Effects of a Novel Class III Antiarrhythmic Agent, NIP-142, on Canine Atrial Fibrillation and Flutter

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The effects of a new benzopyran derivative, NIP-142, on atrial fibrillation (AF) and flutter (AFL) and on electrophysiological variables were studied in the dog. NIP-142 (3 mg/kg) was administered intravenously to pentobarbital-anesthetized beagles during vagally-induced AF and during AFL induced after placement of an intercaval crush. Isolated canine atrial tissues were studied using standard microelectrode technique. NIP-142 terminated AF in 5 of 6 dogs after an increase in fibrillation cycle length (CL) and prevented reinitiation of AF in all 6 dogs. NIP-142 terminated AFL in all 6 dogs without any appreciable change in flutter CL, and prevented reinitiation of AFL in all 6 dogs. NIP-142 prolonged atrial effective refractory periods ($11\pm5\%$, $3\pm3\%$, $12\pm3\%$, and $10\pm5\%$ from the baseline value at basic CLs of 150, 200, 300, and 350 ms, respectively) without changes in intraatrial conduction time. The prolongation of the atrial effective refractory period was greater in the presence of vagal stimulation. NIP-142 decreased action potential phase-1 notch and increased phase-2 plateau height without making any changes in the action potential duration, although it did reverse carbachol-induced shortening of the action potential duration. In conclusion, NIP-142 is effective in treating AFL and vagally-induced AF by prolonging atrial refractoriness. (*Circ J* 2002; **66**: 185–191)

Key Words: Antiarrhythmic agent; Atrial fibrillation; Atrial flutter; NIP-142; Refractoriness

Most class III drugs block the rapid component of the delayed rectifier current (I_{Kr}), increase the action potential duration (APD) and prolong the QT interval, all of which can lead to torsades de pointes! In addition, the efficacy of I_{Kr} blockers as treatment for tachyarrhythmias is limited because of the reverse use dependency of the class III effect; the effect on APD could be attenuated at shorter cycle lengths as seen in atrial fibrillation (AF).² For these reasons, the efforts in developing new class III antiarrhythmic drugs has been directed toward more effective compounds but with low proarrhythmic risk.

NIP-142 ((3*R**,4*S**)-4-cyclopropylamino-3,4-dihydro-2,2-dimethyl-6-(4-methoxyphenylacetylaminomethyl)-7-nitro-2*H*-1-benzopyran-3-ol) is a newly developed benzopyran derivative (Fig 1), which terminates aconitine-induced atrial tachyarrhythmia in isolated guinea pig hearts and anesthetized canine hearts³ and binds to the batrachotoxin-sensitive Na^+ channel site 2 receptor to which aconitine binds, but not to the tetrodotoxin-sensitive Na^+ channel site 1 receptor.⁴ In anesthetized dogs, the drug decreases heart rate in spite of transient hypotension³ and inhibits both L- and T-type calcium currents (I_{Ca}) in isolated guinea pig ventricular myocytes.⁵ NIP-142 prolongs atrial but not ventricular refractoriness,⁴ does not prolong the QT interval³ and has little effect on the action potential variables.⁵ A preliminary study suggests this new compound blocks the acetylcholine-activated potassium current ($I_{K,ACh}$) in the

dog, but does not bind to the muscarinic receptors expressed in Chinese hamster ovary cell (unpublished data), which further suggests that NIP-142 may be effective in terminating the AF caused by increased vagal tone without causing ventricular proarrhythmias. However, the effects of this drug on other types of atrial tachyarrhythmias and atrial action potential characteristics have not been explored thoroughly. We, therefore, investigated the effects of NIP-142 on AF induced by vagal stimulation and on atrial flutter (AFL) caused by reentry induced by intercaval obstacle, as well as its effects on electrophysiological variables, in the dog. We also investigated its effects on the atrial action potential characteristics in isolated canine atrial tissue by using a standard microelectrode technique.

Methods

Animal Preparation

Thirty-three beagles (Kasho, Tokyo, Japan) of either sex weighing 10–17 kg were anesthetized with sodium pentobarbital (25 mg/kg, iv). Animal handling procedures were performed in accordance with the 'Guideline for Animal Experiment' at the university. Dogs were ventilated with room air via an endotracheal tube connected to a volume-

(3*R**,4*S**)-4-cyclopropylamino-3,4-dihydro-2,2-dimethyl-6-(4-methoxyphenylacetylaminomethyl)-7-nitro-2*H*-1-benzopyran-3-ol

Fig 1. Molecular structure of NIP-142.

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cycled respirator (607E, Harvard Apparatus, Millis, USA). A left femoral vein was cannulated to infuse 0.9% saline for replacement of spontaneous fluid losses and also to inject drugs. After median sternotomy, the heart was exposed and suspended in a pericardial cradle. The chest was kept warm by 2 operating lamps and covering it with a plastic sheet.

Vagally Induced AF Model

As in a previous report⁶ both vagus nerves were isolated in the neck of 6 dogs, ligated twice and then severed. Bipolar Teflon-coated stainless steel wire hook electrodes with 1.5-cm non-coating distal ends were embedded through a 27-gauge needle within and parallel to the shaft of each vagus to stimulate the efferent vagal nerve. Both ansae subclaviae were isolated at the exit of the stellate ganglia, ligated twice and severed. Octapolar electrode catheters (electrode interval 2.5 mm) were inserted into bilateral atrial appendages to record the atrial electrograms. A bipolar electrode (interelectrode distance 2 mm) was sewn onto the epicardial surface of the right atrial free wall for stimulation. Atrial electrograms and electrocardiogram (ECG) lead II were recorded simultaneously on a thermal recorder (RTA-1200M, Nihon Kohden, Tokyo, Japan) at a paper speed of 100 mm/s and stored in a digital data recorder (RD-130TE, TEAC, Tokyo, Japan) for later analyses. An oscilloscope (VC-11, Nihon Kohden) was also used to monitor real time electrograms.

Stimulation of both the vagi was performed with separate isolated constant current sources (SS-202J, Nihon Kohden) driven by a programmable stimulator (SEN-7203, Nihon Kohden). The current strength was set to prolong sinus cycle length by 100% with right vagal stimulation and to produce a 2:1 atrioventricular (AV) block with left vagal stimulation by using 2-ms rectangular pulses at 50 Hz.⁹ In some dogs, left vagal stimulation produced complete AV block without producing the 2:1 block, and in these dogs, the current strength that produced complete AV block was used under ventricular backup pacing at 120 beats/min by a stimulator (DPS-1300D, Dia Medical System, Tokyo, Japan). Between interventions, the vagi were stimulated to ensure that the sinus and AV nodal responses remained constant.

During bilateral vagal stimulation, AF was induced by atrial extrastimulation using a digital programmable stimulator (SEC-2102, Nihon Kohden). AF lasting more than 10 min was repeatedly induced in each dog. Two minutes after

induction of AF, a bolus intravenous injection of NIP-142 (3 mg/kg) was given (this dosage was selected according to a previous report³). If AF terminated within 5 min of the injection, induction of AF was repeated 2–5 min later. If AF was sustained for more than 5 min after the injection, vagal stimulation was stopped in order to terminate AF, and induction of AF was also repeated.

To determine the atrial cycle length (CL) during AF, atrial electrograms of 30-s duration recorded from the right atrial appendage during AF were analyzed by fast Fourier transformation using a waveform analysis program (BIMUTAS II, Kissei Comtec, Tokyo, Japan). Each 30-s interval was divided into six 5-s segments and from an average of the maximal powers of fast Fourier transformation of 4 simultaneous recordings from consecutive bipolar electrodes, a fibrillation CL was obtained. Next, the fibrillation CL of 6 segments were then averaged to represent each dog. This analysis was made before and after administration of NIP-142 during AF.

To analyze the possible mechanism of termination of vagally induced AF, the atrial effective refractory period (ERP) was determined at a basic cycle length (BCL) of 200 ms before and during vagal stimulation, and repeated after injection of NIP-142 (see later).

AFL With Anatomical Obstacle Model

In 6 dogs, after the chest was opened through lateral thoracotomy at the fourth right intercostal space, an anatomical obstacle was produced by ligation of the intercaval atrial tissue according to the method of a previous study⁷ (Fig 2). If AFL was not induced with atrial pacing after placing one intercaval obstacle ($n=3$), an additional obstacle was created toward the right atrial appendage. Multipolar electrodes were placed on the epicardial surface of both atria to record atrial electrograms. AFL was repeatedly induced by burst right atrial pacing at cycle lengths of 100–115 ms. AFL was defined as a regular atrial rhythm at cycle lengths of 110–150 ms, as in the previous study⁷. This AFL could be terminated by atrial pacing. Six consecutive atrial cycle lengths were averaged to represent the flutter CL in each experiment.

After AFL was initiated, a 10-min period was allowed to elapse to ensure that AFL was sustained. NIP-142 was injected intravenously at a dose of 3 mg/kg. If AFL was terminated within 5 min of the injection, initiation of AFL was attempted. Venous blood samples were obtained just after the termination of AFL to determine plasma drug concentrations.

Measurements of Electrophysiological Variables

Eleven beagles were used to determine the effects of NIP-142 on electrophysiological variables. For this purpose, the vagi and sympathetic nerves were kept intact during the experiment. Two bipolar electrodes (interelectrode distance 2 mm) were sewn onto the epicardial surface of the right atrial free wall and the right ventricle for stimulation, and another 2 bipolar hook electrodes with an interhook distance of 1 mm were inserted into the right and left atrial free walls to record atrial electrograms. The tip of an octapolar electrode catheter (interelectrode distance 2.5 mm) was positioned at the noncoronary cusp of the aortic valve through the right carotid artery to record the His bundle potential. Atrial and ventricular electrograms and ECG lead II were recorded on a thermal recorder, and stored in a digital data recorder.

Fig 2. Diagram of the method used in the atrial flutter model. Atria are seen from the back, and the intercaval crush (a) between the superior (SVC) and inferior (IVC) venae cavae and (b) toward the right atrial appendage (RAA) is shown. Numbers indicate sites and numbers of recording electrodes corresponding to electrograms shown in Fig 4. A star indicates the site of the pacing electrode for programmed stimulation. LAA, left atrial appendage; PV, pulmonary veins.

Before and 10 min after administration of NIP-142 (3 mg/kg, bolus iv), the electrophysiological variables were determined. Programmed stimulation was performed with 2-ms rectangular pulses at twice the diastolic threshold using a digital programmable stimulator. The shortest CL of atrial pacing that maintained 1:1 atrial capture (1:1 SA) or 1:1 atrioventricular conduction (1:1 AV) was determined by shortening the pacing CL in 10-ms steps. The conduction time (CT) between the right atrial stimulation and either the right (intraatrial CT) or the left (interatrial CT) atrial potentials was determined at an atrial pacing cycle length of 150 ms. Atrio-His (AH) and His-ventricular (HV) intervals were determined at an atrial pacing CL of 300 ms. Atrial and ventricular ERPs were determined with the extrastimulus method. A train of 10 basic stimuli (S1) was followed by a premature extrastimulus (S2) that produced a propagated response. The S1S2 interval was shortened in steps of 2 ms until S2 failed to produce a propagated response. ERP was defined as the longest S1S2 interval at which S2 failed to produce a propagated response. Measurements were repeated to ascertain the reproducibility of ERP. BCLs ranged from 150 to 350 ms to determine the use-dependency of effects of the test drug on ERP.

In Vitro Experiments

Ten beagles were killed by venesection under deep anesthesia induced by intravenous injection of an overdose of sodium pentobarbital. The right atrial muscles were dissected and isolated from adherent fat and connective tissues. The preparations were pinned to the silicon block in an organ bath, superfused continuously with physiologic salt solution equilibrated with 95% O₂ and 5% CO₂, and maintained at 36.5±0.5°C. The preparations were stimulated with a programmable stimulator (SEN-3301, Nihon Kohden) at a constant frequency of 1.0 Hz by rectangular 3-ms pulses delivered through a pair of bipolar platinum electrodes. Output was set at 1.5 times the diastolic threshold voltage. Transmembrane action potentials were recorded by a conventional technique with glass microelectrodes. The bath solution contained 118.4 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L CaCl₂, 1.2 mmol/L MgSO₄, 1.2 mmol/L KH₂PO₄, 25.0 mmol/L NaHCO₃, and 11.1 mmol/L glucose (pH adjusted to 7.4 at 37°C). The internal glass electrode solution contained 3 mol/L KCl.

Action potentials were amplified by an amplifier (Intra 767, World Precision Instruments, Sarasota, USA), AD converted, and recorded in a computer (PC8901 DA, NEC, Tokyo, Japan). Data were analyzed by an analysis program (CAPA, Physio-Tech, Tokyo, Japan). The maximal upstroke velocity of the action potential (dV/dt max) was determined by an electronic differentiator. After an equilibration time of 60 min or more, the baseline evoked action potentials were recorded in 6 beagles. Then 0.3 µmol/L carbachol, a muscarinic receptor agonist, was added to the solution to shorten the APD. After 10 min, when the APD became stable, NIP-142 was added cumulatively (1, 10 and 100 µmol/L). In another 4 beagles, as a control study, the APDs were determined before and after application of NIP-142 (1, 10 and 100 µmol/L) without carbachol. The APDs were determined at 20% (APD₂₀), 50% (APD₅₀) and 90% (APD₉₀) of repolarization.

In Vivo Solutions

For the in vivo experiments, NIP-142 (60 mg) was dissolved in 1 ml of a solvent (polyethylene glycol 400:

Fig 3. A representative example of termination of atrial fibrillation with NIP-142. Electrocardiogram (ECG) leads II and III, and atrial electrograms recorded from 4 right atrial sites are arranged from top to bottom. (A) Baseline atrial fibrillation before drug injection (B) Termination of atrial fibrillation. Before termination of atrial fibrillation with NIP-142, the fibrillation cycle length became longer at each recording site as compared with the baseline recording. RR intervals were also prolonged by NIP-142 before termination of atrial fibrillation.

Fig 4. A representative example of termination of atrial flutter with NIP-142. Electrocardiogram (ECG) lead II and atrial electrograms recorded from 6 atrial sites are arranged from top to bottom. (A) Baseline atrial flutter before drug injection. (B) Termination of atrial flutter with NIP-142. NIP-142 did not change the flutter cycle length.

ethanol: 1 mol/L HCl = 2:3:5). The concentration of the solvent (0.05 ml/kg) had no influence on the electrophysiological variables. Neither AFL nor AF was terminated by an injection of this concentration of the solvent in our preliminary study.

Data Analysis

Results are presented as mean±SEM. The analysis of data for significance was performed by an analysis of vari-

Table 1 Electrophysiological Effects of NIP-142

	<i>n</i>	Baseline	NIP-142	<i>p</i> value
<i>Atrial ERP (ms)</i>				
BCL 150 ms	11	95±4	104±4	0.032
BCL 200 ms	11	116±4	120±5	0.189
BCL 300 ms	11	126±4	140±5	0.002
BCL 350 ms	6	145±9	157±7	0.073
<i>Ventricular ERP (ms)</i>				
BCL 200 ms	10	142±4	142±6	0.466
BCL 250 ms	10	157±5	161±5	0.018
BCL 300 ms	10	169±6	175±7	0.025
BCL 350 ms	3	178±8	180±13	0.404
1:1 SA (ms)	9	121±5	132±5	<0.001
1:1 AV (ms)	9	224±9	264±6	<0.001
Intraatrial CT (ms)	5	20±4	20±4	NS
Interatrial CT (ms)	5	38±1	38±1	NS
AH (ms)	5	85±7	116±12	0.004
HV (ms)	5	27±3	27±3	NS

Data expressed as mean values±SEM. ERP, effective refractory period; BCL, basic cycle length; 1:1 SA, the shortest atrial pacing cycle length that maintained 1:1 atrial capture; 1:1 AV, the shortest atrial cycle length that maintained 1:1 atrioventricular conduction; CT, conduction time (BCL 150 ms); AH, the interval between the atrial potential and the His bundle potential (BCL 300 ms); HV, the interval between the His bundle and the ventricular potential (BCL 300 ms). In some dogs atrial and ventricular ERPs were not determined at longer BCL because their sinus cycle lengths were shorter than 350 ms.

Fig 5. Relationships between basic cycle length (BCL) and NIP-142-induced changes in atrial (open circles) and ventricular (solid circles) effective refractory periods (ERP). Data expressed as mean values±SEM. NIP-142-induced prolongation of ERP showed reverse use-dependency at BCLs between 300 and 200 ms in the atrium as well as in the ventricle. At a BCL of 200 ms, NIP-142 did not significantly change either atrial or ventricular ERPs. However, NIP-142 prolonged atrial ERP at the shortest BCL (150 ms).

ance and multiple comparisons were tested by Bonferroni's test. Mean values of 2 groups were compared by Student's *t*-test. Statistical significance was set at *p*<0.05.

Results

Effects on AF and AFL

AF was terminated in 5 of 6 dogs 283±50 s after bolus injection of NIP-142 (Fig 3). NIP-142 increased the mean fibrillation CL from 99±10 (baseline) to 132±10 ms (just before termination, *p*<0.01). After termination of AF with NIP-142, sustained AF (>10 s) was not able to be induced in any of the 6 dogs.

AFL was terminated with NIP-142 in all 6 dogs in 77±7 s (Fig 4). NIP-142 did not change flutter CL (from 126±4 to 128±4 ms just before termination of AFL). In 2 dogs, AFL degenerated into AF before termination. After drug administration AFL was not inducible in any of the 6 dogs. The plasma concentration of NIP-142 just after termination of AFL was 1.70±0.71 µg/ml.

In Vivo Electrophysiological Determination

The electrophysiological effects of NIP-142 at different BCLs are detailed in Table 1. The relationships between BCL and drug-induced changes in atrial and ventricular ERP (expressed as a percentage of the baseline values) are shown in Fig 5. The prolongation of ERP showed reverse use-dependency at BCLs between 300 to 200 ms in the atrium, as well as in the ventricle, in the baseline state without vagal stimulation. However, further shortening of the BCL to 150 ms led to prolongation of atrial ERP. Ventricular ERP was not determined at a BCL of 150 ms. Prolongation of ERP with NIP-142 was greater in the atrium than in the ventricle (12±3% vs 3±1%, BCL 300 ms, *p*<0.05). NIP-142 also significantly prolonged 1:1 SA, 1:1 AV and AH intervals. In contrast, NIP-142 did not change the intraatrial and interatrial CTs, or HV interval.

NIP-142 significantly prolonged atrial ERP at a BCL of 200 ms during vagal stimulation from 70±8 to 121±11 ms (*p*<0.01). Prolongation of ERP with NIP-142 was greater in the presence of vagal stimulation than in the control state (79±15% vs 10±4%, at BCL 200 ms, *p*<0.05).

In Vitro Experiments

NIP-142 (100 µmol/L) decreased action potential phase-1 notch, and increased the height of phase-2 plateau without changes in duration or maximal upstroke velocity of the atrial action potential in the control state (Fig 6, Table 2). Carbachol (0.3 µmol/L) significantly shortened APDs at 20, 50 and 90% of repolarization, and the shortening was attenuated by NIP-142 in a concentration dependent manner (Fig 7, Table 3).

Discussion

The major findings of the present study are as follows. First, NIP-142 terminated both AF induced under vagal stimulation and AFL caused by macro-reentry in dogs. Second, NIP-142 prolonged atrial ERP with a unique rate-dependent property and 1:1 SA interval. This compound prolonged ventricular ERP, but the change was smaller as compared with atrial ERP. Third, NIP-142 did not affect

Fig 6. A representative action potential recorded at a stimulation frequency of 1 Hz. NIP-142 (100 μ mol/L) decreased the action potential phase-1 notch and increased the height of the phase-2 plateau without a change in action potential duration at 90% of repolarization.

Table 2 Effects of NIP-142 on Action Potential Parameters of Canine Atrial Muscle

	APD ₂₀ (ms)	APD ₅₀ (ms)	APD ₉₀ (ms)	dV/dt max (V/s)
Control	30 $\pm$ 4	70 $\pm$ 3	154 $\pm$ 5	207 $\pm$ 27
NIP-142 (μ mol/L)				
1	30 $\pm$ 4	68 $\pm$ 3	152 $\pm$ 4	205 $\pm$ 35
10	29 $\pm$ 4	69 $\pm$ 2	158 $\pm$ 3	198 $\pm$ 30
100	29 $\pm$ 5	69 $\pm$ 6	155 $\pm$ 10	190 $\pm$ 17

Data expressed as mean values $\pm$ SEM. APD₂₀, APD₅₀ and APD₉₀, action potential duration at 20, 50 and 90% of repolarization level, respectively (stimulation frequency of 1 Hz); dV/dt max, the maximal upstroke velocity of action potential. *p* value was not significant for each concentration vs control.

Fig 7. Representative results of effects of carbachol (CCh, 0.3 μ mol/L) and additional NIP-142 (100 μ mol/L) on action potential of the canine atrium. Carbachol-induced shortening of action potential duration was reversed with NIP-142 (right panel).

Table 3 Effects of NIP-142 on Action Potential Parameters of Canine Atrial Muscle Pretreated With Carbachol

	APD ₂₀ (ms)	APD ₅₀ (ms)	APD ₉₀ (ms)	dV/dt max (V/s)
Control	28 $\pm$ 4	69 $\pm$ 5	147 $\pm$ 5	239 $\pm$ 12
Carbachol 0.3 μ mol/L	14 $\pm$ 2**	40 $\pm$ 5**	116 $\pm$ 7*	261 $\pm$ 10
Carbachol 0.3 μ mol/L + NIP-142 (μ mol/L)				
1	14 $\pm$ 2	39 $\pm$ 5	114 $\pm$ 8	271 $\pm$ 14
10	14 $\pm$ 2	41 $\pm$ 6	121 $\pm$ 10	258 $\pm$ 21
100	17 $\pm$ 3	51 $\pm$ 7†	141 $\pm$ 9††	222 $\pm$ 31

Data expressed as mean values $\pm$ SEM. Abbreviations as in Table 2. **p*<0.05, ***p*<0.001 vs control; †*p*<0.01, ††*p*<0.001 vs carbachol 0.3 μ mol/L.

either atrial CT or HV intervals, but prolonged both the AH and 1:1 AV intervals. Fourth, NIP-142 decreased the action potential phase-1 notch and increased the height of phase-2 plateau without a change in the APD in the baseline condition. This compound attenuated carbachol induced shortening of the APD in a concentration dependent manner. These findings strongly suggest that this investigational drug has a class III antiarrhythmic effect that is different from other class III antiarrhythmic drugs.

Effects of NIP-142 on the Vagally Induced AF Model

Based on our preliminary (unpublished) data of the blocking effect of NIP-142 on $I_{K,ACh}$, we determined whether NIP-142 could terminate vagally induced AF in the dog, and as expected, it effectively terminated AF in 83% of

dogs. Class III drugs with a selective I_{Kr} blocking effect, including d-sotalol[§], dofetilide⁹ and nifekalant (MS-551)⁶ terminated vagally induced AF in 87.5%, 50% and 75% of dogs, respectively. Other class III drugs that suppress both I_{Kr} and the slow component of the delayed rectifier current (I_{Ks}), such as azimilide⁹ and ambasilide⁸ also terminated vagally induced AF in 93% and 100% of dogs, respectively. NIP-142 was as effective as these class III drugs in terminating vagally induced canine AF.

The prolongation of atrial ERP with NIP-142 was greater in the presence of vagal stimulation than in the control state, possibly because of its blocking effect on $I_{K,ACh}$. The effect of class III antiarrhythmic drugs on vagally induced shortening of the atrial ERP differs from drug to drug. D-sotalol and ambasilide have greater effects on atrial ERP in

the presence of vagal stimulation^{8,10} whereas azimilide does so in the absence of vagal stimulation. In contrast, dofetilide has an almost similar effect on atrial ERP in the presence and in the absence of vagal stimulation⁹. These different results on atrial ERP under vagal stimulation are possibly dependent on different blocking effects on potassium currents. Ambasilide¹¹ and dl-sotalol¹² inhibit $I_{K,ACH}$ in guinea pig atria, but d-sotalol¹¹ does not. The effects of dofetilide and azimilide on $I_{K,ACH}$ are not known yet.

Preliminary ligand analysis revealed that NIP-142 did not directly bind to muscarinic receptors (unpublished data). The effectiveness of NIP-142 in terminating and preventing vagally induced AF and its greater effect on ERP under vagal stimulation are consistent with suppression of $I_{K,ACH}$. This was also supported by our in vitro experiment in which NIP-142 attenuated carbachol induced shortening of the APD in a concentration dependent manner.

Effects of NIP-142 on the AFL With Anatomical Obstacle Model

Based on the efficacy of NIP-142 for vagally induced AF, we also tested whether this new compound could terminate AFL induced after placing an anatomical obstacle. This AFL model did not depend on vagal stimulation. Unexpectedly, AFL was terminated without any appreciable increases in flutter CL and was not inducible after the injection of NIP-142. Class III and Ic drugs are effective in terminating canine AFL caused by reentry.¹³ Prolongation of flutter CL is less when treated with class III drugs as compared with class Ic drugs; this finding suggests that flutter CL depends on conduction velocity but not on refractoriness in the anatomical obstacle model of reentry.^{13,14} The present result is consistent with a class III antiarrhythmic effect of NIP-142. Before termination of AFL with NIP-142, AFL degenerated into AF in 2 dogs as is sometimes seen with class III drugs.^{15,16} Selective I_{Kr} blockers, d-sotalol¹⁴ and E-4031,¹³ have 86% and 100% termination of AFL, and 29% and 100% of prevention of AFL, respectively, in the canine model with intercaval crush. Another new class III drug, azimilide, which blocks both I_{Kr} and I_{Ks} , terminated AFL and suppressed re-initiation of AFL in 100% of dogs in a sterile pericarditis model.¹⁷

The plasma concentration of NIP-142 just after termination of AFL was 1.7 µg/ml or 3.9 µmol/L. In vitro data from the carbachol-treated atrial muscle suggest that this concentration of NIP-142 would produce little effect on atrial ERP even in the presence of high vagal tone. NIP-142 might be deactivated rapidly in vivo; but this needs to be clarified.

Electrophysiological Effects of NIP-142

The mechanism of NIP-142's ability to terminate and prevent AFL caused by macroreentry can be explained by its effect on ionic currents other than $I_{K,ACH}$. The prolongation of atrial ERP by NIP-142 decreased as the BCL shortened from 300 to 200 ms. However, the prolongation by NIP-142 became greater at the shortest BCL of 150 ms (Fig 5) and this was reflected in the significant increase in the 1:1 SA interval. These results indicate that NIP-142 has unique use dependency for atrial ERP as compared with many other class III antiarrhythmic drugs.^{8,9} The mechanism of the biphasic effect of NIP-142 on the use dependency for atrial ERP is not clarified yet, but might involve frequency dependent changes in the balance of action on the inward currents such as the sodium current (I_{Na}) and I_{Ca} vs the outward currents such as $I_{K,ACH}$, the transient outward current

(I_{to}), the ultrarapid component of delayed rectifier current (I_{Kur}), I_{Kr} and I_{Ks} .

A previous study showed that NIP-142 prolonged guinea pig atrial but not ventricular ERP.⁴ In contrast, in the present study NIP-142 slightly prolonged both atrial and ventricular ERP. This difference between the guinea pig and dog could be attributable to a species-specific distribution of cardiac ionic currents. The in vitro study showed that NIP-142 did not change the APD of atrial cells in the absence of carbachol. The discrepancy between the effects of NIP-142 on atrial ERP in vivo and the lack of a change in atrial APD in vitro may be explained by the presence of the intact vagi in the in vivo study. The resting basal vagal tone could have some effects on atrial ERP in vivo through $I_{K,ACH}$.

In the present study we observed a loss of phase-1 notch, an increase in the height of the plateau and no change in the APD at a stimulation frequency of 1 Hz. Mathematical model analysis¹⁸ suggests that the changes in action potential configuration induced with NIP-142 could be explained through suppression of I_{to} and I_{Kur} . The prolongation of ERP by NIP-142 was greater in the atrium than in the ventricle (Table 1, Fig 5), a finding suggestive of suppression of canine I_{Kur} , and suppression of I_{to} with NIP-142 might account for the slight increase in the ventricular ERP. The effects of NIP-142 on ionic currents have been studied by several investigators. Matsuda et al studied the effect of NIP-142 on cloned human cardiac K channel Kv1.5 (hKv1.5) using whole-cell voltage-clamp methods.¹⁹ The hKv1.5 channel is the molecular identity of the I_{Kur} in the human heart. NIP-142 inhibited the hKv1.5 current in a concentration-dependent manner but independent of frequency. Seki et al evaluated the effect of NIP-141 (hydrochloride of NIP-142) on human atrial myocytes using whole-cell voltage-clamp methods²⁰ and found that NIP-141 inhibited both I_{to} and I_{Kur} in a dose-dependent manner. These findings are consistent with ours.

Significant prolongation of the AH interval and 1:1 AV interval with NIP-142 could be caused by suppression of I_{Ca} .⁵ In the clinical setting, suppression of the AV node through inhibition of I_{Ca} is useful for slowing the ventricular response during atrial tachyarrhythmias. In the present study, Na dependent conduction (ie, inter- and intra-atrial conduction) was not affected by NIP-142 at a pacing cycle length of 150 ms. Furthermore, the flutter CL of AFL induced with an anatomical obstacle, which is dependent on the circuit length and the conduction velocity, was not prolonged by NIP-142. These suggest that termination of AF and AFL by NIP-142 is not attributable to suppression of I_{Na} .

Study Limitations

First, experimental efficacy can not be directly extrapolated to the clinical setting. There are some differences in atrial electrophysiological properties between species; I_{Kur} in the dog has different properties from that in the human.^{20,22} Furthermore, the mechanisms of AF and AFL in the present study might not be identical to those of clinical AF and AFL. The properties of AF induced by vagal stimulation in dogs could be different from those in the diseased, dilated atria seen in chronic AF. However, this canine model of AF may resemble paroxysmal AF occurring under increased vagal tone in selected patients with relatively normal atria.^{23,24} Second, the dose-response effects of NIP-142 on cardiac electrophysiologic variables were not determined. Third, NIP-142 reversed carbachol induced short-

ening of APD, suggesting inhibition of $I_{K,ACh}$, but direct determination of ionic currents was not performed. Finally, action potential properties were determined only at a stimulation CL of 1 s, so combinations at shorter CL could have revealed more detailed rate dependent effects of NIP-142.

Conclusion

Although the present study was limited for the reasons just outlined, NIP-142 is a promising, new antiarrhythmic drug with a unique rate dependent effect on atrial ERP. It has less effect on ventricular refractoriness compared with the atrium and suppresses AH conduction, suggesting a potential effectiveness in treating clinical AF and AFL with low risk of ventricular proarrhythmia.

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