp technique. The I_{sK} currents were inhibited by the new class III antiarrhythmic drugs NE-10064 and NE-10133. These compounds were equally potent in inhibiting a slowly activating potassium current (I_{Ks}) in guinea pig ventricular myocytes. No effects of these compounds could be observed on several other cloned delayed rectifier potassium channels, nor did they affect the inward rectifier current, I_{K1}, in guinea pig cardiac myocytes at the concentrations tested. The blockade of I_{sK} channels may contribute to the class III antiarrhythmic efficacy of these novel antiarrhythmics.

The slow component of the delayed rectifier potassium channel, I_{Ks}, makes a significant contribution to action potential repolarization under certain physiological conditions (i.e. high heart rate or ß-adrenergic tonus). Inhibitors of this channel could thus be extremely useful for antiarrhythmic therapy (1). Certain class III antiarrhythmic drugs such as sotalol and its structural analogue E-4031 have no direct effect on I_{Ks}, but inhibit a more rapidly activating component (I_{Kr}) of the delayed rectifier potassium channel (2). Potential disadvantages of a selective inhibition of I_{Kr} is a loss in efficacy after an increase in heart rate or ß-receptor stimulation, and a possible induction of early after-depolarizations (EAD) at low heart rates (3,4). The basis for the reduced efficacy of I_{Kr} inhibitors at high heart rates or ß-adrenergic

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tonus is an increase in I_{Ks} under such conditions or the activation of a cAMP- and time-dependent Cl^- current (4-6).

A cDNA encoding the I_{sK} protein was first cloned from rat kidney and subsequently from rat, mouse, guinea pig and human heart (7-11). Expression of the I_{sK} protein after injection of its cRNA in *Xenopus* oocytes results in a voltage-dependent potassium current, I_{sK}, with electrophysiological properties similar to the slow component of the delayed rectifier potassium current, I_{Ks}, in guinea pig ventricular myocytes. It was recently shown that the I_{sK} protein underlies the slow component of the delayed rectifier potassium current in guinea pig heart (10). This conclusion is supported by the immunolocalization of the I_{sK} protein in guinea pig heart (12).

A new chlorophenylfuranyl compound (NE-10064) that is structurally unrelated to the methanesulfonanilide class III antiarrhythmic drugs such as sotalol, was found to have class III antiarrhythmic effects in calf heart muscle (13) and antifibrillatory actions in an acute ischemic model in conscious dogs (14). This report describes the properties of two new chlorophenylfuranyl antiarrhythmic compounds (NE-10064 and NE-10133) on I_{sK} expressed in *Xenopus* oocytes and on I_{Ks} in guinea pig ventricular myocytes. Parts of this paper have been presented in abstract form (15).

MATERIALS AND METHODS

The two-microelectrode voltage-clamp configuration was used to record from *Xenopus* oocytes that were previously injected with cRNA encoding the I_{sK} protein (16). Recordings were performed at room temperature. The superfusing solution contained (mM): NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5 (pH 7.4). Chemicals were added from stock solutions into the perfusion solution as indicated. Dissociated cardiocytes (17) were placed in a 0.5 ml recording chamber and continuously superfused with Tyrode solution. Whole cell currents were measured with an Axopatch 1c amplifier (Axon Instruments, USA). The patch pipettes filled with the internal solution had tip resistance of 1.5 to 3 mΩ. Approximately 80% of the series resistance was compensated electronically. After formation of the whole cell recording configuration the holding potential in voltage clamp mode was set to the resting membrane potential recorded in current clamp mode (-72 to -77 mV). Recordings were performed at 31° C. The composition of the Tyrode solution (mM) was: NaCl , 145; KCl, 5; $CaCl_2$, 1.8; $MgCl_2$, 1; glucose, 10; HEPES, 10; the pH was adjusted to 7.35 with NaOH. The internal solution contained (mM): K_2-phosphocreatine, 10; Na_2ATP, 5; K-aspartate, 90; KCl, 39.6; $MgCl_2$, 5.7; and HEPES, 10, 0.1 mM EGTA (free $[Ca^{2+}]_i$: 25 nM), pH of 7.1. Chemicals used were: Isoproterenol (Isuprel), NE-10064: 1-[[[5-(4-chlorophenyl)-2-furanyl] methylene]-amino]-3-[4-(4-methyl-1-piperazinyl)-butyl]-2,4-imidazolidine-dione dihydro-chloride; NE-10133: 1-[[[5-(4-chlorophenyl)-2-furanyl]methylene]-amino]-3-[3-[4-(2-hydr-oxyethyl)-1-piperazinyl]-propyl]-2,4-imidazolidinedione dihydrochloride.

RESULTS AND DISCUSSION

The I_{sK} protein was expressed in *Xenopus* oocytes and the resulting ionic current, I_{sK}, was measured with the two-microelectrode voltage-clamp configuration (16). Application of NE-10064 resulted in a concentration-dependent inhibition of I_{sK}, which reversed only partially during a 30 min washout (Fig. 1A, B). The estimated EC_{50} was 1.8 ± 0.25 μM (n = 4). The compound NE-10133 had similar effects, but was less potent, having an EC_{50} of 12.0 ± 0.24 μM (n = 4; Fig. 1C). In contrast, neither of the two compounds affected the

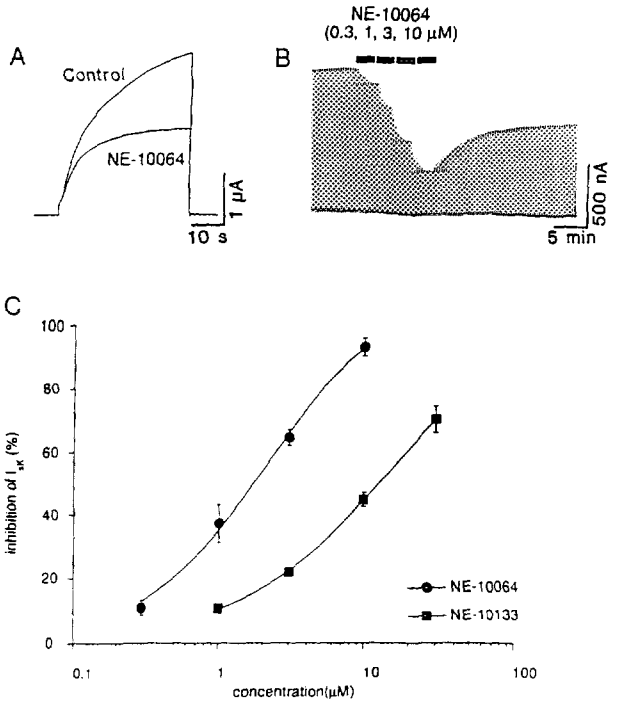

Fig. 1. Effects of NE-10064 on I_{sK} expressed in *Xenopus* oocytes. **A)** Superimposed current traces of 30 s steps to 40 mV, before and 5 min after application of NE-10064 (3 µM). **B)** The upward deflections represent the I_{sK} current amplitude evoked with a 4 s voltage step to 40 mV every 30 s. NE-10064 was applied accumulatively as indicated. **C)** Inhibition curves for NE-10064 and NE-10133 on I_{sK} expressed in *Xenopus* oocytes.

delayed rectifier potassium channels RBK1, RBK2 or RGK5 (18,19) expressed in *Xenopus* oocytes at tested concentrations up to 20 µM (n=5 for each potassium channel; data not shown).

In guinea pig ventricular myocytes the voltage- and time-dependent outward currents I_{Kr} and I_{Ks} have different biophysical properties. I_{Kr} activates more rapidly and its current-voltage relation has a negative slope at potentials positive to 0 mV (2). The slowly activating current I_{Ks} was consequently electrophysiologically isolated with long voltage steps (5 s) to 60 mV. At this potential the contribution of Na^+- or Ca^{2+}-inward currents were minimal. Further, E-4031 (5 µM) had no inhibitory effects on the outward current amplitude under these conditions, confirming that I_{Kr} does not contribute to the outward current at very positive potentials (2).

Figure 2A shows that superfusion with NE-10064 decreased I_{Ks}. The outward current in control solutions increased slowly throughout the 5 s pulse to 60 mV. However, in the presence of NE-10064, the outward current relaxed slightly towards the end of the pulse after reaching a plateau. This relaxation could indicate an open channel block by NE-10064, which is consistent with preliminary findings on human I_{sK} channels expressed in *Xenopus*

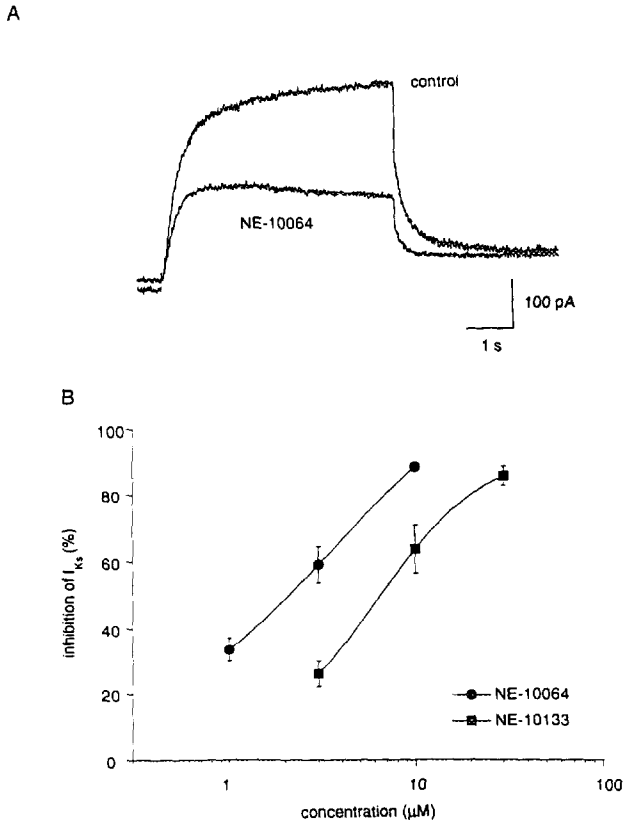

Fig. 2. Effects of NE-10064 on I_{Ks} in guinea pig cardiac myocytes. **A)** I_{Ks} was evoked with 5 s voltage steps to 60 mV from a holding potential of -77 mV, conditions where I_{Kr} does not contribute to the current amplitude. The tail currents were measured at -35 mV. Steady state inhibition with NE-10064 (3 μM) was reached after 5 min of application. **B)** Inhibition curves for NE-10064 and NE-10133 for I_{Ks} in guinea pig cardiac myocytes.

oocytes, where a use- and voltage-dependence of NE-10064 blockade could be demonstrated (20). The dose response of inhibition of I_{Ks} by NE-10064 and NE-10133 is shown in Figure 2B. The estimated EC_{50} was 1.9 ± 0.2 μM (n = 5) and 11.1 ± 1.6 μM (n = 4) for NE-10064 and NE-10133, respectively. The inhibitory potency of these antiarrhythmics for I_{Ks} in ventricular myocytes and for I_{sK} expressed in *Xenopus* oocytes was not significantly different.

The inward rectifying current, I_{K1}, was recorded with fast voltage ramps from -100 to -55 mV (Fig. 3). Superfusion with 3 μM NE-10064 did not inhibit I_{K1} (n=4; Fig. 3).

Action potential prolongation by I_{Kr} inhibitors in guinea pig ventricular myocytes is antagonized by isoproterenol (4). The effect of NE-10064 on I_{Ks} stimulated by ß-adrenergic agonists was thus evaluated. Application of isoproterenol (1 μM) increased I_{Ks} 2.3 fold as previously described (n=5; 17). Subsequent application of NE-10064 (3μM) decreased I_{Ks} by 55 ± 3% (n=5). This shows that the sensitivity of I_{Ks} to blockade by these compounds is

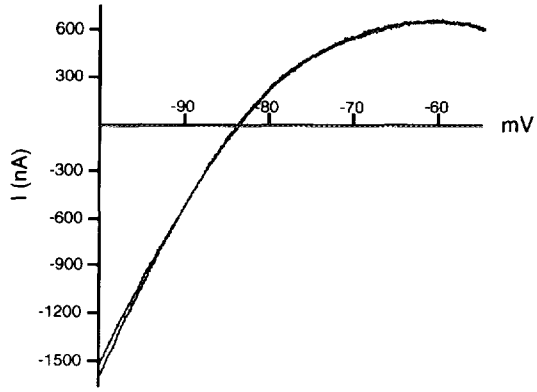

Fig. 3. Lack of effect of NE-10064 (3 μM) on I_{K1}. I_{K1} was evoked with a fast voltage ramp (4.5 mV/10 ms) from -100 to -55 mV. The traces for I_{K1} are identical for control and I_{K1} after superfusion with NE-10064.

the same regardless of the degree of ß-adrenergic tone, a feature which could be of significant therapeutic importance.

The class III antiarrhythmic agents, NE-10064 and NE-10133, are demonstrated to block rat I_{sK} in a *Xenopus* oocytes model and to block the slowly activating component of the delayed rectifier potassium channel, I_{Ks}, in guinea pig cardiac myocytes with similar potency. These results further support the hypothesis that the I_{sK} protein underlies I_{Ks} in guinea pig ventricular myocytes. Further, preliminary results suggest that human I_{sK} channels are blocked with a similar potency by NE-10064 (20). To our knowledge this is the first report of potent I_{Ks} blockers. It will be an important goal to investigate the specificity of these novel class III antiarrhythmics. To this end, we have no indication that NE-10064 does inhibit other potassium conductances, such as the classical delayed rectifier or the inward rectifier, I_{K1}. However, the potassium conductance I_{Kr} was too small in our preparations to test for effects of NE-10064 on this conductance. The potent blockade of the slow component of the delayed rectifier potassium channel in heart by NE-10064 and NE-10133 may play a role for their antiarrhythmic efficacy.

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