Experimental Studies

Potassium Channel Openers Antagonize the Effects of Class III Antiarrhythmic Agents in Canine Purkinje Fiber Action Potentials
Implications for Prevention of Proarrhythmia Induced by Class III Agents

Masahiko Kondo, MD, Takeshi Tsutsumi, MD, and Saburo Mashima, MD

SUMMARY
We studied the effects of potassium channel openers (PCOs) on frequency dependent prolongations of action potential duration (APD), triggered activities and oscillatory action potentials (OSC) induced by E-4031 and dofetilide.

The action potentials of canine Purkinje fibers were recorded by a glass microelectrode technique. The effects of E-4031 (10⁻⁶ M) as well as that of additional nicorandil (2 × 10⁻⁵ M) on the APD were examined. When abnormal automaticity was observed under perfusion of E-4031 (10⁻⁵ M) or dofetilide (10⁻⁵ M), action potentials were recorded continuously to estimate the sequential effects of additional perfusion of nicorandil (6 × 10⁻⁵ M) or Y-26763 (10⁻⁵ M) on triggered activities and OSC.

APD prolongation by E-4031 at slower stimulation rates (cycle lengths ≥1000 msec) was suppressed by nicorandil in a dose dependent manner. Both nicorandil and Y-26763 abolished the train of early afterdepolarization (EAD) due to E-4031 or dofetilide with a shifting of the resting membrane potential to a more negative level. PCOs also normalized dofetilide induced abnormal automaticities (EAD, OSC).

The antagonistic actions of PCOs on changes in action potential induced by class III antiarrhythmic agents may prevent the development of proarrhythmias produced by these agents. (Jpn Heart J 1999; 40: 609–619)

Key words: Class III antiarrhythmic agents, Reverse frequency dependence, Potassium channel opener, Early afterdepolarization, Triggered activity

CLASS III antiarrhythmic agents are characterized by prolongation of the QT interval and the ventricular refractory period, which is known to be associated with the potential to induce torsades de pointes (TdP) and other ven-
tricular tachyarrhythmias or sudden death.  

On the other hand, potassium channel openers (PCOs) which have been clinically used as antihypertensive agents and coronary vasodilators, are also known to have antiarrhythmic and cardioprotective actions.\textsuperscript{2-4} It was reported that PCOs abolished APD prolongation and early afterdepolarization (EAD) induced by Class III agents and other related substances.\textsuperscript{5-8} While Class III agents are known to exert a more remarkable effect at slower rates, the relationship between the action of PCOs and so-called reverse frequency dependence (RFD) has not been elucidated in detail. Moreover, different membrane mechanisms have been suggested for the action potential prolongation and RFD under the action of Class III antiarrhythmic agents.\textsuperscript{9}

In this study, we evaluated the effects of PCOs semi-quantitatively on APD changes due to class III agents at different stimulation rates in canine Purkinje fibers. The antagonistic actions of PCOs to abnormal automaticities due to class III agents were also studied. The results indicate somewhat different behaviors of APD changes at fast and slow stimulation rates, as well as the prevention by PCOs of Tdp due to high concentrations of class III antiarrhythmic agents.

**Methods**

Six mongrel dogs (8–12 kg) were used. The heart was isolated under pentobarbital anesthesia and immersed in 4°C cooled Tyrode’s solution (mM; NaCl 137.0, NaH\(_2\)PO\(_4\) 1.8, NaHCO\(_3\) 12.0, MgCl\(_2\) 0.5, KCl 2.7, CaCl\(_2\) 1.8, Glucose 5.5; pH 7.3 ± 0.5) gassed with 95% O\(_2\) 5% CO\(_2\). Free running false tendons were dissected from the right ventricle and mounted in a lucite tissue bath with an effective volume of 4 ml. Preparations were perfused at 8 ml/min with gassed Tyrode’s solution at 37 ± 0.5°C. Bipolar suction electrodes (tip OD 0.5 mm of less) were connected to a stimulator with an isolated output. Stimuli were 2 msec in duration and the current was 2 times threshold. Purkinje fibers were impaled with glass microelectrodes selected for tip resistances of 20–50 MΩ and filled with 3M KCl. The microelectrodes were connected through silver-silver chloride connections to an operational amplifier (model-750, WPI Inc, New Haven, USA) with variable input capacitances. The bath was grounded through a similar 3M KCl-silver-silver chloride junction. The amplified transmembrane potential signal was displayed on a dual beam oscilloscope (SS-6611, Iwatsu Inc, Tokyo) and recorded on a thermal array recorder (RTA-1100, Nihon Kohden Co., Ltd., Tokyo). The signal from the amplifier and a sawtooth signal (200 V/sec) were input into an analog differentiator whose output was linear in the 100–1000 V/sec range. The oscilloscope screen was photographed and the action potential duration at 90% repolarization (APD90) and maximum upstroke
velocity of phase zero ($V_{max}$) were measured manually.

The specimens were stimulated at 1 Hz for about 60 minutes until the action potential characteristics had stabilized. To record the cycle length (CL)-dependent alterations of the action potential, CL was switched successively to 300, 500, 700, 1000, 2000, and 4000 msec. After the control data were recorded, Tyrode's solution containing E-4031 ($10^{-6}$ M) was applied and the action potential was recorded in the same manner as the controls. Following these observations, nicorandil ($2, 6 \times 10^{-5}$ M) was added in the perfusate and CL dependent APD changes were examined again. The concentration of nicorandil was selected with reference to the clinically used dose in Japan. If triggered activity was present under class III agents (E-4031 or dofetilide; $10^{-5}$ M), nicorandil ($2$ or $6 \times 10^{-5}$ M) or Y-26763 ($10^{-5}$ M), a benzopyran derivative PCO$_{10}$ was added to the perfusate until triggered activity disappeared. The larger dose ($10^{-3}$ M) of class III agents is considered to be the toxic level since abnormal automaticities can be induced in all experiments under low potassium (3.0 meq or less) condition.

**Drugs:** E-4031 was obtained from Eisai Co. Ltd. (Tokyo), Nicorandil was obtained from Chugai Pharmaceutical Co. Ltd. (Tokyo), and 4 and 12 mg vials intravenously administered in coronary care unit were used. Dofetilide was provided by Pfizer Pharmaceuticals Inc. (Tokyo), and Y-26763 was obtained from Japan Tobacco Inc. (Osaka, Japan).

**Data analysis:** A standard linear regression method was used to analyze the relationships between CL and APD$_{90}$. The slope of every group was calculated and compared using two way analysis of variance. Student's paired t-test was used to analyze differences between control APD$_{90}$ versus APD$_{90}$ under drugs. All values are presented as mean ± SD.

**Results**

**Action potential duration:** The changes in action potential of the Purkinje fibers are shown in Figure 1. The upper tracings show, as control, APD shortening with increasing stimulation rates. When E-4031 was added to the perfusate, APDs were prolonged, particularly at slower stimulation rates (CL > 1000 msec) as shown in the middle tracings of Figure 1. APD$_{90}$ with CL of 1000 msec was 343 msec before and 495 msec after application of E-4031.

The marked prolongation of APD at the slower stimulation rates under E-4031 indicates that E-4031 has a reverse frequency dependent action. APD$_{90}$ with CL of 300 msec was 174 msec in the control and 275 msec after E-4031. APD$_{90}$ at higher stimulation rates was found to be longer under E-4031 than that in the control. The lower tracings in Figure 1 show the effect of nicorandil on
Figure 1. Cycle length dependent changes in action potential under the perfusion of E-4031 and E-4031 plus nicorandil. C = control, E-4031: E-4031; 1 μM, E-4031 + nicorandil: addition of nicorandil (20 μM) to E-4031 (1 μM). The values on the bottom indicate the cycle length.

Table I. Cycle Length Dependent Changes in APD_{90} Produced by E-4031 and Nicorandil

<table>
<thead>
<tr>
<th>BCL (msec)</th>
<th>4000</th>
<th>2000</th>
<th>1000</th>
<th>700</th>
<th>500</th>
<th>400</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 8)</td>
<td>396.2</td>
<td>406.2</td>
<td>378.8</td>
<td>346.3</td>
<td>303.8</td>
<td>266.3</td>
<td>230.0</td>
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<tr>
<td></td>
<td>± 36.4</td>
<td>± 17.1</td>
<td>± 18.3</td>
<td>± 21.2</td>
<td>± 18.0</td>
<td>± 9.9</td>
<td>± 13.2</td>
</tr>
<tr>
<td>E-4031 (n = 5)</td>
<td>660.2</td>
<td>588.4</td>
<td>528.0</td>
<td>460.8</td>
<td>382.8</td>
<td>330.6</td>
<td>288.0</td>
</tr>
<tr>
<td></td>
<td>± 67.3*</td>
<td>± 75.6*</td>
<td>± 71.9*</td>
<td>± 46.4*</td>
<td>± 22.8*</td>
<td>± 14.1*</td>
<td>± 22.8*</td>
</tr>
<tr>
<td>E-4031 + nicorandil (2 × 10^{-5} M)</td>
<td>548.3</td>
<td>508.3</td>
<td>453.3</td>
<td>403.3</td>
<td>348.3</td>
<td>311.7</td>
<td>255.8</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>± 60.3'</td>
<td>± 43.0'</td>
<td>± 33.3'</td>
<td>± 28.8'</td>
<td>± 20.4'</td>
<td>± 16.0'</td>
<td>± 14.9'</td>
</tr>
<tr>
<td>E-4031 + nicorandil (6 × 10^{-5} M)</td>
<td>315</td>
<td>312.5</td>
<td>302.5</td>
<td>287.5</td>
<td>260.0</td>
<td>237.5</td>
<td>215.0</td>
</tr>
<tr>
<td>(n = 4)</td>
<td>± 12.9'</td>
<td>± 22.2'</td>
<td>± 31.6'</td>
<td>± 26.3'</td>
<td>± 21.6'</td>
<td>± 20.6'</td>
<td>± 17.3'</td>
</tr>
</tbody>
</table>

All values are given as mean ± SD. APD_{90} = action potential duration at 90% repolarization; CL = cycle length (msec) p < 0.01 control vs E-4031, p < 0.01 E-4031 vs E-4031 + nicorandil (2 × 10^{-5} M).

APD prolongation induced by E-4031. As shown in these tracings, APDs were markedly shortened in a CL dependent manner. The results of the measurements of APD_{90} are summarized in Table I. APD_{90} under E-4031 alone was significantly longer (p < 0.01) than the control at all CLs. When nicorandil (2 × 10^{-5} M) was added, APD_{90} was markedly shortened at all CLs. Higher concentrations of nicorandil produced pronounced APD_{90} shortening, resulting in an APD_{90}
shorter than control. Throughout the experiment, resting membrane potentials were not significantly different from the control.

The relation between APD<sub>90</sub> and CL is plotted on the semi-logarithmic scale in Figure 2. The degree of the prolongation of APD by class III agents is more marked at slower heart rates, and declines as the pacing rate increases. As shown in Figure 2, APD<sub>90</sub> as a function of CL is somewhat different between lower and higher stimulation rates. Hence, we analyzed curves in Figure 2 separately at slower (CL ≥ 1000 msec) and faster (CL ≤ 1000 msec) stimulation rates. Under E-4031 perfusion, CL dependent APD<sub>90</sub> prolongation became more marked at the slower stimulation rates. Under the perfusion of E-4031 combined with a smaller dose of nicorandil (2 × 10<sup>-5</sup> M), APD<sub>90</sub> was shortened in all CLs. The larger dose of nicorandil (6 × 10<sup>-5</sup> M) produced a more marked decrease in APD in both ranges of stimulation rates. The values of parameters obtained from separate analysis of regression lines of high and lower stimulation rates with and without drugs are summarized in Table II. There is a significant difference between the values of the slope in the control and after E-4031 (10<sup>-6</sup> M). E-4031 perfusion markedly increased the slope, especially at slower stimula-
Table II. Parameters from Regression Lines between Cycle Length and Action Potential Duration (APD₉₀)

<table>
<thead>
<tr>
<th></th>
<th>BCL ≤ 1000 msec</th>
<th>BCL ≥ 1000 msec</th>
</tr>
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<tr>
<td></td>
<td>r</td>
<td>slope</td>
</tr>
</tbody>
</table>
| Control (n = 8)  | 0.92 | 0.208 | 380.1 *  | 0.28 | 0.006 * | 184.5 *
| E-4031 (n = 5)   | 0.91 | 0.345 | 491.6 NS | 0.63 | 0.04 | 197.3
| E-4031 + nicorandil (2 × 10⁻³ M) (n = 6) | 0.93 | 0.270 | 433.3 * | 0.64 | 0.03 | 197.8 *
| E-4031 + nicorandil (2 × 10⁻³ M) (n = 6) | 0.80 | 0.123 | 301.3 | 0.22 | 0.004 | 189.3

BCL = basic cycle length; r = correlation coefficient; slope = slope of regression line; Incep = intercept of regression line. * < p = 0.05; ** < p = 0.01; NS = not significant.

Figure 3. Induction of early afterdepolarization after the perfusion of E-4031 St = electrical stimulation; Vₘₐₓ = maximum upstroke velocity of phase zero; E = electrogram; OFF = no electrical stimulation; EAD = early afterdepolarization. The changes in Purkinje fiber action potentials after perfusion of E-4031 are shown in panel A-D. After the perfusion of E-4031 (B), APDs were markedly prolonged (panel B-D) and EAD was elicited in panel C. The repetitive firings triggered from normal automaticity appear in panel D.

...tion rates. Nicorandil (2 × 10⁻³ M) reduced the values of slope. With a larger dose of nicorandil (6 × 10⁻³ M), the slope returned nearly to the control value at slower rates, and was also more markedly reduced than control level at higher rates.

Early afterdepolarization and oscillatory potential: Tracings from Purkinje fiber in Figure 3 clearly show that E-4031 induced EAD. Several minutes after
the perfusion of E-4031 (2 × 10⁻⁶ M), the action potential was prolonged at slower stimulation rates (CL ≥ 2000 msec). EAD was induced in 15 of 18 experiments when APD90 exceeded 500 msec or more, as shown in panel C in Figure 3. Take off potential of EAD was −45 to −55 mV. The repetitive firings of EAD were frequently observed when the spontaneous firing rate was relatively slow (0.2 Hz or less). V_{max} was only slightly reduced after E-4031 perfusion. Simultaneous records of external electrogram (E) with successive firings of EADs in panels C and D suggested that EAD was conducted through the Purkinje-muscle junction to ventricular myocardium. Ten to fifteen minutes after E-4031 perfusion, Purkinje fiber action potential was prolonged and slow normal automaticity was observed. Subsequently the train of EAD triggered from the prolonged action potential appeared as shown in panel B in Figure 4. Panel C in Figure 4 shows that the firing rate of EAD decreased immediately after adding Y-26763 to E-4031, and then the take off potential was abruptly restored to a more negative level and EAD was rapidly abolished as the resting membrane potential was normalized. Several minutes after adding Y-26763, the automatic activities were completely suppressed as in panel D. Figure 5 shows the effects of doxetilide and
Figure 5. Early afterdepolarization and oscillatory potential induced by dofetilide and the effects of niconandil on the abnormal automaticities. S = electrical stimulation; Vmax = maximum upstroke velocity of phase zero; OSC = oscillatory potential; EAD = early afterdepolarization. After the perfusion of dofetilide (10^{-3} M), APD prolongation (panel A) and slow diastolic depolarization were observed (panel B). Following the appearances of OSCs or delayed afterdepolarization (DAD), action potentials triggered from OSC appeared, some of which were accompanied by EADs (panels B and C). Additional perfusion of niconandil (5 x 10^{-5} M) rapidly abolished EAD (panel D). Amplitude of action potential and resting membrane potential were gradually normalized. Several minutes after niconandil, action potential was normalized despite the presence of dofetilide (panel E).

Additional PCO (niconandil) on the action potential alterations induced by dofetilide (10^{-5} M). About twenty minutes after perfusion of dofetilide, APD was prolonged and slow diastolic depolarization was observed in the early half recording of panel B, and then the automatic activity was provoked. In the latter half of panel B, after the driving stimuli were off, OSCs or delayed afterdepolarization (DAD) appeared from -10 to -15 mV depolarized from the resting membrane potential. Subsequently, automatic firing appeared when the amplitude of OSC reached the threshold level, and was accompanied by EADs as indicated by the arrows in panel B. One of these EADs recorded with higher paper speed is shown in panel C. Panel D shows the effect of niconandil on the dofetilide induced abnormal automatic activities. The application of niconandil (6 x 10^{-5} M) to the perfusate rapidly abolished the dofetilide induced EAD. The
amplitude of the action potential and resting membrane potential were then gradually normalized with simultaneous recovery of $V_{\text{max}}$. Several minutes after nicorandil, the action potential was normalized despite the presence of dofetilide (panel E).

**DISCUSSION**

**APD and K-channel opener:** It is known that action potential prolongation due to class III antiarrhythmic drug is more marked at slower heart rates, which characterizes the RFD of the drug action. Different manners of drug action have been suggested at fast and slow heart rates. In this study, we examined the effect of PCO on action potential prolongation due to class III drugs separately at faster and slower heart rates. The results indicated that nicorandil shortened the prolonged action potential under E-4031 perfusion, especially at slower rates. Nicorandil also normalized the slope of regression lines of action potential duration and the heart rate, which was steepened by E-4031.

E-4031 and dofetilide are considered to be specific blockers of IKr, although there is still controversy on the mechanism for the RFD of these drugs. It has been proposed that they act on the open channel state and the unblocking process is slower at slower heart rates. Elevated extracellular potassium concentration with tachycardia is supposed to reduce the IKr blocking action. On the other hand, the target channel of nicorandil has been reported to be IKATP.

Hence, our observations can be explained as a result of increased total repolarizing current due to nicorandil. At slower heart rates, a more marked decrease in IKr will exaggerate the effect of additional outward currents.

**Triggered activities and oscillatory potentials:** The present study demonstrated experimentally that repetitive firing from EAD was inhibited by nicorandil and Y-26763, as a result of deepening the firing membrane potential to the normal level. Previous studies have reported a suppressive effect of PCO on EAD of Purkinje fibers due to Bay K 8644, quinidine, and clofilium. This effect of PCO is manifested through enhancement of repolarization, common with cromakalim, pinacidil and nicorandil, regardless of their structural differences. In addition to confirming the reported enhancement of repolarization, the results of this study also indicate a hyperpolarizing action for PCO, which is similar with observations in vascular smooth muscle fibers.

In this study, the IKr blocker dofetilide showed remarkable changes in the Purkinje action potential, including depolarization of the resting membrane potential by 10 to 15 mV, DAD like oscillatory potentials and EAD at the same time (Figure 5). Abnormal automaticity of Purkinje fibers has been reported under high extracellular Ca, Ba, ketanserine, norepinephrine, and
strophanthinide,\textsuperscript{13,15} often accompanied by a decrease in the resting membrane potential. One of the reasons for DAD and oscillatory potentials in Figure 5, which were observed only after 20 to 30 min of the application of the drug, seems to be enhancement of Ca entry during prolonged action potential due to IKr blockers. Abnormal automaticities were all inhibited by Y-26763. Pumping out of Ca by means of Na/Ca exchange mechanism may play a role, which is accelerated by the hyperpolarizing effect of Y-26763.\textsuperscript{14} In summary, PCO worked as a sort of physiological antagonist against abnormalities of cardiac action potentials induced by toxic effects of class III antiarrhythmic agents.

**Clinical implications:** Class III antiarrhythmic agents sometimes cause a kind of polymorphic ventricular tachycardia called Tdp, through QT prolongation and bradycardia.\textsuperscript{1,15} The present study suggests that abnormal automaticity in Purkinje fibers can be the focus of ventricular tachycardia induced by class III antiarrhythmic agents. This is in accord with the results of a previous study by El-Sherif \textit{et al.}, which showed the endocardial origin of polymorphic tachycardias with rapid rate by means of three dimensional analysis of ventricular activation pattern.\textsuperscript{16}

On the other hand, increased dispersion of the action potential duration observed in congenital and acquired long QT syndromes has been considered to be a contributing factor for the reentrant arrhythmias.\textsuperscript{1,15} The rate of repetitive firing due to E-4031 was about 30/min in our experiments. Similar rates have been reported in previous reports with clofilium\textsuperscript{7} and quinidine.\textsuperscript{30} Far more rapid rates are experienced in clinical cases. Hence, EAD may trigger tachycardias but the continuation of arrhythmias seems to be not the result of repetitive firing of EAD, but rather involve reentrant mechanisms related to the QT prolongation. The results of the present study showed that nicorandil shortened the QT interval especially at slower rates, suggesting an inhibitory action of PCO to the reentry as well, in addition to the suppression of abnormal automaticities. As a result, PCO is considered to be effective for proarrhythmias due to class III drugs, especially for bradycardia dependent ventricular arrhythmias, as well as for ventricular arrhythmias in congenital long QT syndrome.\textsuperscript{15}

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**References**

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