The Cellular Electrophysiologic Effect of A New Amiodarone Like Antiarrhythmic Drug GYKI 16638 in Undiseased Human Ventricular Muscle: Comparison With Sotalol And Mexiletine


a Department of Pharmacology & Pharmacotherapy, b Department of Cardiac Surgery, c Department of Surgery, Albert Szent-Györgyi Medical and Pharmaceutical Center, University of Szeged, Hungary
d Division of Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged, Hungary
e Department of Organic Chemistry, Semmelweis Medical University, Budapest, Hungary

Abstract: The cellular electrophysiologic effect of GYKI 16638, a new antiarrhythmic compound was studied and compared with that of sotalol and mexiletine in undiseased human right ventricular muscle preparation by applying the conventional microelectrode technique.

GYKI 16638 (5 µM), at stimulation cycle length of 1000 ms, lengthened action potential duration (APD90) from 338.9 ± 28.6 ms to 385.4 ± 24 (n = 9, p < 0.05). This APD lengthening effect, unlike that of sotalol (30 µM), was rate-independent. GYKI 16638, contrary to sotalol and like mexiletine (10 µM), exerted a use-dependent depression of the maximal rate of depolarization (Vmax) which amounted to 36.4 ± 11.7 % at cycle length of 400 ms (n = 5, p < 0.05) and was characterised with an offset kinetical time constant of 298.6 ± 70.2 ms.

It was concluded that GYKI 16638 in human ventricular muscle shows combined Class IB and Class III antiarrhythmic properties, resembling the electrophysiological manifestation seen after chronic amiodarone treatment.

INTRODUCTION

Many new antiarrhythmic drugs have been introduced into the therapy but the pharmacological treatment of ventricular arrhythmias is not always successful and it is often associated with life-threatening proarrhythmic complications. One type of proarrhythmia relates to the impairment of impulse conduction due to the block of the fast inward sodium channels and was attributed to the increased mortality after oral flecainide and encainide treatment reported in the CAST trial [6]. The other type of proarrhythmia was noticed with drugs like sotalol, which lengthens repolarization in a reverse rate-dependent manner and can induce early afterdepolarization (EAD) [25] leading, in certain patients, to torsade de pointes chaotic ventricular tachycardia [16]. This latter was postulated to explain the increased mortality after d-sotalol treatment in the SWORD [28] trial. However, amiodarone, a drug with multiple modes of action [12] affecting several receptors and transmembrane ionic channels, has been successfully used in patients to control cardiac arrhythmias [12, 14]. Amiodarone, although lengthens ventricular repolarization and blocks inward sodium channels, has relatively low proarhythmic activity [14], suggesting that combination of these two effects is not necessarily harmful. Amiodarone, however, has several and some serious extracardiac side effects, likely associated to its specific chemical structure. This limits the long-term use of the drug in many patients. Therefore, great effort is being made world-wide to developed new amiodarone-like antiarrhythmic drugs free from serious side effects. In the Drug Research Institute, in the course of an intensive chemical synthetic program, new compounds were synthetized, which electrophysiologically resembled amiodarone in the sense that they had combined Class IB and Class III antiarrhythmic properties without structural relationship with amiodarone. In this study we describe the effect of one of this compounds, GYKI 16638 (Fig. 1), on the action potentials in undiseased human ventricular muscle in comparison with sotalol and mexiletine.

METHODS

Trabecular and small (diameter < 2 mm) papillary muscle preparations were obtained from undiseased human
Fig. (1). The chemical structure of GYKI 16638.

donor hearts used for homograft valve transplantation surgery. The experimental protocol complied with the Declaration of World Medical Association proclaimed in Helsinki and was approved by the Ethical Review Board of the Albert Szent-Györgyi Medical University (No. 51-57/1997 OEj). 16 preparations were used from 16 patients (mean age 40.0 ± 0.3 years, 8 females and 8 males). The patients did not receive medication other than dobutamine, furosemide or plasma volume expanders. Before preparation, the hearts were perfused with cardioplegic solution and were kept cool (4-6 °C) for 2-4 hours. Than the trabecular and papillary muscles were excised and mounted in a tissue chamber (volume = 50 ml) in oxygenated (95% O₂ + 5% CO₂) modified Tyrode’s solution containing (in mM): NaCl, 115; KCl, 4; CaCl₂, 1.8; MgCl₂, 1; NaHCO₃, 20; and glucose, 11 at 37 °C. The pH of this solution was 7.35 to 7.45.

Each preparation was initially stimulated at a basic cycle length of 1000 ms (frequency = 1 Hz), using 2 ms long rectangular constant voltage pulses isolated from ground and delivered via bipolar platinum electrodes in contact with the preparation by a EMG 4747 type stimulator. At least 1 hour was allowed for each preparation to equilibrate while they were continuously superfused with the Tyrode’s solution. Transmembrane potentials were recorded using the conventional microelectrode recording technique. Microelectrodes filled with 3 M KCl and having tip resistances 5-20 MΩ were connected to the input of a high impedance electrometer (Biologic VF102), which was referenced to the ground. The first derivative of the transmembrane potentials (Vmax) was electronically derived by a Biologic DV-140 Differentiator. The voltage outputs from all amplifiers were connected after digitalisation (ADA 3300 AD board) to an IBM 386-486 microprocessor base personal computer for data acquisition and also displayed on a dual beam memory oscilloscope (Tektronix 2230). The resting membrane potential (RP), action potential amplitude (APA) and action potential duration (APD) measured at 50% and 90% repolarization (APD50-90), were obtained by APES HSE software developed in our Department. The stimulator was also controlled by the software.

After measuring the action potential parameters (maximal sampling rate = 50 kHz) at the basic cycle length of 1000 ms, 9 different constant stimulation cycle lengths (300, 400, 500, 700, 1000, 1500, 2000, 3000 and 5000 ms) were applied. Action potential parameters were measured at each cycle length following “quasi steady state” adaptation (25 beats) to the new pacing cycle length. To determine the recovery (offset) kinetics of Vmax, extra stimuli (S2) were applied with gradually increasing coupling interval from the basic cycle length (S1-S1) of 1000 ms after every 20th basic stimulus (S1). Diastolic interval was determined as a time interval corresponding to the difference between the timing of the extra stimulus (S1-S2) and the time point of the APD90 of the preceding basic action potential.

After control measurements drugs were superfused for 40-60 minutes and measurements were repeated. When impalements were lost, reimpalements were attempted. If the new impalement deviated more than 5 % from the preceding one, the experiment was excluded from the evaluation. D-sotalol (Bristol-Myers Co. Wallingford, CT, USA) and Mexiletine (IDR Ltd. Budapest, Hungary) were prepared daily from aqueous stock solutions (30 mM and 10 mM) to obtain the final drug concentration in the tissue bath. GYKI 16638 (IDR Ltd. Budapest, Hungary) was diluted similarly but from 10 mM stock solution containing 50 % DMSO. At concentrations less than 0.2 % DMSO did not affect action potential parameters established in separate measurements.

Student’s t test for paired data was used to compare the results statistically. Results were considered significant when P was found to be less than 0.05.

RESULTS

The effect of 5 µM GYKI 16638 on the basic action potential parameters was studied at 1 Hz stimulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>RP (mV)</th>
<th>APA (mV)</th>
<th>APD50 (ms)</th>
<th>APD90 (ms)</th>
<th>Vmax (V/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>-89.7 ± 0.9</td>
<td>119.2 ± 2.0</td>
<td>233.0 ± 17.4</td>
<td>301.8 ± 19.7</td>
<td>230.8 ± 21.3</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>-88.8 ± 1.5</td>
<td>119.2 ± 2.4</td>
<td>281.0 ± 21.0*</td>
<td>387.0 ± 27.6*</td>
<td>250.0 ± 21.1</td>
</tr>
<tr>
<td>Sotalol 30 µM</td>
<td>6</td>
<td>-87.7 ± 1.0</td>
<td>113.8 ± 1.9</td>
<td>205.0 ± 19.2</td>
<td>271.3 ± 24.3</td>
<td>296.2 ± 33.3</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>-87.7 ± 0.9</td>
<td>110.8 ± 2.7</td>
<td>186.6 ± 18.2*</td>
<td>254.2 ± 22.7*</td>
<td>288.3 ± 28.9</td>
</tr>
<tr>
<td>Mexiletine 10 µM</td>
<td>6</td>
<td>-87.3 ± 1.0</td>
<td>114.4 ± 2.2</td>
<td>248.6 ± 22.0</td>
<td>338.9 ± 28.6</td>
<td>289.4 ± 11.2</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>-87.1 ± 1.2</td>
<td>111.6 ± 2.4</td>
<td>242.6 ± 20.9</td>
<td>385.4 ± 24.0*</td>
<td>268.2 ± 17.3</td>
</tr>
<tr>
<td>GYKI-16638 5 µM</td>
<td>9</td>
<td>-87.1 ± 1.2</td>
<td>111.6 ± 2.4</td>
<td>242.6 ± 20.9</td>
<td>385.4 ± 24.0*</td>
<td>268.2 ± 17.3</td>
</tr>
</tbody>
</table>

RP = resting potential; APA = action potential amplitude; APD50-90 = 50 and 50 % repolarization time; Vmax = maximal rate of depolarization; * = p < 0.05
Fig. (2). Action potential recordings before and after 30 µM sotalol, 10 µM mexiletine and 5 µM GYKI 16638 superfusion in undiseased human right ventricular muscle preparations. The stimulation frequency was 1Hz.

frequency and compared with that of 30 µM sotalol and 10 µM mexiletine. Results of representative experiments are shown in Fig. (2) and summarized in table 1. GYKI 16638 lengthened APD$_{90}$ but not APD$_{50}$, without significantly changing other action potential parameters. Sotalol (30 µM) lengthened both APD$_{50}$ and APD$_{90}$, while mexiletine (10 µM) significantly shortened repolarization measured as both APD$_{50}$ and APD$_{90}$.

The frequency dependent effect of GYKI 16638, sotalol and mexiletine was investigated on the APD$_{90}$ and V$_{\text{max}}$ in the range of stimulation cycle length between 300 and 5000 ms. Results are shown in Fig. (3A). Sotalol (30 µM) increased APD considerably more at long cycle lengths than at short ones, i.e. it induced strong reverse rate-dependent APD prolongation. In contrast, mexiletine (10 µM) shortened APD. This APD shortening was minimal and statistically not significant at high stimulation frequencies i.e. at short cycle lengths, while it proved to be significant at longer cycle lengths. The magnitude of the APD lengthening effect of GYKI 16638 (5 µM) was marked but less than that of sotalol. This APD prolongation, however, unlike that induced by sotalol, was apparently independent of the rate of stimulation i.e. the drug elicited similar increase of the time for repolarization in the whole range of the studied cycle lengths (400-5000 ms).

The frequency dependent effect of GYKI 16638, sotalol and mexiletine on the V$_{\text{max}}$ was also studied. Sotalol (30 µM), as expected from previous animal studies [21, 22], did not change significantly V$_{\text{max}}$ at any stimulation cycle lengths (Fig. 3B), while both 10 µM mexiletine and 5 µM GYKI 16638 exerted rate dependent V$_{\text{max}}$ depression i.e. the higher stimulation rate was associated with more V$_{\text{max}}$ block (Fig. 3B). The characteristics of this frequency dependent V$_{\text{max}}$ block was very similar with mexiletine and GYKI 16638 i.e. both drugs depressed V$_{\text{max}}$ only at cycle lengths shorter than 1000 ms. This rate-dependent V$_{\text{max}}$ block was the consequence of the slowing of the recovery of sodium channels from inactivation, which was studied by applying second stimuli with increasing coupling intervals during the diastole from a basic cycle length of 1000 ms. In control condition i.e. before drug administration and also after sotalol superfusion, V$_{\text{max}}$ value returned, within 30-40 ms during early diastole, almost to its value characteristic at the basic cycle length. In marked contrast, after mexiletine and GYKI 16638 administration the recovery of V$_{\text{max}}$ was considerably delayed and could be characterised with
recovery time constant of 310.4±71.5 ms (amplitude 96.9±12.7 V/s, n=6) and 298.6±70.2 ms (amplitude 74.9±13.7 V/s, n=8), respectively.

**DISCUSSION**

The purpose of the present experiments was to examine the cellular electrophysiological effects of GYKI 16638, in comparison with sotalol and mexiletine, in isolated undiseased human ventricular muscle. The most important result of this study is that GYKI 16638, unlike sotalol, lengthened repolarization in a frequency independent manner, while like mexiletine, it decreased V_max only at heart rates faster than the normal range.

The two drugs, sotalol and mexiletine, which were used for comparison, were investigated previously intensively on the ventricular action potential in different mammalian preparations, including in some experiments ventricular trabecular muscle obtained from explanted endstage heart failure human heart [13], but the effects of these drugs on the action potential characteristics have never been established directly in undiseased human ventricular muscle. Sotalol, which has been classified as Class III antiarrhythmic drug [21] has been reported to lengthen APD in a reverse rate-dependent manner without affecting V_max [22]. In this study we confirmed these earlier observations made in animal preparations and in vivo clinical electrophysiological measurements [11]. Mexiletine, a drug which has been classified as a Class IB antiarrhythmic [4], according to earlier investigations performed also in animal preparations (5, 8, 24, 25) and in vivo clinical electrophysiological measurements [9], shortened or did not change APD significantly, but induced V_max block with fast recovery kinetics [5].

The effect of GYKI 16638 on the action potential and transmembrane ionic currents is currently under investigation. Preliminary results showed that it lengthened APD and blocked V_max in rabbit cardiac papillary muscle [2] and depressed the rapidly activating delayed rectifier potassium (I_{Kr}) and inward rectifier potassium (I_{K1}) currents in rabbit ventricular myocytes [2]. In addition, in dog, rabbit and rats the compound exerted in vivo antiarrhythmic effect comparable to that of sotalol [2].

The purpose of the chemical synthesis of the GYKI compounds was to develop an antiarrhythmic drug, which would be devoid of the proarrhythmic potential of the selective Class III and Class I drugs, and does not exhibit serious extracardiac side effects. To achieve this latter goal, the chemical structure of GYKI does not resemble that of amiodarone, but shows similarities with the structure of both sotalol and mexiletine (Fig. 1). Combining Class IB and Class III antiarrhythmic effects was reported to attenuate reverse rate-dependent prolongation of APD [25] and abolished EAD formation [25] commonly seen after administration of pure Class III drugs. It was also reported that erythromycin induced torsade de pointes arrhythmia was successfully abolished with mexiletine in a patient [10]. Although some clinical reports [27] suggested strong antiarrhythmic effect of the combination of Class IB and Class III drugs, no large scale clinical trial has been initiated to establish the real therapeutic value of such combination.

Amiodarone, however, is a drug, which has strong antiarrhythmic efficacy in patients, while showing promising
results regarding mortality in multicenter clinical trials [7]. In addition, amiodarone treatment is associated with low incidence of proarrrhythmic complication [14]. Amiodarone, besides other effects, possesses strong rate-independent Class III [18] and Class IB [18, 23] properties. These favourable effects of amiodarone can be, at least partly, attributed to its peculiar cellular electrophysiologic properties. Amiodarone induced strong rate dependent $V_{\text{max}}$ block [15, 23] with fast offset kinetics [18, 23]. Also, chronic amiodarone treatment lengthened repolarization rate-independently (18) and decreased $I_{Kr}$ density in rabbit ventricular muscle [26]. It is important to note that both acute [18] and chronic [20] amiodarone administration abolished EAD formation in dog Purkinje fibers [18] and M cells [20] alike. This latter effect can be attributed to the depression of the slowly inactivating [3] or window [1] sodium current, which would limit the undesirable consequence of $I_{Kr}$ block, resulting in rate-independent APD prolongation and minimal increase of dispersion of repolarization. Based on its cellular electrophysiologic effects, a similar mechanism can be postulated with GYKI 16638.

The present has, however, some possible limitations. We have applied only one drug concentration. Taking into consideration the diffusion barrier and the time course of the experiments, the selected drug concentrations were somewhat higher, but still close to the therapeutic plasma concentrations measured in patients with sotalol [11, 16, 29] and mexiletine [9, 17] and established with GYKI 16638 (1-2 µM) in pharmacokinetic investigations. Also, we applied only right ventricular endocardial preparations, which may not well represent the whole heart [19]. These limitations are explained by the difficulty in obtaining human ventricular preparations for experimental purpose.

In conclusion, in human ventricular muscle GYKI 16638 shows combined Class IB and Class III antiarhythmic properties, resembling the electrophysiologic manifestation seen after chronic amiodarone treatment. GYKI 16638 can therefore be a useful new antiarhythmic drug candidate for further studies in animal experiments and also in patients.

ACKNOWLEDGEMENTS

This work was supported by grants from the Hungarian National Research Foundation (OTKA T 031910, T-032558 and T-035018), Hungarian Ministry of Health (ETT 532/2000 and 536/2000), National Committee for Technological Development (OMFB, No 1025), Hungarian Ministry of Education (FKFP 1025/1997) from the Technological Development (OMFB, No 1025), Hungarian National Research Foundation (OTKA T 031910, T-032558 and T-035018), Hungarian Ministry of Health (ETT 532/2000 and 536/2000), Hungarian Ministry of Education (FKFP 1025/1997) from the Technological Development (OMFB, No 1025), Hungarian National Research Foundation (OTKA T 031910, T-032558 and T-035018), Hungarian Ministry of Health (ETT 532/2000 and 536/2000), Hungarian Ministry of Education (FKFP 1025/1997) from the Technological Development (OMFB, No 1025), Hungarian National Research Foundation (OTKA T 031910, T-032558 and T-035018), Hungarian Ministry of Health (ETT 532/2000 and 536/2000), Hungarian Ministry of Education (FKFP 1025/1997) from the Technological Development (OMFB, No 1025), Hungarian National Research Foundation (OTKA T 031910, T-032558 and T-035018), Hungarian Ministry of Health (ETT 532/2000 and 536/2000), Hungarian Ministry of Education (FKFP 1025/1997) from the Technological Development (OMFB, No 1025), Hungarian National Research Foundation (OTKA T 031910, T-032558 and T-035018), Hungarian Ministry of Health (ETT 532/2000 and 536/2000). This work was supported by grants from the Hungarian National Research Foundation (OTKA T 031910, T-032558 and T-035018), Hungarian Ministry of Health (ETT 532/2000 and 536/2000), National Committee for Technological Development (OMFB, No 1025), Hungarian Ministry of Education (FKFP 1025/1997) from the Hungarian Academy of Sciences and by János Bolyai Research Scholarship (for L. V.).

LIST OF ABBREVIATIONS

| APA | = | Action potential amplitude |
| APD | = | Action potential duration |
| $\text{APD}_{50}$ and $\text{APD}_{90}$ | = | Action potential durations at 50% and 90% of repolarization |
| EAD | = | Early afterdepolarisation |
| $I_{k1}$ | = | Inward rectifier potassium current |
| $I_{Kr}$ | = | Rapid component of the delayed rectifier potassium current |
| RP | = | Resting membrane potential |
| $V_{\text{max}}$ | = | Maximal rate of depolarisation |

REFERENCES


