Cardiac Electrophysiological and Antiarrhythmic Effects of N-n-butyl Haloperidol Iodide

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Key Words
N-n-butyl haloperidol • Arrhythmia • Electrophysiology • Ionic currents • Ventricular myocytes

Abstract
Aims: N-n-butyl haloperidol (F2), a novel compound of quaternary ammonium salt derivatives of haloperidol, was reported to antagonize myocardial ischemia/reperfusion injuries. The antiarrhythmic potential and electrophysiological effects of F2 on rat cardiac tissues were investigated. Methods and Results: In Langendorff-perfused rat hearts, the ventricular arrhythmias were induced by left anterior descending coronary artery of rat heart ligated for 20 min before the release of the ligature. F2 provided some inhibitive effects against ischemia- and reperfusion-induced ventricular arrhythmias. In His bundle electrogram and epicardial ECG recordings, the drug produced bradycardia, delayed the conduction through the atrioventricular node and prolonged the Wenckebach cycle length and atrioventricular nodal effective refractory period. In whole-cell patch-clamp study, F2 primarily inhibited the L-type Ca2+ current (IC50 = 0.17 µM) with tonic blocking properties and little use-dependence. And the drug also decreased the Na+ current (IC50 = 77.5 µM), the transient outward K+ current (IC50 = 20.4 µM), the steady-state outward K+ current (IC50 = 56.2 µM) and the inward rectifier K+ current (IC50 = 127.3 µM). Conclusion: F2 may be a promising drug for the treatment of ischemic heart disease with cardiac arrhythmia.

Introduction
Patients with ischaemic heart disease often experience events of ventricular tachyarrhythmia that may even culminate in sudden cardiac death. However, the CAST investigation [1, 2] found that some class Ic antiarrhythmics significantly increased postinfarction mortality. Moreover, the SWORD study documented that d-sotalol, a “pure” class III agent, caused torsade de pointes arrhythmias, and even might increase mortality in subsets of patients with myocardial infarction and lowered ejection fraction [3]. Prevention of ventricular arrhythmias and sudden cardiac death remains a continuing challenge in drug development despite intensive research in recent years.
N-n-butyl haloperidol (F2), a novel compound of quaternary ammonium salt derivatives of haloperidol, was found to maintain the effect of coronary artery relaxation but have no extrapyramidal side reactions like haloperidol [4]. Our previous studies showed that F2 could antagonize myocardial injury induced by ischemia/reperfusion in rat and rabbit [5, 6], and its cardioprotective mechanism might be associated with the inhibition of Ca2+ overload by blocking calcium channels of ventricular myocytes [7] and the suppression early growth response (Egr)-1 gene overexpression induced by myocardial ischemia/reperfusion [8]. As we know, myocardial ischemia/reperfusion can give rise to ventricular tachyarrhythmia, and arrhythmia usually got involved with ion-channel dysfunction. Due to the blocking effect of F2 on myocardial calcium channels, if F2 possess the anti-arrhythmic effects, the combination of cardioprotective and antiarrhythmic effects of F2 may be advantaged in treatment of ischaemic heart disease. We have therefore evaluated the potential of F2 in prevention of ischaemia/reperfusion induced arrhythmias and its electrophysiological and mechanical actions.

Methods and Materials

Animals
Adult Sprague-Dawley rats of either sex (220-300 g) were used in our experiments. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All experimental protocols were approved by the Laboratory Animal Ethics Committee of our institution (No. 2007101501).

Ischaemia- and reperfusion-induced arrhythmias
Rats were anaesthetized with sodium pentobarbitone (50 mg·kg−1, i.p.), were given heparin (250 units, i.p.), and then were killed by cervical dislocation. The heart was rapidly excised and mounted on a Langendorff apparatus and perfused via the aorta with oxygenated normal Tyrode solution (37°C). Perfusion pressure was constant, with equivalent 70 cm H2O. ECG involved recording from two silver wire electrodes placed on the aorta and the ventricular apex. Electrical signals were continuously monitored and recorded by computer after digitization by use of a biological data acquisition and analysis system (BL-420, TME Technology Co., Chengdu, China) at a sampling frequency of 1 kHz.

Rat hearts (n = 12 per group) were perfused for an initial 5 min with normal Tyrode solution, then the solution was switched in a randomized, blinded fashion to one of 5 solutions modified by the addition of stock solution to contain 0, 0.1, 0.3, 1.0, or 3.0 µM F2. The 0 µM stock solution contained only dimethylsulfoxide (DMSO), a solvent of F2. After a further 5 min of perfusion, the left anterior descending coronary artery was ligated for 20 min before the release of the ligature. The establishment of ischaemia/reperfusion was ascertained by the amount of coronary effluent [9]. Successful occlusion was confirmed by 30% - 40% reduction in coronary flow as compared with pre-ischaemic values.

In previous experiments we found that F2 produced sinus bradycardia. To examine the role of bradycardia in modulating the antiarrhythmic actions of F2, we performed additional studies in paced rat hearts (n = 12 per group). Methods were as for unpaced hearts (above) except that F2 was investigated at one concentration only (1.0 µM). Hearts were paced at a frequency of 5 Hz with the inner-installed stimulator of the BL-420 recording system with use of the bipolar atrial and ventricular electrodes (twice threshold current, 1 ms pulse width) [10].

Arrhythmias were defined according to the Lambeth Conventions [11]. When one type of arrhythmia converts into another type without an intervening period of sinus rhythm, each type of arrhythmia is recorded as having been present during the period of assessment.

His bundle electrogram (HBE) and epicardial ECG
In the Langendorff-perfused heart model, the self-made bipolar cannula electrodes, combining the functions of Langendorff-perfusion aortic cannula and recording electrodes, were inserted into the aortic root for HBE recording [12]. The HBE and epicardial ECG signals were continuously monitored and recorded in a computer after digitization through a multichannel physiological signal recording system (RM6240BD, Chengdu Instrument Factory, Chengdu, China). High right atrial pacing electrode was placed near the junction of the superior vena cava and right atrium, and ventricular pacing electrode was placed on the pericardium near the right-ventricular apex. A pacing stimulus of 1 ms duration with an intensity of twice the threshold current was delivered to the heart preparation through the bipolar atrial or ventricular electrodes.

The right atrium was paced at a constant rate (4 Hz) that is slightly faster than the spontaneous heart rate. At this constant rate pacing, the intra-atrial conduction time (SA), atrioventricular nodal conduction time (AH), His-Purkinje conduction time (HV) and ventricular repolarization time (VRT) were measured. The 3 following stimulating protocols were introduced for electrophysiological studies [9]. (1) Incremental right atrial pacing was used to determine the Wenckebach cycle length (WCL). The atrial pacing cycle length was decreased (every 5 s) in steps of 10 ms until a stable 1:1 atrioventricular conduction pattern was lost. The cycle length at which a 1:1 atrioventricular conduction just started to fail was defined as the WCL. (2) After a train of 8 stimuli (S1) of constant-rate atrial pacing, a single premature stimulus (S2) was introduced for unpaced hearts (above) except that F2 was investigated at one concentration only (1.0 µM). The coupling interval (S1S2) between the last S1 and S2 was progressively shortened in 10-ms steps after every train of stimuli until S2 did not evoke an atrial depolarization wave A2. The following data were obtained: atrial effective refractory period (AERP), the S S2 interval in which the S2, just started to fail to evoke an atrial depolarization wave A2; atrioventricular
nodal effective refractory period (AVNERP), the S1S2 interval in which the evoked A1 just started to fail to evoke a His bundle depolarization wave H2; and His-Purkinje functional refractory period (HPFRP), the shortest conducted V1V2 interval with increasing pacing rate. (3) The ventricular extrastimulation study protocol was similar to protocol one by applying incremental ventricular pacing. The ventricular effective refractory period (VERP) was defined as the pacing cycle length that just started to fail to evoke a premature ventricular depolarization.

The electrophysiological parameters of rat hearts (n = 10) were recorded with initially perfusion of normal Tyrode solution, then recorded after cumulative application of F2 (0.1-3.0 µM).

Whole-cell patch-clamp recording

Single ventricular myocytes were isolated from the hearts of adult rats by enzymatic dissociation as previously described [7]. Myocytes were perfused with HEPES-buffered Tyrode solution in a recording chamber at room temperature. Membrane potential and currents were recorded by the tight-seal whole-cell configuration with use of an Axopatch 200B amplifier with low-pass filtering at 2 kHz, digitized by DigiData 1322A and processed by PCLAMP 8.2 software (Axon Instruments, Foster City, CA, USA). The electrode capacitance and whole-cell capacitance currents were maximally compensated by use of the amplifier. The series resistance was compensated by 60% to 80%. The liquid junction potential between pipette and bath medium was not corrected.

Solutions and drugs

The normal Tyrode solution contained (in mM): NaCl 137.0, KCl 5.4, MgCl2 1.0, NaH2PO4 0.33, CaCl2 1.8, glucose 10.0 and HEPES 10.0, titrated to pH 7.4 with NaOH. The internal pipette filling solution contained (in mM): KCl 140.0, MgCl2 1.0, Na2-ATP 2.0, EGTA 10.0, and HEPES 5.0, adjusted to pH 7.2 with KOH. Na+ current (INa) was measured in low extracellular sodium ([Na+]o=10 mM, with NaCl replaced by Choline-Cl) solution to reduce INa and improve voltage control. To separate ion currents, we chose to add 30 µM Tetrodotoxin (for elimination of INa) and/or 0.2 mM CdCl2 (for elimination of ICa) into the bathing solution, and/or to substitute Cs+ for K+ in the bathing and pipette solution (for elimination of IK). F2 (synthesized by our lab and identified by Shanghai Organic Chemistry Institute of the Chinese Academy of Sciences; purity greater than 98%) was prepared as 0.1 M stock solution in DMSO and diluted to the desired drug concentration with bathing solution before each experiment, with DMSO less than 1% at the highest F2 concentration used. At this concentration DMSO by itself had no effect on the cells. Tetrodotoxin was purchased from Fisheries Science and Technology Development Company of Hebei Province, China.

Data analysis and statistics

Gaussian distributed variables are expressed as mean ± SEM. Statistical comparison of data was performed using one-way ANOVA followed by Tukey test for individual significant differences or paired Student’s t-test where appropriate. Binomially distributed variables were compared by chi-square test with Yates’ correction as appropriate. A P < 0.05 was considered significantly different. In the patch-clamp study, concentration-response curves were fitted by the Hill equation: 

\[ \frac{I_{\text{drug}}}{I_{\text{control}}} = \frac{1}{1 + (C/IC50)^h} \]

where \( I_{\text{drug}} \) and \( I_{\text{control}} \) are the current amplitudes in the...
presence of the drug at concentration $C$ and absence of the drug, respectively; $IC_{50}$ is the concentration for half-maximal block and $H$ is the Hill coefficient. The inactivation curves of the channel current were fitted by the Boltzmann equation:

$$I / I_{\text{max}} = \frac{1}{1 + \exp\left(\frac{V_m - V_h}{k}\right)}$$

where $I$ gives the current amplitude and $I_{\text{max}}$ its maximum, $V_m$ the potential of prepulse, $V_h$ the half-maximal inactivation potential, and $k$ the slope factor. The activation curves of the channel current were also fitted by the Boltzmann equation:

$$G / G_{\text{max}} = \frac{1}{1 + \exp\left(\frac{V_h - V_m}{k}\right)}$$

where conductance of channel ($G$) was calculated from the current-voltage relationship according to the following equation:

$$G = I / (V_m - V_{\text{rev}})$$

where $V_{\text{rev}}$ is the reversal potential of the current. The curve fitting was performed by use of OriginLab 7.5 (OriginLab Co., Northampton, MA, USA) and Clampfit 8.2 (Axon).

### Results

**Inhibition of ischaemia- and reperfusion-induced arrhythmias**

In the unpaced rat heart, F2 significantly reduced the incidence of ischemia- and reperfusion-induced arrhythmias in a concentration-dependent manner. At the highest drug concentration, ventricular tachycardia (VT) and ventricular fibrillation (VF) were completely abolished ($P < 0.05$), and ventricular premature beats (VPB) incidence was significantly reduced (Fig. 1A and B). F2 significantly widened RR and PR intervals before and during ischemia but had no effect on QT intervals (Table 1). F2 still possessed its antiarrhythmic actions on ischemia- and reperfusion-induced arrhythmias when hearts were paced at 5 Hz (Fig. 1C and D).

**Modification of the electrophysiological properties of the cardiac conduction system**

Representative electrograms for determining the electrophysiological properties of the cardiac conduction system are shown in Fig. 2. Changes in the electrophysiological parameters of the cardiac conduction system in 10 rats after cumulative application of F2 (0.1-3.0 µM) are summarized in Table 2. The conduction interval

<table>
<thead>
<tr>
<th>Group [F2 (µM)]</th>
<th>RR (ms) I-10</th>
<th>I-1</th>
<th>I+10</th>
<th>PR (ms) I-10</th>
<th>I-1</th>
<th>I+10</th>
<th>QT (ms) I-10</th>
<th>I-1</th>
<th>I+10</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>238±11</td>
<td>260±12</td>
<td>304±16</td>
<td>42±1</td>
<td>44±1</td>
<td>52±2</td>
<td>82±5</td>
<td>95±7</td>
<td>115±11</td>
</tr>
<tr>
<td>0.1</td>
<td>251±13</td>
<td>281±18</td>
<td>318±19</td>
<td>46±2</td>
<td>47±2</td>
<td>60±3*</td>
<td>91±9</td>
<td>94±9</td>
<td>114±16</td>
</tr>
<tr>
<td>0.3</td>
<td>225±9</td>
<td>274±16</td>
<td>319±17</td>
<td>40±2</td>
<td>46±2</td>
<td>64±4*</td>
<td>81±5</td>
<td>89±5</td>
<td>109±20</td>
</tr>
<tr>
<td>1.0</td>
<td>239±11</td>
<td>317±17*</td>
<td>382±24*</td>
<td>42±1</td>
<td>52±2*</td>
<td>71±6*</td>
<td>83±5</td>
<td>105±12</td>
<td>127±19</td>
</tr>
<tr>
<td>3.0</td>
<td>246±12</td>
<td>328±21*</td>
<td>408±27*</td>
<td>45±2</td>
<td>58±3*</td>
<td>76±7*</td>
<td>85±6</td>
<td>108±11</td>
<td>136±22</td>
</tr>
</tbody>
</table>
through the atrial tissue (SA interval) was not significantly affected. However, the conduction through the atrioventricular node (AH interval) was significantly lengthened by F$_2$ in a concentration-dependent manner. The conduction through the His-Purkinje system (HV interval) and ventricular repolarization time (VRT) was lengthened by F$_2$ only at a high concentration (3.0 µM). After 5-min washout of F$_2$, the lengthened intervals did not recover completely to the interval before treatment. It suggested that F$_2$ may have high affinity to the myocardial tissues. F$_2$ concentration-dependently prolonged WCL, AVNERP and HPERP. At high concentrations (1.0, 3.0 µM), AERP and VERP were also prolonged.

Effect of F$_2$ on action potential

Action potential was elicited in current-clamp mode by 3-ms, 2-nA current injections at 1 Hz. Fig. 3 illustrates the concentration-dependent effects of F$_2$ on the action potential waveforms in a rat ventricular myocyte. The resting membrane potential was not significantly affected by F$_2$. At the highest concentration (100 µM), the action potential amplitude (APA) and the maximal upstroke velocity of depolarization ($V_{\text{max}}$) were decreased, and the action potential duration at 50% and 90% repolarization (APD$_{50}$ and APD$_{90}$) was prolonged. The effect of F$_2$ was reversed after 5-min washout with control solution (Table 3).

**Table 2.** Concentration-related effect of F$_2$ on the conduction system of rat isolated perfused hearts. Values are means ± SEM ($n=10$). SA, sinoatrial conduction interval; AH, atrio-His bundle conduction interval; HV, His-ventricular conduction interval; VRT, ventricular repolarization time interval; WCL, Wenckebach cycle length; AERP, atrial effective refractory period; AVNERP, atrioventricular nodal effective refractory period; HPFRP, His-Purkinje system functional refractory period; VERP, ventricular effective refractory period. *$P<0.05$ vs control group.

<table>
<thead>
<tr>
<th>F$_2$ (µM)</th>
<th>SA (ms)</th>
<th>AH (ms)</th>
<th>HV (ms)</th>
<th>VRT (ms)</th>
<th>WCL (ms)</th>
<th>AERP (ms)</th>
<th>AVNERP (ms)</th>
<th>HPFRP (ms)</th>
<th>VERP (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>10 ± 1</td>
<td>40 ± 1</td>
<td>14 ± 1</td>
<td>74 ± 5</td>
<td>129 ± 6</td>
<td>49 ± 2</td>
<td>97 ± 4</td>
<td>130 ± 6</td>
<td>66 ± 3</td>
</tr>
<tr>
<td>0.1</td>
<td>10 ± 1</td>
<td>42 ± 2</td>
<td>15 ± 1</td>
<td>76 ± 5</td>
<td>130 ± 7</td>
<td>51 ± 2</td>
<td>108 ± 5</td>
<td>132 ± 3</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>0.3</td>
<td>11 ± 1</td>
<td>44 ± 2*</td>
<td>15 ± 2</td>
<td>80 ± 7</td>
<td>143 ± 6*</td>
<td>53 ± 3</td>
<td>120 ± 5*</td>
<td>172 ± 6*</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>1.0</td>
<td>10 ± 1</td>
<td>54 ± 4*</td>
<td>17 ± 2</td>
<td>83 ± 4</td>
<td>161 ± 7*</td>
<td>58 ± 3*</td>
<td>150 ± 5*</td>
<td>190 ± 4*</td>
<td>107 ± 4*</td>
</tr>
<tr>
<td>3.0</td>
<td>12 ± 1</td>
<td>58 ± 7*</td>
<td>21 ± 2*</td>
<td>90 ± 6*</td>
<td>194 ± 9*</td>
<td>74 ± 4*</td>
<td>178 ± 6*</td>
<td>221 ± 7*</td>
<td>112 ± 7*</td>
</tr>
<tr>
<td>Washout</td>
<td>12 ± 1</td>
<td>56 ± 5*</td>
<td>17 ± 2</td>
<td>92 ± 7*</td>
<td>174 ± 10*</td>
<td>59 ± 4*</td>
<td>148 ± 5*</td>
<td>190 ± 4*</td>
<td>103 ± 7*</td>
</tr>
</tbody>
</table>

**Table 3.** Effect of F$_2$ on action potential parameters in rat ventricular myocytes. Values are means ± SEM ($n=6$). RMP, resting membrane potential; APA, action potential amplitude; $V_{\text{max}}$, maximum upstroke velocity of depolarization; APD$_{50}$ and APD$_{90}$, action potential duration measured at 50% and 90% of repolarization, respectively. *$P<0.05$ vs control group.

<table>
<thead>
<tr>
<th>F$_2$ (µM)</th>
<th>RMP (mV)</th>
<th>APA (mV)</th>
<th>$V_{\text{max}}$ (V/s)</th>
<th>APD$_{50}$ (ms)</th>
<th>APD$_{90}$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>71.1 ± 0.8</td>
<td>119.0 ± 4.6</td>
<td>136.4 ± 13.7</td>
<td>7.8 ± 0.8</td>
<td>21.1 ± 1.4</td>
</tr>
<tr>
<td>0.1</td>
<td>72.2 ± 0.9</td>
<td>121.2 ± 5.5</td>
<td>148.1 ± 16.2</td>
<td>7.2 ± 0.8</td>
<td>20.4 ± 1.5</td>
</tr>
<tr>
<td>1</td>
<td>72.9 ± 1.1</td>
<td>116.7 ± 4.5</td>
<td>139.3 ± 16.4</td>
<td>7.2 ± 0.6</td>
<td>19.8 ± 1.0</td>
</tr>
<tr>
<td>10</td>
<td>72.6 ± 1.2</td>
<td>103.3 ± 4.5*</td>
<td>125.6 ± 12.5</td>
<td>7.7 ± 0.6</td>
<td>21.5 ± 1.2</td>
</tr>
<tr>
<td>100</td>
<td>71.6 ± 1.0</td>
<td>79.8 ± 6.4*</td>
<td>84.4 ± 11.3*</td>
<td>9.4 ± 0.9*</td>
<td>24.1 ± 1.6*</td>
</tr>
<tr>
<td>Washout</td>
<td>73.0 ± 1.3</td>
<td>109.5 ± 6.6</td>
<td>130.1 ± 12.8</td>
<td>7.5 ± 0.6</td>
<td>22.0 ± 1.3</td>
</tr>
</tbody>
</table>

**Fig. 3.** Effect of F$_2$ on action potential waveforms in a rat ventricular myocyte.
Effect of F₂ on Na⁺ currents (I_{Na})

I_{Na} was elicited by 30 ms step depolarization from a holding potential of -100 mV to potentials ranging from -80 to +20 mV in 10 mV increments at 1 Hz (Fig. 4A). F₂ reduced I_{Na} amplitude at all potentials tested, without changes in either threshold potential or peak potential (Fig. 4B). The concentration-dependent effect of F₂ on I_{Na} at -30 mV was fitted by the Hill equation to reveal a concentration for half-maximal block (IC₅₀) of 77.5 µM and a Hill coefficient (H) of 0.61 (Fig. 4C).

To study the effect of F₂ on the voltage-dependent steady-state I_{Na} inactivation and test current traces under control conditions (middle) and after 5-min superfusion with 10 µM F₂ (right). (E) Voltage dependence of steady-state I_{Na} activation and inactivation in the absence and presence of 10 µM F₂.

Effect of F₂ on K⁺ currents (I_{K⁺})

I_{K⁺} was elicited by 30 ms step depolarization from a holding potential of -100 mV to potentials ranging from -80 to +20 mV in 10 mV increments at 1 Hz (Fig. 5A). F₂ reduced I_{K⁺} amplitude at all potentials tested, without changes in either threshold potential or peak potential (Fig. 5B). The concentration-dependent effect of F₂ on I_{K⁺} at -30 mV was fitted by the Hill equation to reveal a concentration for half-maximal block (IC₅₀) of 77.5 µM and a Hill coefficient (H) of 0.61 (Fig. 5C).

To study the effect of F₂ on the voltage-dependent I_{K⁺} availability, a double-pulse experiment was applied with a 20 ms test pulse to -30 mV following a 1000 ms conditioning prepulse from the holding potential of -100 mV to potentials ranging from -150 to -50 mV in 10 mV steps (Fig. 5D). The steady-state Na⁺ channel inactivation curves were obtained by normalizing I_{Na} amplitudes to their maximum value and were plotted as a function of prepulse membrane potential. F₂ appeared to block the Na⁺ current by causing a negative shift of the steady-state inactivation curve without affecting the slope factor. On average (n = 3), V₀ = -91.9 ± 3.9 mV and k = 7.7 ± 1.8 mV under control conditions, and V₀ = -104.9 ± 5.3 mV (P < 0.05) and k = 8.4 ± 2.3 mV (P > 0.05) with 10 µM F₂. The Na⁺ channel activation curves were obtained by normalizing conductance of the Na⁺ channel to their maximum value and were plotted as a function of membrane potential (Fig. 5E). F₂ did not affect the voltage dependence for activation.

Fig. 5. Effect of F₂ on K⁺ currents. (A) Families of current traces elicited by a series of 400-ms long hyperpolarizing or depolarizing pulses ranging from -140 to +60 mV from a holding potential of -80 mV in the absence and presence of 1 µM F₂. (B) and (C) Averaged I-V relationship for I_{peak} and I_{SS} observed in the absence and presence of F₂. (D) Concentration-response curve for the effect of F₂ on I_{K₁}, I_{K₂} and I_{SS}.
Fig. 6. Effect of F2 on I_{Ca,L} (A) The original records of I_{Ca,L} elicited by 300-ms step depolarizations from a holding potential of -40 mV to potentials ranging from -30 to +70 mV under control conditions and after superfusion with F2. (B) I-V relationship for I_{Ca,L}. (C) The original superimposed records of I_{Ca,L} elicited by 300-ms step depolarization from -40 to +10 mV under control conditions and after superfusion with F2. (D) Concentration-response curve for the inhibition of F2 on I_{Ca,L}. (E) Effect of F2 on the voltage dependence of steady-state I_{Ca,L} activation and inactivation. Top: The clamp protocol (left) for voltage-dependent steady-state I_{Ca,L} inactivation and test current traces under control conditions (middle) and after 5-min superfusion with 1 µM F2 (right). (F) Effects of F2 on the recovery of I_{Ca,L} from inactivation. I_{Ca,L} completely recovered from inactivation within 500 ms under control conditions. Inset: The double pulse protocol and superimposed records of I_{Ca,L} elicited by the conditioning and test pulse in the presence of 1 µM F2. (Time axes were broken and the consecutive traces were recorded after 2000-, 3000-, 4000- and 5000-ms recovery interval). (G) Tonic and use-dependent inhibition of I_{Ca,L} by F2. Superimposed current records of the 1st and 15th I_{Ca,L} obtained on the repetitive depolarizing pulses from -40 to +10 mV at the frequency of 0.5 and 1 Hz during control conditions and after drug superfusion. (Ga) Relation between peak I_{Ca,L} and number of pulses applied at different rates under the control and F2-application conditions. (Gb) Ratio of peak I_{Ca,L} in presence of F2 and in control conditions, used as an estimator of the fraction of unblocked channels.

On average (n = 5), \( V_h = -44.3 \pm 3.6 \) mV and \( k = 5.2 \pm 0.6 \) mV under control conditions, and \( V_h = -44.7 \pm 4.3 \) mV and \( k = 4.7 \pm 0.8 \) mV (\( P > 0.05 \) for \( V_h \) and \( k \)) with 10 µM F2.

**Effect of F2 on K+ currents**

Typical current traces recorded in response to a series of 400 ms hyperpolarizing and depolarizing clamp steps to test potentials between -140 and +60 in 20 mV increments from a holding potential of -80 mV at 0.2 Hz are shown in Fig. 5A. The addition of F2 (1 µM) to the superfusion solution reduced the amplitude of peak outward K+ currents (mainly I_{to}) and peak inward K+ currents through the inward rectifier K+ channel (mainly I_{K1}). The steady-state K+ outward currents (I_{SS}) at the end of 400 ms clamp steps were also inhibited. Fig. 5B and C show the current-voltage relation for the peak K+ currents and I_{SS} before and after the addition of 0.1, 1 and 10 µM F2. The concentration-dependent effect of F2 on I_{to} and I_{SS} at +60 mV and on I_{K1} at -140 mV is...
shown in Fig. 5D. The IC$_{50}$ values calculated from the concentration-response curve were 20.4, 56.2 and 127.3 µM, and the Hill coefficients were 0.70, 0.72 and 0.63 for $I_{\text{to}}$, $I_{\text{SS}}$ and $I_{\text{Ki}}$, respectively.

Effect of F$_2$ on L-type Ca$^{2+}$ current ($I_{\text{Ca,L}}$)

To activate $I_{\text{Ca,L}}$, we delivered 300 ms pulses to potentials ranging from -30 to +70 mV in 10 mV increments from a holding potential of -40 mV (to inactivate $I_{\text{Na}}$ and T-type Ca$^{2+}$ current) at 0.2 Hz. Representative current traces obtained from a ventricular myocyte and the current-voltage relationships in the absence and presence of F$_2$ are shown in Fig. 6A and B. F$_2$ reduced $I_{\text{Ca,L}}$ amplitude with deceleration of the activation of $I_{\text{Ca,L}}$ (Fig. 6C). The activation of currents during a depolarizing pulse (from -40 to +10 mV) was well fitted to a mono-exponential function with activation time constants ($\tau_a$) in the absence and presence of F$_2$. On average ($n = 5$), the $\tau_a$ of $I_{\text{Ca,L}}$ was 5.4 ± 1.4 ms during control, and 5.6 ± 1.7 (P > 0.05), 10.3 ± 2.6 (P < 0.05) and 18.5 ± 4.2 ms (P < 0.05) after 0.1, 1 and 10 µM F$_2$ application, respectively. However, the inactivation of $I_{\text{Ca,L}}$ was unaffected. The decay of currents was well fitted to a biexponential function with fast and slow inactivation time constants ($\tau_f$ and $\tau_s$). The calculated $\tau_a$ and $\tau_s$ revealed no significant differences between the absence and presence of F$_2$. $\tau_a = 12.7 ± 3.4$ ms (control), 13.6 ± 3.8 ms (0.1 µM F$_2$), 14.0 ± 4.7 ms (1 µM F$_2$), and 14.5 ± 4.9 ms (10 µM F$_2$), and $\tau_s = 123.9 ± 39.4$ ms (control), 116.4 ± 37.8 ms (0.1 µM F$_2$), 144.3 ± 43.1 ms (1 µM F$_2$), and 153.6 ± 47.4 ms (10 µM F$_2$). Fig. 6D shows the concentration-dependent curve fitted by the Hill equation with an IC$_{50}$ of 0.17 µM, a Hill coefficient of 1.28 and maximal inhibition $E_{\text{max}}$ of 62.4%.

The voltage dependence of steady-state inactivation and activation curves of $I_{\text{Ca,L}}$ are shown in Fig. 6E. F$_2$ (1 µM) caused a small positive-shift of the steady-state inactivation relationship. $V_{h} = -31.2 ± 5.8$ mV and $k = 4.7 ± 0.9$ mV under control, and $V_{h} = -26.2 ± 6.0$ mV and $k = 5.0 ± 1.1$ mV ($n = 5$, P > 0.05) for $V_{h}$ and $k$ in the presence of F$_2$. However, 1 µM F$_2$ caused an increase of the slope factor of the steady-state activation curve. The values of $V_{h}$ were -6.3 ± 0.7 mV and -7.2 ± 1.0 mV ($n = 5$, P > 0.05), and the values of $k$ were 6.6 ± 0.6 mV and 9.1 ± 0.9 mV ($n = 5$, P < 0.05) under control and F$_2$ treatment, respectively.

Recovery of $I_{\text{Ca,L}}$ from inactivation was evaluated by a standard double pulse protocol. A 1-s conditioning pre-pulse was applied from -80 to +10 mV to inactivate $I_{\text{Ca,L}}$, followed by a 200 ms test-pulse from -80 to +10 mV at intervals (Δt) between 10 ms and 8 s. The frequency of stimulation was 0.1 Hz. The current for each test pulse was normalized to that for the prepulse and plotted as a function of recovery time (Fig. 6F). In control conditions, $I_{\text{Ca,L}}$ completely recovered from inactivation within 500 ms, and the time course of recovery from inactivation could be well fitted by a mono-exponential function, with the time constant of recovery $\tau_{\text{rec}} = 190.1 ± 9.1$ ms ($n = 5$). In the presence of 1 µM F$_2$, recovery of $I_{\text{Ca,L}}$ was decelerated and became biexponential, with fast time constant $\tau_{\text{rec,f}} = 150.6 ± 8.4$ ms and slow time constant $\tau_{\text{rec,s}} = 746.1 ± 11.7$ ms ($n = 5$).

To study the tonic and use-dependent block, a train of 15 depolarizing pulses from -40 to +10 mV for 200 ms was applied at frequencies of 0.5 and 1 Hz. The amplitude of $I_{\text{Ca,L}}$ induced by each successive pulse before and after the addition of 1 µM F$_2$ is plotted in Fig. 6Ga. Tonic blockade was assessed as the difference between the $I_{\text{Ca,L}}$ amplitude of the first pulse in the control condition and after drug exposure [13]. In the presence of F$_2$ (1 µM), the amplitude of $I_{\text{Ca,L}}$ evoked by the first pulse in the pulse train was reduced from the control value of 9.54 ± 1.36 pA·pF$^{-1}$ to 4.78 ± 0.94 pA·pF$^{-1}$ ($n = 4$, $P < 0.05$) at 0.5 Hz and from 9.76 ± 1.41 pA·pF$^{-1}$ to 4.93 ± 0.98 pA·pF$^{-1}$ ($n = 4$, $P < 0.05$) at 1 Hz. The ratio of $I_{\text{Ca,L}}$ in the presence of F$_2$ to that in control (Fig. 6Gb), which can be used as an approximate parameter of the fraction of unblocked $I_{\text{Ca,L}}$ channels [14], did not significantly decrease during the 15 pulses. The ratio of $I_{\text{Ca,L}}$ between the first and fifth pulse decreased only by 3.5% (0.5 Hz) and 7.2% (1 Hz). The data suggest that (a) F$_2$ showed tonic blocking properties and therefore showed some affinity for binding to the resting state of the $I_{\text{Ca,L}}$ channel, and (b) inhibition of $I_{\text{Ca,L}}$ by F$_2$ exhibited little use-dependence.

Discussion

The present study examined the potential of F$_2$ in prophylaxis of ischemia- and reperfusion-induced arrhythmias. The antiarrhythmic action may be mainly mediated through blockade of the Ca$^{2+}$ channels and partly through the Na$^+$ and K$^+$ channel. Consequently, F$_2$ could prolong the atrio-His bundle conduction intervals and the refractoriness of the cardiac conduction system.

An important consequence of both myocardial ischaemia and reperfusion is the occurrence of various

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disturbances of cardiac rhythm, including the potential lethal condition of VF [15]. Some anti-ischemic actions can also produce an indirect antiarrhythmogenic action. However, our results showed that F₂ might possess the direct antiarrhythmogenic effect via the influence of cardiac electrophysiology. First, anti-ischemic interventions delay only the onset of ischemia- and reperfusion-induced VF susceptibility [16] but do not suppress arrhythmic rate throughout the time course of ischemia, in contrast to the F₂ findings in the rat (Fig. 1C). Second, pacing rat hearts failed to reverse the protective effects of the drug, as would be expected if anti-ischemic actions contributed to the antiarrhythmic effects [10].

The results showed that F₂ might exert antiarrhythmic activity chiefly via blocking the Ca²⁺ channels. The IC₅₀ of F₂ blocking the L-type Ca²⁺ channel was close to its effective antiarrhythmic concentration; however, F₂ had little effect on the Na⁺ and K⁺ channels at the concentration range used in the present antiarrhythmic study. Drugs that block the L-type Ca²⁺ channel may preferentially slow conduction and increase the refractory period of slow response fibres [17], which are consistent with the observation of PR interval widening and prolongation of AV nodal conduction time, as well as AVNERP and WCL, by F₂ in experiments.

According to the modulated receptor hypothesis, affinity of the drug for the receptor varies with the state of the channel (resting, activated, or inactivated) by the separate rate constants [18, 19]. The results of experiments indicated that F₂ might have higher affinity for binding to the resting state of the L-type Ca²⁺ channel because of its tonic-blocking properties and little use-dependence. The affinity of F₂ to the inactivated- and resting-state Ca²⁺ channels could be estimated by the following equation:

\[ \Delta V_h = k \times \ln \left( \frac{1 + D/K_R}{1 + D/K_I} \right) \]

where \( \Delta V_h \) is the shift in the midpoint of the steady-state inactivation curve, \( k \) is the slope factor, \( D \) is the drug concentration, and \( K_R \) and \( K_I \) are the dissociation constants for resting and inactivated Ca²⁺ channels, respectively. The positive shift of \( \Delta V_h \) and \( k \) produced by 1 µM F₂ (Fig. 6E), along with the value for \( K_R \) from Fig. 6D, gives a value of \( K_I = 0.70 \mu M \) for binding to the inactivated Ca²⁺ channels, which is slightly greater than \( K_R = 0.17 \mu M \). The deceleration of activation of \( I_{Ca,L} \) and no influence on the inactivation of \( I_{Ca,L} \) also showed that F₂ could interact with the resting Ca²⁺ channel and had low affinity for binding to the open state. In this study, the recovery process was slowed by F₂ being better described by a double exponential, the slower phase possibly indicating a slow dissociation of drug molecules from the inactivated Ca²⁺ channels [20, 21]. This finding is consistent with F₂ possessing some affinity for binding to the inactivated Ca²⁺ channels. Our results are different from that of Huang, et al. [7], who found that F₂ had a high affinity to the inactivated Ca²⁺ channels from F₂ inducing a negative shift of steady-state inactivation curve and slowing down the recovery from inactivation of \( I_{Ca,L} \). The discrepancy with our results could be attributed to the different clamp protocol used and no compensation for capacity or leaky currents in the authors’ experiments.

Although F₂ had lower potency in blocking the Na⁺ and K⁺ channels than the L-type Ca²⁺ channel, it might contribute to synergistic effects on ischemia and reperfusion tachyarrhythmias. F₂ may exert some antiarrhythmic activity by suppression of oscillatory afterpotentials or extrasystole via blocking Na⁺ channels and by prolongation of APD and effective refractory period (ERP) via blocking K⁺ channels. An \( I_{Ca,L} \) blocker will lead to APD₉₀ shortening due to a decline of the plateau phase [22]. However, the present study showed that APD₉₀ and APD₉₀ were not significantly affected by F₂, even prolonged at the highest concentration (100 µM). These results may be related to the inhibition of \( I_K \). Penkoske [23] thought that the cardiac electrophysiology of reperfusion arrhythmia was characterized by refractory period shortening. A pure \( I_{Ca,L} \) blocker will aggravate this tendency; however, it can counter-balance the contradiction by the simultaneous inhibition of \( I_K \). Acquired malignant arrhythmia is often involved in multi-channel changes. Under these conditions, blockade of multi-channels may be more useful than inhibition of a single type of ion channel [24].

There is a potential limitation in this study. The effects on ionic current were obtained at room temperature and the kinetics of F₂ block and unblock may be substantially different at physiological temperature.

In conclusion, F₂, a novel compound of a quaternary ammonium salt derivative of haloperidol, exerts class IV antiarrhythmogenic properties, as well as some blocking K⁺ and Na⁺ channel effects. F₂ has a chemical structure different from other typical Ca²⁺ channel blockers, yet produces strong effects both on cardiac and coronary artery tissues. The combination of cardioprotective and antiarrhythmic effects suggest that F₂ may be a promising drug for the treatment of ischemic heart disease with cardiac arrhythmia.
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