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Acetylcholine-Activated Potassium Channel as a Novel Target for AF Treatment

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1. Introduction

Atrial fibrillation (AF) is the most frequently encountered cardiac arrhythmia in the clinical practice with prevalence of over 2,200,000 and 1,300,000 in the United States and Japan, respectively. The prevalence of AF is strongly age dependent; less than 1% of individuals aged <60 years, but approximately 5% of those older than 65 years and about 8% of those older than 80 years are affected by AF (Furberg et al., 1994; Kannel et al., 1998). AF is not lethal arrhythmia but associated with an increased long-term risk of stroke, heart failure, and all-cause mortality (Stewart et al., 2002). One of the most debilitating consequences of AF is the accompanying risk of stroke, which occurs in an estimated 60,000 patients with AF per year in the United States (Go et al., 2001).

Antiarrhythmic drug therapy remains a cornerstone to restore and maintain sinus rhythm for patient both with paroxysmal and persistent AF. However, conventional drugs such as sodium channel blockers (class I antiarrhythmic drugs) and potassium channel blockers (class III antiarrhythmic drugs) have limited efficacy, especially in persistent AF patients. Clinical studies have shown that the potassium channel blockers can terminate AF in only 30% of cases (Ellenbogen et al., 1996; Falk et al., 1997). Sodium channel blockers are more effective than potassium channel blockers, but a potent sodium channel blocker, pilsicainide demonstrated a modest efficacy in converting AF to sinus rhythm, with a conversion rate of 45% within 90 min in patients with recent-onset AF compared with 8.6% on placebo and was ineffective in chronic AF (Okishige at al., 2006). In addition, these conventional drugs have severe problematic risks of ventricular proarrhythmia, such as *torsade de pointes* (TdP) through excessive delay of ventricular replarization (Waldo et al., 1996; Trop-Pederson et al., 1999) and left ventricular contractile dysfunction. Thus, the development of new antiarrhythmic agents which have more effective and safer profile is highly desirable. These days, the pharmaceutical industry has focused on "atrial-specific antiarrhythmic drug" in the search for safer drugs to terminate or prevent AF (Wirth et al., 2003; Vos 2004; Goldstein & Stambler 2005).

The acetylcholine-activated potassium channel (I_{KACh} channel) is one of the most promising targets for atrial-specific antiarrhythmic agent, because the channel is more abundantly expressed in the atrial than ventricle myocytes (Krapivinsky et al., 1995). In this article, the pathophysiolosical roles of I_{KACh} channel in AF are summarized. In

addition, the in vitro and in vivo profiles of I_{KACh} blockers and the usefulness for AF treatment are also discussed.

2. Pathophysiological role of I_{KACh} channel in AF

The cardiac I_{KACh} is a ligand-gated inward rectifier potassium current. The cardiac I_{KACh} channel is heterotetramer of two distinct homologous proteins encoded by GIRK1 and GIRK4 (Kubo et al., 1993; Krapivinsky et al., 1995). The current amplitude is small under basal condition, but it is activated by acetylcholine secreted from the vagal nerve or by adenosine through muscarinic M_2 receptor or adenosine A_1 receptor, respectively. Activated M_2 receptor and A_1 receptor stimulate I_{KACh} via $G_{\beta\gamma}$ protein activation (Fig. 1). Vagal nerve activation, acetylcholine or adenosine treatment shortens atrial effective refractory period (ERP), increases atrial ERP dispersion and promotes AF (Kabell et al., 1994; Liu and Nattel 1997), possibly through the I_{KACh} activation. A report with GIRK4 knockout mice showed that the I_{KACh} channel plays a crucial role in the initiation of AF (Kovoor et al., 2001). In addition, it is well known that the enhancement of vagal nerve tone can lead to paroxysmal AF in human (Chen et al., 1998).

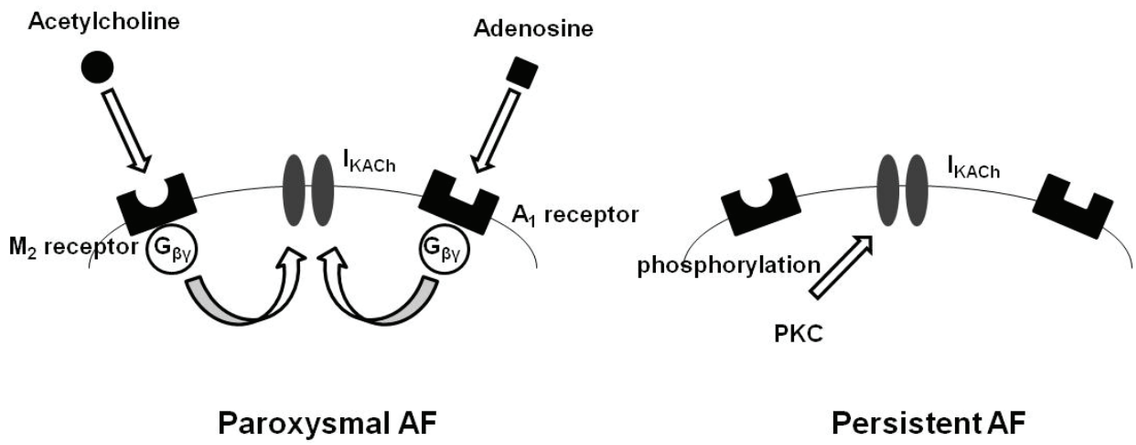

Fig. 1. The different mechanism of I_{KACh} activation mechanism between paroxysmal AF and persistent AF

Although the enhancement of I_{KACh} could be responsible for the initiation of paroxysmal AF in human (Chen et al., 1998), the pathophysiological role of I_{KACh} in patient with persistent AF remains unclear. Dobrev et al. (2001, 2002) showed that the activation of I_{KACh} by muscarinic receptor stimulation is smaller in atria from AF patients compared to those in patients with sinus rhythm. The total acetylcholine-induced inward current in atrial myocytes from AF patients reported to be larger than those from patient with sinus rhythm. However, this increase was due to the increased density of the inward rectifier K^+ current (I_{K1}); the density of I_{KACh} was rather decreased in AF patients than SR patients (Bosch et al., 1999). In addition, reduced muscarinic receptor-related activation of I_{KACh} could be explained by decreased expression of the channel subunits (Dobrev et al., 2001; Brundel et al., 2001a, 2001b). Therefore, it had been thought that the I_{KACh} channel is down-regulated in atrium of persistent AF patients and the effects of the channel blockers might have less effective in persistent AF patients.

However, recent studies suggested that a constitutively active component of I_{KACh} channel exists and it may be upregulated in persistent AF patients. Tertiapin, a selective I_{KACh} blocking peptide, concentration-dependently blocked the whole cell I_{KACh} in persistent AF patients with a higher potency than that in patient with paroxysmal AF or sinus rhythm (Dobrev et al., 2005). The report clearly showed that the basal inward rectifying potassium current was larger in persistent AF patients, although the muscarinic receptor-activated component of I_{KACh} was larger in patients with sinus rhythm or paroxysmal AF. In addition, single channel analysis revealed the presence of constitutively active I_{KACh} channels in persistent AF patients but not in patients with sinus rhythm or paroxysmal AF (Dobrev et al., 2005). Such a tertiapin-sensitive and constitutively active current component contributing to basal outward current was detected in canine left atria and pulmonary vein, and the component was upregulated in preparations from tachycardia-remodeled atria (Ehrlich et al., 2004). In addition, tertiapin prolonged the atrial action potential duration (APD) and prevented AF development in canine tachycardia-remodeled atria (Cha et al., 2006). These results suggest that the receptor-independent I_{KACh} is activated and plays a crucial role in persistent AF patients.

The molecular mechanism underlying constitutive I_{KACh} channel activation in remodeled atria remains unknown. The regulation of I_{KACh} is complex and the channel activity is modulated by levels of intracellular Na^+ concentration (Sui et al., 1996), ATP (Sui et al., 1996; Kim et al., 1997) phosphatidylinositol biphosphate (Huang et al., 1998), and fatty acid (Kim and Pleumsamran, 2000). Recently, Voigt et al. (2007) reported that abnormal PKC function might play important role in the occurrence of constitutive I_{KACh} activation in clinical AF. Although further work will be needed to define the precise signaling mechanisms that enhance the channel activity, modified phosphorylation dependent channel regulation may contribute to the development of constitutive I_{KACh} activity (Fig. 1).

3. In vitro and in vivo profiles of selective I_{KACh} blockers

Several class III antiarrhythmic drugs have been shown to block I_{KACh} in atrial cell (Guillemare et al., 2000; Mori et al., 1995). The blocking effects on I_{KACh} might work additively for their antiarrhythmic efficacy. However, the effects of these drugs on I_{KACh} are not so potent. In addition, the drugs block other cardiac potassium current, including the rapid rectifier potassium current, I_{Kr}. Thus, investigations using more potent and selective blocker are needed to clarify the therapeutic potential, atrial selectivity and safety of I_{KACh} blockers. In this session, the pharmacological profiles of potent I_{KACh} blockers, tertiapin, NIP-141 and NIP-151 are discussed.

3.1 Pharmacological profile of tertiapin, a selective I_{KACh} blocking peptide

Tertiapin is a 21 amino acid residue peptide with two disulfide bonds, isolated from venom of the European honey bee. The peptide was shown to block I_{KACh} potently in cardiac myocyte (Jin and Lu 1998). This peptide inhibits, in a concentration-dependent manner (IC_{50} = ~8 nmol/L), acetylcholine or GTP analogue-induced whole cell current in rabbit atrial myocyte (Kitamura et al., 1994). For the information of selectivity of the peptide, Drici et al. (2000) reported that tertiapin selectively inhibited I_{KACh} at IC_{50} of about 30 nmol/L without significant effects at 1 μmol/L on I_{K1}, I_{Kr}, the transit owtward current (I_{to}), the ultra-rapid

delayed rectifier K+ current (I_{Kur}), the slow component of the delayed rectifier K+ current (I_{Ks}), fast sodium current (I_{Na}) and L-type calcium current (I_{Ca}). Tertiapin was the first reported selective I_{KACh} blocker, so we used this peptide to clarify the pathophysiological role of I_{KACh} in AF.

dose (nmol kg^{-1})	*VNS-induced AF*			*aconitine-induced AF*	
	termination of AF	time to AF termination (s)	AF reinduction	termination of AF	time to AF termination (s)
vehicle	0/4	–	–	0/4	–
4	1/5	102	1/1	1/5	137
12	5/5**	88 ± 22	1/5	3/5	49 ± 5
41	n.t.	–	–	4/4*	41 ± 6

n.t. means not tested. Time of AF termination data were expressed as mean ± S.E.M.
**P<0.01, *P<0.05 vs vehicle-treated group

Table 1. Efficacy of tertiapin in terminating vagal nerve stimulation (VNS)-induced atrial fibrillation (AF) and aconitine- induced AF in dogs (Hashimoto et al., 2006)

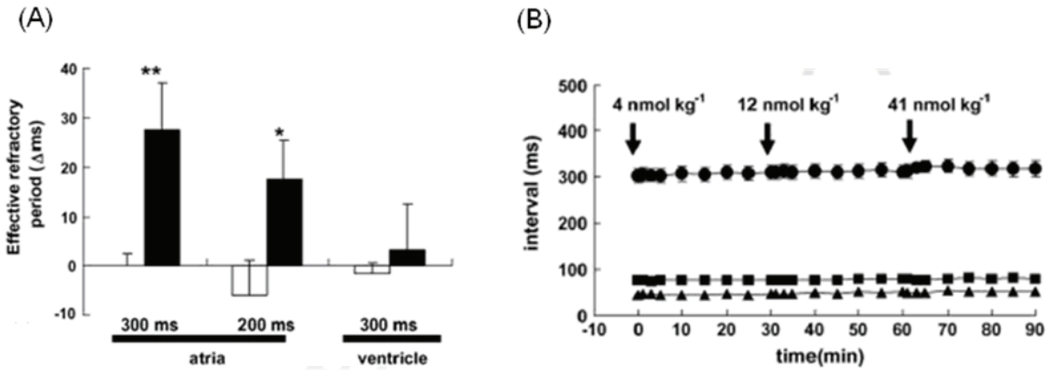

Fig. 2. (A) Effects of tertiapine (12 nmol/kg; solid column) and vehicle (saline; blank column) on atrial and ventricular effective refractory period with vagal nerve stimulation in dogs. Data are expressed as mean ± S.E.M. (n=4) $^{*}P<0.05$, $^{*}P<0.01$ compared with vehicle treated group. (B) Effects of tertiapin (4-41 nmol/kg) on ECG parameters: PQ(■), QRS(▲) intervals and corrected QT value (QTc; ●) in anesthetized beagle dogs. Data are expressed as mean ± S.E.M. (n=4; Hashimoto et al., 2006).

We examined the effects of tertiapin on the two canine AF models: vagal nerve stimulation (VNS)-induced and aconitine-induced AF models, to clarify the antiarrhythmic potential of I_{KACh} blockade. VNS-induced model and aconitine-induced model have been known as different type of paroxysmal AF models; a micro-reentry type AF model and ectopic-focus induced AF model, respectively. Intravenous injection of tertiapin (4-41 nmol/kg) terminated AF in both canine AF models (Hashimoto et al., 2006, Table 1). Tertiapin (12 nmol/kg) significantly prolonged atrial ERP at the basic cycle length of 200 and 300 ms without affecting conduction velocity in atria under VNS condition (Fig. 2A). The result suggests that AF termination by tertiapin is associated with atrial ERP prolongation.

Alternatively, tertiapin at the effective dose ranges (4-42 nmol/kg) had no significant effects on ECG parameters such as PQ, QRS and corrected QT (QTc) intervals and ventricular ERP in anesthetized dogs (Fig. 2B; Hashimoto et al., 2006). These results suggest that I_{KACh} has a crucial role in the maintenance of paroxysmal AF, but has no contribution in ventricular repolarization.

3.2 Pharmacological profile of NIP-142, a multiple channel blocker

The studies using tertiapin clearly suggests that I_{KACh} blocker is effective in some types of AF patients. AF is chronic cardiac disease and the patients have to take medicine for a long term. Thus, the orally active small molecule would be good for the AF treatment. Tertiapin is a peptide and cannot be applied for oral administration. Thus, we conducted the screening of the blocker and found a candidate compound. NIP-142, (3R*, 4S*)-4-cyclopropylamino-3,4- dihydro-2,2-dimethyl-6-(4-methoxyphenylacetylamino)-7-nitro-2H-1-benzopyran-3-ol is synthesized by Nissan Chemical Industries, LTD. (Tanaka & Hashimoto, 2007). The compound has inhibitory effects on multiple potassium channels including I_{KACh} (GIRK1/GIRK4) channel (Table 2). The bioavailability of NIP-142 is about 25-50% in rats and more than 75% in dogs (Tanaka & Hashimoto, 2007), which suggests that the compound would be orally active.

channel	host cell	effect of NIP-142 (IC_{50})	reference
Kv1.5	HEK293	4.8 M	Matsuda et al. 2001
	Xenopus oocyte	100 M	-
GIRK1/GIRK4	HEK293	0.64 M	Matsuda et al. 2006
	Xenopus oocyte	10 M	Matsuda et al. 2005
hERG	HEK293	44 M	Matsuda et al. 2004
KCNQ1/KCNE1	HEK293	12 M	-
Kv4.3, Kv4.2	*Xenopus* oocyte	>100 M	-

Table 2. Inhibitory effects of NIP-142 on membrane currents

3.2.1 Effect of NIP-142 on the membrane currents

Effects of NIP-142 on various outward membrane currents have been studied by voltage clamp analyses (Table 2). The delayed rectifier current has three components: the rapid component, I_{Kr}, the slow component, I_{Ks} and the ultrarapid component, I_{Kur}. The effects on I_{Kr}, I_{Ks} and I_{Kur} were examined with HEK293 cells expressing the human ether a-go-go (hERG) channel, the human KCNQ1/KCNE1 channel and human K_v 1.5 channel, respectively (Matsuda et al., 2001, 2006). NIP-142 concentration-dependently inhibited these three channel currents. The IC_{50} on I_{Kr}, I_{Ks} and I_{Kur} were 44, 12 and 4.8 μmol/L, respectively (Matsuda et al., 2001; 2006). NIP-142 also inhibited the mouse Kv4.2 and Kv4.3 currents but the potency was very low. This compound had a slight effect on I_{K1}: about 40% inhibition was observed with 10 μmol/L NIP-142.

The effect of NIP-142 on I_{KACh} was examined in HEK293 cells (Matsuda et al., 2006) and Xenopus oocytes (Matsuda et al., 2005) expressing the GIRK1/GIRK4 channel. Concentration- dependent inhibition of the current was observed in both types of cells: the inhibitory effect was 8-10 times potent than those on IKur in both cells (Table 2). The

inhibition of GIRK1/GIRK4 current amplitude in HEK293 cells by 0.1, 1 and 10 μmol/L NIP-142 was 28.9 ± 5.3%, 58.4 ± 6.2% and 76.8 ± 8.4%, respectively (Matsuda et al., 2006). The inhibition was independent from the voltage dependency of the voltage clamp pulse. Among the repolarizing potassium channel currents, NIP-142 inhibited the primarily the GIRK1/GIRK4 channel current which is I_{KACh} (Table 2).

Among other ion currents, NIP-142 at 10 μmol/L inhibited the peak inward current by about 50% and 25% for the L-type and T-type voltage-dependent calcium current in isolated guinea pig ventricular myocytes, respectively. At the same concentration, NIP-142 had no effects on the hyperpolarization- activated inward current (I_f) in isolated rabbit sinoatrial node cells.

3.2.2 Effects of NIP-142 on the refractory period and action potential of isolated myocardium

Effects of NIP-142 on the refractory period were studied in isolated guinea pig atrial and ventricular preparations. At 10 and 100 μmol/L, NIP-142 concentration-dependently prolonged the refractory period in atrium but not in ventricle (Matsuda et al., 2005). The action potential was measured with standard glass microelectrode techniques. NIP-142 concentration-dependently prolonged APD only in the atrium. No significant changes in other action potential parameters, such as resting membrane potential, action potential amplitude and maximum rate of rise (Vmax), were observed (Matsuda et al., 2005).

The effects of NIP-142 on refractory period and action potential were different from those of conventional class III agents. An I_{Kr} blocker E-4031 prolonged APD both in the atrium and the ventricle. In contrast, NIP-142 showed atrial selective prolongation of APD. The prolongation was mimicked by a selective I_{KACh} blocking peptide, tertiapin (Matsuda et al., 2005). These data suggested that the prolongation by NIP-142 was due to the I_{KACh} blockade. Stimulation of the muscarinic receptor in the mammalian atria leads to a shortening of APD through activation of I_{KACh}. NIP-142 (100 μmol/L) completely reversed the carbachol-induced shortening of APD in canine (Nagasawa et al., 2006) and guinea-pig (Matsuda et al., 2006) atrium. Such reversal also observed when NIP-142 was applied after the APD was shortened by adenosine (Matsuda et al., 2006), which also activates I_{KACh} independent of muscarinic receptor.

One of the major problems with existing antiarrhythmic drugs through prolongation of refractory period and APD is “reverse use-dependency”, the effect of the drugs are weakened under high stimulation frequency. Such effect is observed both in vitro and in vivo and may explain the ineffectiveness of the drug in high frequency in atrial fibrillation (Nattel, 1999). In guinea-pig atrium, E-4031 at 1 μmol/L prolonged APD when it was applied at pacing frequency less than 1 Hz, but not at frequencies higher than 2 Hz. In contrast, NIP-142-induced prolongation of atrial APD was unaffected by stimulation frequency (Matsuda et al., 2005), suggesting that the APD prolonging effect of the drug may be maintained in atrial fibrillation.

3.2.3 Effects of NIP-142 on AF models

In animal studies, NIP-142 terminated AF in various types of AF models (Table 3). Injection of NIP-142 (3 mg/kg) terminated AF after increase in fibrillation cycle length and prevented reinitiation of AF in VNS-induced model. This compound terminated macroreentry type of atrial flutter induced after placement of an intercaval crush without

affecting atrial flutter cycle length (Nagasawa et al., 1999). NIP-142 prolonged atrial ERP by about 10% without affecting intraatrial and interatrial conduction times in these models, which suggested that the antiarrhythmic effects were achieved by the atrial ERP prolongation. We reported NIP-141 (the hydrochloride salt form of NIP-142) at 10 mg/kg terminated AF in aconitine-induced AF model (Hashimoto et al., 2007). Although the mechanism of AF termination in this model remains unclear, the I_{KACh} blocking effect would be mainly involved, because tertiapin was also effective in this model (Hashimoto et al., 2006).

model	effective dose of NIP-142	reference
vagally-induced AF model	3 mg/kg	Nagasawa et al., 1999
intercaval crush-induced AFL model	3 mg/kg	Nagasawa et al., 1999
aconitine-induced AF model	10 mg/kg	Hashimoto et al., 2007
atrial rapid pacing model	2.5 mg/kg/10 min + 2 mg/kg/hr	Hashimoto et al., 2008

Table 3. Effects of NIP-142 in models of atrial fibrillation (AF) and flutter (AFL)

The ERP prolongation by NIP-142 was greater in the presence of VNS than normal condition (Nagasawa et al., 1999). We also reported that this compound at 3 mg/kg prolonged atrial ERP by about 20 ms under VNS, but at doses up to 10 mg/kg it had no significant atrial ERP prolongation effect without VNS (Hashimoto et al., 2007). These findings suggest that the NIP-142-induced atrial ERP prolongation is due mainly to the inhibition of I_{KACh}.

3.2.4 Low proarrhythmic risk with NIP-142

NIP-141 (1-10 mg/kg) produced no significant prolongation of ventricular ERP in the pentobarbital-anesthetized dogs (Hashimoto et al., 2007). NIP-142-induced prolongation of atrial ERP was greater than that of ventricular ERP in AF models (Nagasawa et al., 1999). Because ventricular ERP or QT prolongation is not suitable for drug-induced TdP (Thomsen et al., 2003, 2004), we also evaluated the proarrhythmic risk of NIP-142 using the methoxamine-sensitized rabbit model, known as Carlsson model (Carlsson et al., 1990). By intravenous infusion, NIP-142 (30 mg/kg over 30 min) induced no ventricular arrhythmia in eight rabbits. In contrast, an I_{Kr} blocker, clofilium (15 mg/kg over 30 min) induced TdP in six of eight rabbits. These results suggests that NIP-142 has little effects on ventricular repolarization and not likely to cause proarrhythmia.

In conclusion, NIP-142 preferentially blocks I_{KACh} and has atrial selective ERP prolonging effects and antiarrhythmic effects in some AF models. This compound appeared to be, therefore, a potential therapeutic agent for the treatment and prevention of AF with lower risk of causing ventricular proarrhythmia than conventional drugs. However, NIP-142 at effective dose in AF model significantly decreased blood pressure. Because tertiapin had no significant effects on blood pressure, it was caused by the effects on other target except I_{KACh}. NIP-142 has some blocking effects on I_{CaL} and I_{Na} (Hashimoto et al., 2007), so the effect on blood pressure might be due to the blockade of these channels.

3.3 Pharmacological profile of NIP-151, a novel I_{KACh} channel blocker

We modified the chemical structure of NIP-142 and found a more potent and selective I_{KACh} channel blocker, NIP-151. The compound showed good bioavailability in dogs and rats,

almost same as that of NIP-142. The pharmacological profiles of NIP-142 and NIP-151 are shown in Table 4. NIP-151 blocks I_{KACh} 400 times more potently than NIP-142, but the effects on I_{CaL} and I_{Na} were less and about 7 times more potent than those of NIP-142, respectively. As a result, NIP-151 had no significant effects on blood pressure at the dose (0.3 mg/kg) that the compound prolonged atrial ERP by 38 ms under VNS. In contrast, NIP-142 decreased blood pressure by about 40% at the dose (3 mg/kg) that the compound prolonged ERP by 20 ms under VNS.

Compound	Effects on ion channels (IC_{50}; μmo/L)			Effects on anesthetized dogs	
	I_{KACh}	I_{Na}	I_{CaL}	Atrial ERP prolongation	Blood pressure
NIP-142	0.65	45	44	20 ms (3 mg/kg)	-38%
NIP-151	0.0016	6.6	>100	38 ms (0.3 mg/kg)	-5%

Table 4. The pharmacological profile of NIP-142 and NIP-151

3.3.1 Effects of NIP-151 on ion channels

The effects of NIP-151 on I_{KACh} and I_{Kr} were examined using HEK293 cells expressing human GIRK1/GIRK4 channel and hERG channel, respectively by voltage clamp analyses (Hashimoto et al., 2008). NIP-151 at 0.1-100 nmol/L concentration-dependently inhibited the GIRK1/GIRK4 channel current (Fig. 3): NIP-151 at 0.1, 1, 10 and 100 nmol/L inhibited the current at -120 mV by 13.7 ± 24.8%, 42.0 ± 12.2%, 73.1 ± 4.1% and 87.2 ± 1.5%, respectively. The IC_{50} value for the inhibition of GIRK1/GIRK4 channel current was 1.6 nmol/L. NIP-151 at 1, 10 and 100 μmol/L inhibited the tail current of hERG-channel current on repolarization by 15.3 ± 1.5%, 23.9 ± 3.4% and 73.3 ± 2.4%, respectively. The IC_{50} value for the inhibition of hERG channel current was 57.6 μmol/L. In our preliminary experiments, this compound had little effects (less than 50% inhibition at 10 μmol/L) on other components of the delayed rectifier potassium currents, I_{Kur} and I_{Ks}. NIP-151 was extremely selective for GIRK1/GIRK4 channel current over other repolarizing currents.

The mechanism of I_{KACh} blockade by NIP-151 remains unclear. We evaluated the effects of NIP-151 on muscarinic M_2 receptor and adenosine A_1 receptor using radioligands binding assay. NIP-151 had slight inhibitory effects on radio ligand binding to these receptors, but the effect was much weaker than that on I_{KACh}: the IC_{50} on M_2 and A_1 receptors were 57.1 μmol/L and 89.9 μmol/L, respectively. In addition, NIP-151 blocked I_{KACh} in HEK293 cells or xenopus oocyte expressing GIRK1/GIRK4 only (not coexpressing these receptors). These results suggest that NIP-151 directly blocked I_{KACh} channel or inhibited the downstream signaling pathway of these receptors.

We also evaluated the NIP-151 on the various types of GIRK channel subtypes. Cardiac-type and brain-type brain type GIRK channels are composed of GIRK1/GIRK4 and GIRK1/GIRK2 heteromultimeric subunits, respectively. NIP-151 preferentially blocked GIRK1/ GIRK4 channel current over GIRK1/GIRK2 current. In contrast, tertiapin blocked both currents at almost same potency (Matsumoto et al., 2008). The pathophysiological role of GIRK1/GIRK2 channel remains unclear, but seizure activity has been reported in GIRK2 null mice (Siqnorini et al., 1997). Thus, GIRK1/GIRK2 blocker might induce some

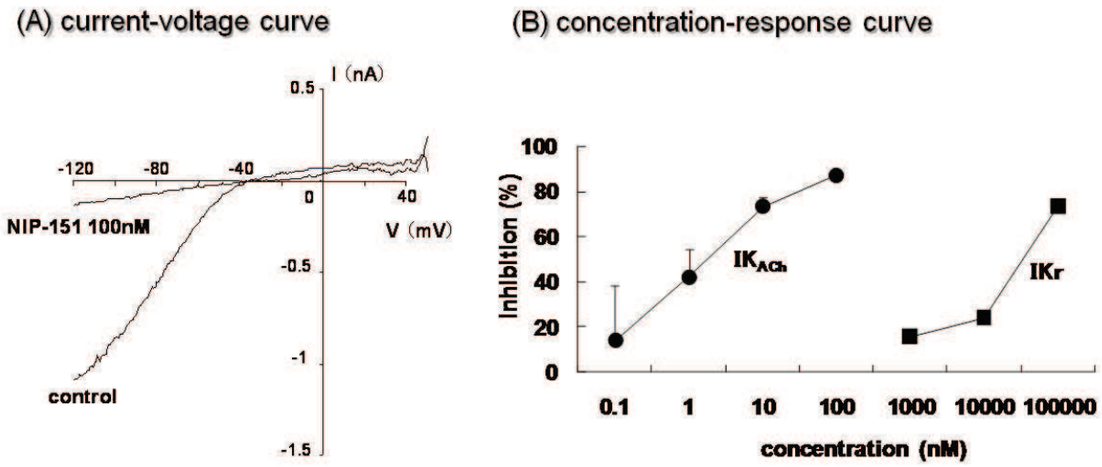

Fig. 3. Effects of NIP-151 on GIRK1/GIRK4 and hERG channel currents. (A) Typical GIRK1/GIRK4-channel current in the absence (control) and presence of NIP-151 at 100 nmol/L. (B) Concentration-response relationship for blockade by NIP-151 of the GIRK1/GIRK1 channel current at 120 mV (solid circle) and the tale current of hERG-channel current (solid square). Data are shown as mean ± SEM from three to five experiments (Hashimoto et al., 2008).

adverse effects on neural activity, such as seizure. Thus, inhibitory effects on GIRK1/GIRK4 channel current over GIRK1/GIRK2 current might be preferable profile for anti AF agents.

3.3.2 Effects of NIP-151 on paroxysmal AF models

We examined the effects of NIP-151 on canine paroxysmal AF models and atrial and ventricular ERP, and compared against those of a standard class III agent dofetilide (Roukoz & Saliba 2007), which is clinically used for the management of AF. NIP-151 (15-30 μg/kg/min) converted AF to sinus rhythm in canine VNS- and aconitine-induced AF models (Table 5, Fig. 4). The compound at the same doses (15-75 μg/kg/min) prolonged atrial ERP significantly, thus the AF termination effects were associated with the atrial ERP prolongation (Hashimoto et al., 2008). In contrast, a selective I_{Kr} blocker dofetilide, which had little effects on GIRK1/GIRK4 channel current, represented significant ERP prolongation both in the atrium and ventricle, but it was not effective in the VNS-induced and aconitine-induced AF models (Table 5).

The reason why dofetilide did not show positive effects on AF models would be mainly due to reverse use dependency. In our preliminary study, dofetilide (0.3 μg/kg) prolonged atrial ERP by 29 ± 7 ms at a basic cycle length of 300 ms, but had slight prolongation (5 ± 5 ms) at a basic cycle length of 200 ms. Nattel et al. (1998) also have shown that dofetilide had limited efficacy in VNS-induced AF model, because of reverse use dependent ERP prolongation, compared with azimilide, which increased atrial ERP in a frequency-independent manner. Alternatively, NIP-151 (0.3 mg/kg) significantly prolonged atrial ERP by 41± 7 ms and 27 ± 5 ms at basic cycle lengths of 300 and 200 ms, respectively. These results suggest that the reverse use dependency of NIP-151 is less significant than that of IKr blockers such as dofetilide and consequently effective under the AF (high frequent) condition.

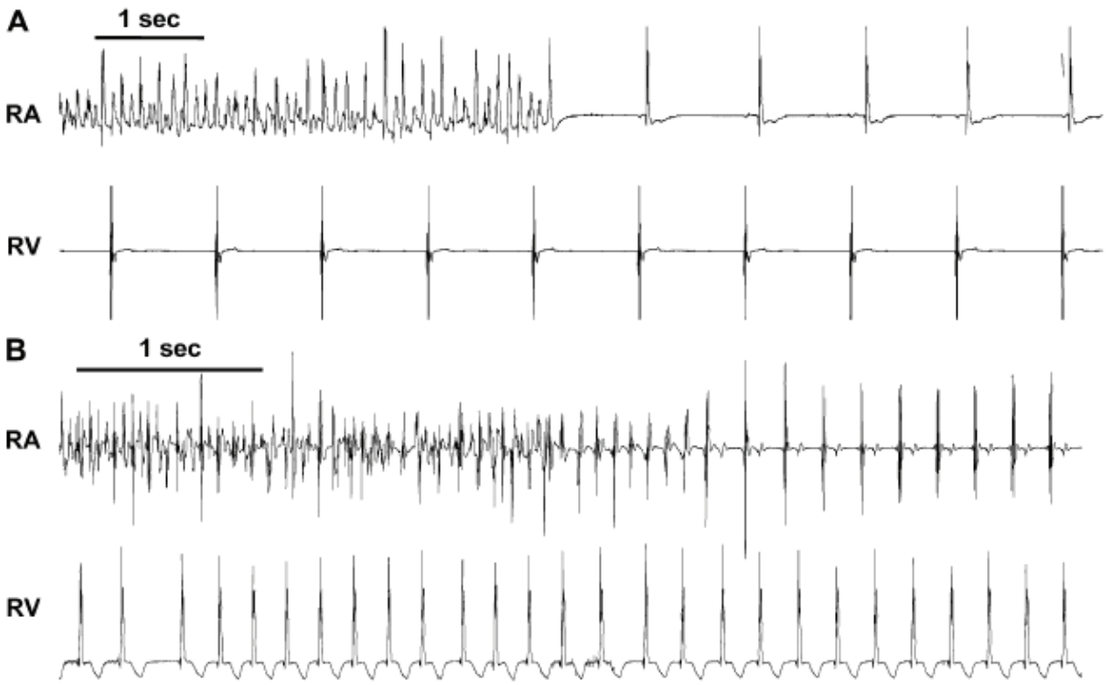

Fig. 4. Representative examples of atrial fibrillation (AF) termination with NIP-151. Electrograms from right atrium (RA) and right ventricular free wall (RV) were recorded. (A) Termination of vagal nerve stimulation-induced AF with NIP-151 at 15 μg/kg/min. (B) Termination of aconitine-induced AF with NIP-151 at 100μg/kg. (Hashimoto et al., 2008)

	VNS-induced AF model				*aconitine-induced AF model*		
	dose (g/kg/min)	termination of AF	time to AF termination(s)	AF reinduction	dose (g/kg)	termination of AF	time to AF termination(s)
vehicle	-	1/10	98	1/1	-	0/5	-
NIP-151	5	4/5*	525 ± 25	3/4	10	0/5	-
	15	5/5**	372 ± 38	1/5	30	4/5*	85 ± 23
					100	5/5**	76 ± 15
dofetilide	3	1/4	793	1/1	100	0/4	-
	10	0/4	-	-			
Time to AF termination data were expressed as mean ±SEM							
**P<0.01, *P<0.05 vs vehicle-treated group							

Table 5. Effects of NIP1-151 and dofetilide in terminating vagal nerve stimulation (VNS)-induced and aconitine-induced atrial fibrillation (AF). (Hashimoto et al., 2008)

We also evaluated the effects of NIP-151 in canine atrial flutter model with Y-shaped right atrial incision. Intravenous infusion of NIP-151 (75 μg/kg/min over 10 min) did not terminated atrial flutter in this model (0 of four dogs), although the compound slightly prolonged atrial flutter cycle length. This result suggests that the I_{KACh} blocker might have weak efficacy on macro-reentry type atrial flutter.

3.3.3 Low proarrhythmic risk with NIP-151

We found that NIP-151 had little effects on hemodynamic (blood pressure, left ventricular systolic pressure and maximum left ventricular developed pressure increased rate) and electrophysiological values (PQ, QRs, QT and corrected QT) in isoflurane-anesthetized dogs, even when the drug was administrated at doses that 20- to 60- fold higher than the effective dose in the VNS-AF model. NIP-151 also represented no changes in action potential parameters (Vmax and APD) in beagle dog ventricular muscle, and no proarrhythmic effects in the methoxamine-sensitized rabbit model (Carlsson model). In contrast, dofetilide prolonged the QTc interval in anesthetized dogs and APD in beagle dog ventricular muscle, and it induced TdP in Carlsson model (Hashimoto et al., 2008). These results suggest that NIP-151 is expected to be devoid of a ventricular proarrhythmic effects rather than a clinically available class III drug.

3.4 The effect of I_{KACh} blockers in remodeled heart

It is clinically observed that the recurrent episode of paroxysmal AF often progresses more persistent forms of AF. Wijffels et al. (1995) reported this phenomenon as "AF begets AF". They found continuous rapid atrial pacing in the goat heart lead to progress shortening of atrial ERP and increased duration of AF once it is induced. This phenomenon was found to be associated with structural and electrical remodeling. Structural remodeling, characterized by fibrosis and hypertrophy, affects excitation pattern of atria and creates a substitute for AF development and maintenance (Ehrlich et al., 2006). Electric remodeling is accompanied by alterations in the expression and/or function of various ion channels in a way that promotes rapid and irregular activation of atria (Wijffels et al., 1995; Nattel et al., 2007). The ion channels affected by electrical remodeling include I_{Na} and I_{Kr}, which are targets of conventional antiarrhthmic agents. The expressions and activities of these channels are significantly decreased after atrial tachypacing, thus the conventional drugs have limited efficacies in the remodeled hearts of persistent AF patients. Thus, the development of a new drug which is effective in persistent AF patients is highly desirable.

We evaluated the I_{KACh} blockers on the canine atrial tachypacing model to predict the efficacy in persistent AF patients. The atrial tachypacing model was established by 7-10 days right atrial pacing at 400 bpm. In this model, the atrial ERP was significantly decreased by about 40 ms at a basic cycle length of 300 ms. Intravenous administration of NIP-151 (0.3 mg/kg) prolonged atrial ERP by 15 ± 2 ms and 44 ± 8 ms at a basic cycle length of 300 ms before and after the atrial tachypacing, respectively. Tertiapin (12 nmol/kg) also prolonged atrial ERP by 7 ± 1 ms and 20 ± 1 ms at a basic cycle length of 300 ms before and after the atrial tachypacing, respectively (Fig. 5). The atrial ERP prolonging effects by these I_{KACh} blockers was significantly potentiated after the atrial tachypacing. Interestingly, the atrial ERP prolongation by these I_{KACh} blockers did not significant show use-dependency. The atrial ERP prolongations by NIP-151 (0.3 mg/kg) after the atrial tachypacing were 40 ± 6 ms, 44 ± 8 ms and 32 ± 5 ms at basic cycle length of 400 ms, 300 ms and 200 ms. A selective I_{Kr} blocker, E-4031 prolonged atrial ERP before and after atrial tachypacing, but the prolonging effect was significantly decreased from 46 ± 7 ms to 25 ± 5 ms after atrial tachypacing (Fig. 5). These results suggest that I_{KACh} blockers might have better efficacy in persistent AF patients than conventional I_{Kr} blockers.

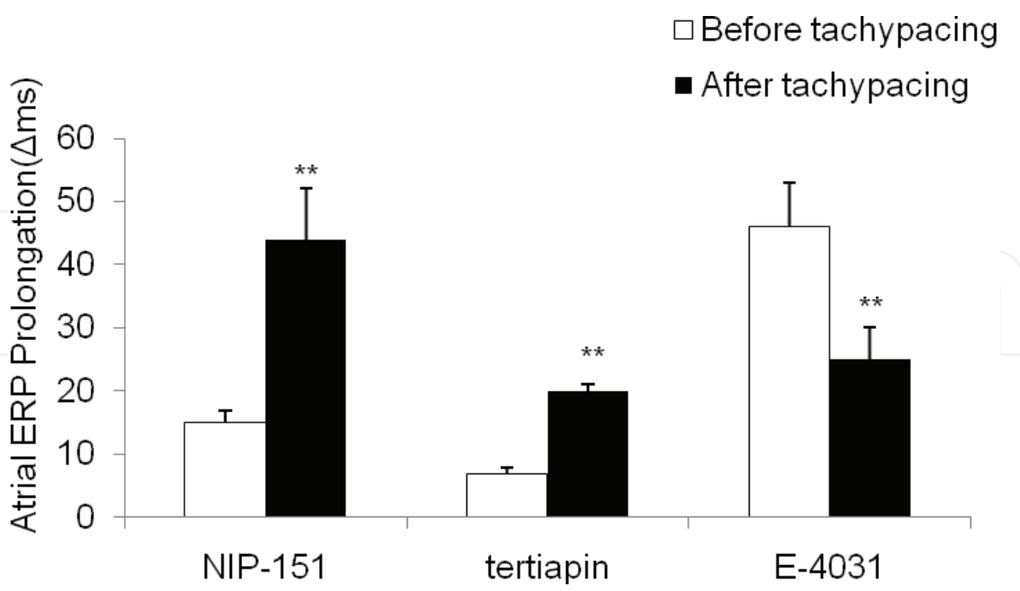

Fig. 5. Effects of NIP-151, tertiapin and E-4031 on atrial effective refractory period (ERP) prolongation in atrial tachypacing model. The ERP was mesuared at the basic sysle length of 300 ms. Data are shown as mean ± s.e.m. (n=4-5). **P<0.01 compared with before tachypacing.

To clarify the activation mechanism of I_{KACh} in this model, we evaluated the effects of a muscarinic M_2 receptor antagonist, AF-DX116 and an adenosine A_1 receptor antagonist, DPCPX. AF-DX 116 (0.3 mg/kg) slightly prolonged atrial ERP (~10 ms) before and after atrial tachypacing, but the prolonging effect was unchanged between before and after the pacing. DPCPX (0.3 mg/kg) had little effects on atrial ERP both before and after the atrial tachypacing. These results suggest the I_{KACh} activation in the model would be associated with non-receptor regulated mechanism. As discussed above, constitutively I_{KACh} is activated in the persistant AF patients and dogs with atrial tachypacing (Dobrev et al., 2005, Ehrlich et al., 2004). The potentiated ERP prolongation by NIP-151 and tertiapin after the atrial tachypacing might be associated with the blockade of constitutively active I_{KACh} channel.

4. Clinical implication

Currently available anti-arrhythmic agents such as sodium channel blockers and potassium channel blockers have not only limited efficacy but also severe side effects such as proarrhythmia and reduced cardiac function (Li et al., 2009). Considerable attention has recently focused on atrial-selective AF drug. I_{KACh} channel has been thought to be a good target for AF treatment, because the channel is important for atrial, but not for ventricular repolarization and AF susceptibility (Nattel & Carlsson, 2006). Paroxysmal AF occurring at night, at rest, and/or consuming meals or alcohol is caused at least partly by the activation of ligand-operated I_{KACh} channel (Coumel, 1999). In addition, our results from atrial tachypacing model suggested that electrical remodeling increased the density of constitutively active I_{KACh} that is activatedd in atrial cells through muscarinic receptor or

adenosine receptor-independent mechanism. The constitutively active I_{KACh} channel is observed in cardiomyocytes from patients with persistent AF (Dobrev et al., 2005). These observations suggest that I_{KACh} blockers would be effective not only in paroxysmal AF but also persistent AF treatment.

Agonist-activated inward rectifier potassium current are lager in left than right atria of patients with paroxysmal AF (Voigt et al., 2010). The left-to-right gradient in inward rectifier background current may contribute to the left-to-right atrium dominant frequency gradients in paroxysmal AF patients, accelerating frequency and stability of reentry-promoting rotors. I_{KACh} blockers might modify not only atrial refractoriness but also AF –promoting reentrant sources in the experimental models described.

The contribution of I_{KACh} channel activation to the development and maintenance of AF in patients remains unknown, because a highly selective I_{KACh} blocker has not been clinically available. As with all animal experiments or human tissue studies, it might be difficult to predict whether the efficacy can be directly extrapolated to patients because the AF is complex and has varied manifestations. From this perspective, the clinical assessment to verify the importance of I_{KACh} channel and to ascertain the therapeutic utility of a selective I_{KACh} blocker is required.

We have shown that the highly safe profile of I_{KACh} blocker in cardiovascular systems, but there is little information about the adverse effects of the blockers on extracardiac organs. GIRK1/GIRK4 channel is mainly located in atrium, but other subtypes such as GIRK1/GIRK2, GIRK1/GIRK3, and GIRK2/GIRK3 are located in some types of neurons (Saenz del Burgo et al., 2008). The physiological roles of these subtypes have not been cleared, but seizure activity has been reported in GIRK2 null mice (Siqnorini et al., 1997). Before conducting clinical study, we have to examine fully the extracardiac effects of I_{KACh} blockers.

5. Conclusion

Recent studies clearly showed that I_{KACh} channel has a crucial role in the development and maintenance of AF. This channel would be a novel target for the treatment both in paroxysmal and persistent AF patients. I_{KACh} blockers terminated AF in some canine AF models with atrial-specific ERP prolongation. This profile is thought to be ideal for anti-AF agent, because the blockers have minimum risk of ventricular proarrhthmia such as TdP. Some drug candidates that block I_{KACh} are under clinical or preclinical stages (Machida et al., 2011; Ehrlich & Nattel, 2009; Voigt et al, 2009). The clinical efficiency of the blockers will be clarified in the near future.

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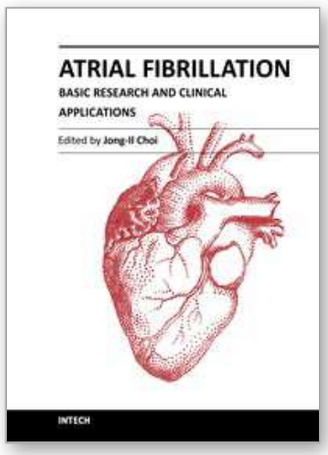

Atrial Fibrillation - Basic Research and Clinical Applications

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Atrial Fibrillation-Basic Research and Clinical Applications is designed to provide a comprehensive review and to introduce outstanding and novel researches. This book contains 22 polished chapters and consists of five sections: 1. Basic mechanisms of initiation and maintenance of atrial fibrillation and its pathophysiology, 2. Mapping of atrial fibrillation and novel methods of signal detection. 3. Clinical prognostic predictors of atrial fibrillation and remodeling, 4. Systemic reviews of catheter-based/surgical treatment and novel targets for treatment of atrial fibrillation and 5. Atrial fibrillation in specific conditions and its complications. Each chapter updates the knowledge of atrial fibrillation, providing state-of-the art for not only scientists and clinicians who are interested in electrophysiology, but also general cardiologists.

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