Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 510 (2005) 161–166

www.elsevier.com/locate/ejphar

# Cellular electrophysiological effect of terikalant in the dog heart

Péter Biliczki[a], Károly Acsai[a], László Virág[a], László Tálosi[a,b], Norbert Jost[a,b], András Biliczki[a], Julius Gy. Papp[a,b], András Varró[a,*]

[a]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Szeged, Hungary
[b]Division of Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged, Hungary

Received 1 July 2004; received in revised form 21 December 2004; accepted 24 December 2004

**Abstract**

The cellular mechanism of action of terikalant, an investigational antiarrhythmic agent known to block the inward rectifier and other potassium currents, has not yet been fully clarified. The aim of the present study was therefore to analyse the in vitro electrophysiological effects of terikalant in canine isolated ventricular muscle and Purkinje fibers by applying the standard microelectrode technique. The effects of terikalant on the duration of action potential at a stimulation cycle length of 1000 ms and on the maximum upstroke velocity of the action potential in right ventricular papillary muscle were examined at 1, 2.5, 10, and 20 μM concentrations. Terikalant significantly prolonged the action potential duration measured both at 50% and 90% of repolarization in concentration-dependent manner. The maximum upstroke velocity of the action potential was unaffected at 1 and 2.5 μM concentrations. However, this parameter was significantly reduced at 10 and 20 μM concentrations of terikalant. In Purkinje fibers terikalant (2.5 μM) also produced a marked action potential lengthening effect. Frequency dependence (cycle length of 300–5000 ms) of the action potential lengthening effect of terikalant was studied at a concentration of 2.5 μM. Prolongation of the duration of action potential occurred in a reverse frequency-dependent manner both in papillary muscle and Purkinje fibers, with a more pronounced frequency-dependence observed in Purkinje fibers. The onset kinetics of the terikalant (10 μM) induced block of the maximum upstroke velocity of the action potential was rapid ($0.6\pm0.1$ beat$^{-1}$, $n$=6) like that of Class I/B antiarrhythmics, and the offset (recovery) kinetics of the drug ($2956\pm696$ ms, $n$=6) best resembled that of Class I/A antiarrhythmic drugs. It was concluded that terikalant, unlike pure Class III antiarrhythmic drugs, has combined mode of action by lengthening repolarization and blocking the inward sodium current in a use-dependent manner.


*Keywords:* Terikalant; Cellular electrophysiology; Antiarrhythmic action; Multichannel block

## 1. Introduction

Lengthening of cardiac action potential duration and prolongation of the refractoriness is an important mode of action of antiarrhythmic drugs (Class III agents, Singh and Vaughan Williams, 1970). Some newly introduced antiarrhythmic drugs (ibutilide, dofetilide) selectively prolong the action potential duration acting via specific inhibition of the rapid component of the delayed rectifier potassium current ($I_{Kr}$) without decreasing conduction velocity as a consequence of inhibition of inward sodium current ($I_{Na}$). Most drugs with pure Class III action prolong the action potential more at slow rates and produce little or no change at fast ones. This phenomenon, termed reverse rate-dependence (Hondeghem and Snyders, 1990) is particularly evident in M cells and Purkinje fibers. (Antzelevitch and Sicouri, 1994). The reverse rate-dependent nature of the action potential lengthening effect of most pure Class III drugs seriously limits the antiarrhythmic efficacy by compromising their ability to prolong the action potential and refractoriness when most needed, namely during tachycardia. In addition, it contributes importantly to the proarrhythmic effect caused by Class III agents because of the

* Corresponding author. Tel.: +36 62 545 682/545 683; fax: +36 62 545 680.
*E-mail address:* dept@phcol.szote.u-szeged.hu (A. Varró).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.12.040

excessive prolongation of repolarization. Emphasis was therefore shifted toward developing compounds with a multifaceted ("hybrid") pharmacological profile (Link et al., 1996; Podrid, 1995) with multiple molecular targets suggested by the "Sicilian gambit" (Task Force of the Working Group on Arrhythmias of the European Society of Cardiology, 1991). Many investigators are now considering that application of drugs with multiple actions like amiodarone would represent a possible effective way to treat both ventricular and supraventricular arrhythmias (Nattel and Talajic, 1988).

RP 58866, 1-[2-(3,4-dihydro-2*H*-1-benzopyran-4-yl)ethyl]-4-(3,4-dimethoxy-phenyl) piperidine, and its enantiomer, terikalant [(*s*)(−)isomer], RP 62719) were first considered as selective blockers of the inward rectifier potassium current ($I_{K1}$), (Escande et al., 1992). These compounds exhibited efficacy in experimental arrhythmias (Rees and Curtis, 1994), suggesting that block of $I_{K1}$ may be a useful antiarrhythmic mechanism with lower incidence of the torsade de pointes ventricular tachycardias (Farkas and Coker, 2002). However, the cellular mechanism of action of terikalant is still controversial and not fully elucidated. Therefore, the aim of the present study was to investigate the in vitro electrophysiological effects of terikalant, this new investigational antiarrhythmic drug with largely unknown cellular mode of action, in canine isolated ventricular muscle and Purkinje fibers, by applying the standard microelectrode technique.

## 2. Materials and methods

All experiments were carried out in compliance with the Guide for the Care and Use of Laboratory Animals (U.S.A. NIH publication No 85-23, revised 1985). The protocols were approved by the Review Board of the Committee on Animal Research of the University of Szeged (54/1999 OEj). Papillary muscles were obtained from the right ventricle, and free-running false tendons of Purkinje fibers were isolated from both ventricles of hearts removed through a right lateral thoracotomy from anesthetized (sodium pentobarbital, 30 mg/kg i.v.) mongrel dogs of either sex weighing 8–20 kg. The preparations were placed in a tissue bath and allowed to equilibrate for at least 2 h while superfused with oxygenated (95% $O_2$ and 5% $CO_2$) Tyrode's solution (flow rate 4–5 ml/min) warmed to 37 °C (pH 7.3±0.5). The composition of Tyrode's solution was (in mM): NaCl 120, KCl 4.0, $NaHCO_3$ 22, $CaCl_2$ 1.0, $MgCl_2$ 1.0, and D-glucose 11.0.

The experiments were carried out by applying the standard intracellular microelectrode technique. During the equilibration period, the tissues were stimulated at a basic cycle length of 1000 ms. Electrical pulses of 2 ms in duration and twice diastolic threshold in intensity ($S_1$) were delivered to the preparations through Teflon-coated bipolar silver electrodes. Transmembrane potentials were recorded with the use of glass capillary microelectrodes filled with 3 M KCl (tip resistance: 5 to 15 MΩ). The microelectrodes were coupled through an Ag–AgCl junction to the input of a high-impedance, capacitance-neutralizing amplifier (Biologic VF-102). The first time derivative of the upstroke of the action potential ($dV/dt$) was obtained by using an electronic differentiator (Biologic DV-140), the output of which was linear between 100 and 1000 V/s. Intracellular recordings were displayed on a storage oscilloscope (Tektronix 2232) and led to a computer system (HSE APES) designed for on-line determination of the following parameters: resting membrane potential, action potential amplitude, action potential duration at 50% and 90% repolarization and the maximal rate of rise of the action potential upstroke ($V_{max}$).

The following types of stimulation were applied in the course of the experiments: stimulation with a constant cycle length of 1000 ms; stimulation with different constant cycle lengths ranging from 300 to 5000 ms (or to 2000 ms in the case of Purkinje fibers to prevent spontaneous diastolic depolarizations at cycle lengths longer than 2000 ms). To determine the recovery kinetics of $V_{max}$, extra test action potentials were elicited by using single test pulses ($S_2$) in a preparation driven at a basic cycle length of 1000 ms. The $S_1$–$S_2$ coupling interval was increased progressively from the end of the refractory period. The effective refractory period was defined as the longest $S_1$–$S_2$ interval at which $S_2$ failed to elicit a propagated response. The diastolic intervals preceding the test action potential were measured from the point corresponding to 90% of repolarization of the preceding basic beat to the upstroke of the test action potential and were increased progressively. To determine the onset kinetics the preparations were continuously stimulated at cycle length of 1000 ms, the stimulation was interrupted for 1 min, and then a train of 40-beat stimuli was applied with a cycle length of 500 ms. After control measurements the preparations were superfused for 30 min with Tyrode's solution containing the compound under study, and then the measurements were resumed.

Terikalant (RP62719, Rhone-Poulenc Rorer, France) was dissolved in dimethyl-sulphoxide (DMSO) at stock solution concentration of 1 or 100 mM. Results were analyzed by using Student's *t*-test for paired and unpaired data. Differences were considered significant when $p<0.05$. Data are expressed as mean±standard error of the mean (S.E.M).

## 3. Results

### *3.1. Effects of terikalant on the action potential parameters in canine ventricular muscle and Purkinje fibers*

The effect of terikalant on the duration of the action potential and on the maximum velocity of the action potential upstroke ($V_{max}$) at a cycle length of 1000 ms was investigated in concentrations of 1, 2.5, 10, and 20 μM

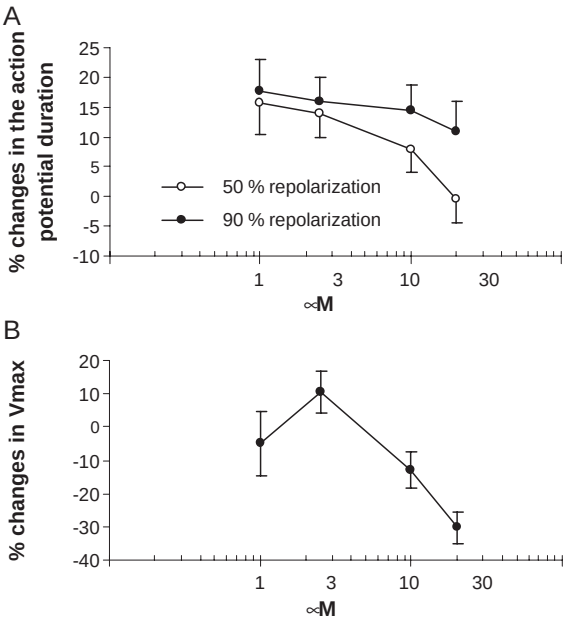


Fig. 1. Concentration–response characteristics of the effect of terikalant on duration of the action potential measured at 50% (○) and 90% (●) of repolarization (A) and the maximum upstroke velocity of the action potential ($V_{max}$; B) at a cycle length of 1000 ms in dog papillary muscles. Terikalant was applied at concentrations of 1, 2.5, 10, and 20 μM ($n$=4–6/concentration).

in right ventricular papillary muscle preparations. Fig. 1A shows that terikalant concentration-dependently lengthened the action potential, for which effect was more pronounced when the duration of the action potential was measured at 50% of repolarization. In Fig. 1B the effect of terikalant on $V_{max}$ at the same concentrations is shown. At low concentrations (1 μM and 2.5 μM) terikalant did not affect the $V_{max}$, although a small but non-significant increase was observed at 2.5 μM concentration, which was likely due to an improved microelectrode–cell connection observed in some experiments with time. However, application of terikalant at 10 μM and 20 μM concentration resulted in a marked and concentration-dependent decrease in $V_{max}$.

A detailed analysis of the action potentials recorded before and after the application of terikalant was carried out at 2.5 μM and 10 μM concentrations. Results are summarized in Table 1, and representative traces of the action potentials and the first derivatives of the action potential upstroke, d$V$/d$t$, recorded before and after administration of terikalant in canine ventricular muscle and Purkinje fibers are shown in Fig. 2. Terikalant, investigated at 2.5 μM concentration, significantly lengthened the action potential durations measured both at 50% and 90% of repolarization from 190.4±7.3 ms to 216.4±8.2 ms ($n$=6, $P$<0.05) and from 224.7±6.9 ms to 259.8±9.6 ms ($n$=6, $P$<0.05), respectively, in papillary muscle without causing considerable change in the maximum diastolic potential, action potential amplitude, and $V_{max}$. However, at 10 μM concentration, in addition to the lengthening of the action potential duration from 209.2±9.4 ms to 224.9±10.3 ms and from 253.9±7.7 ms to 288.9±7.4 ms ($n$=6, $P$<0.05), measured at 50% and 90% of repolarization, respectively, the drug also caused a significant decrease in $V_{max}$ from 211.8±14.1 V/s to 183.3±14.5 V/s ($n$=6, $P$<0.05). In Purkinje fiber 2.5 μM terikalant significantly lengthened the action potential duration measured at 90% of repolarization from 241.9±13.2 ms to 320.8±16.2 ms ($n$=6, $P$<0.05) without affecting the action potential duration measured at 50% of repolarization and the maximum diastolic potential. Also, $V_{max}$ was significantly decreased by the drug from 747.4±68.6 V/s to 586.3±79.2 V/s ($n$=6, $P$<0.05) and a slight but significant reduction in action potential amplitude from 132.6±2.4 mV to 127.6±3.4 mV was also found in Purkinje fibers in the presence of 2.5 μM terikalant (Table 1; Fig. 2C).

### 3.2. Frequency-dependent effects of terikalant in ventricular papillary muscle and Purkinje fiber

Fig. 3A and B show that the drug, at a concentration of 2.5 μM, lengthened the duration of the action potential measured at 90% of repolarization in a reverse frequency-dependent manner both in papillary muscle and Purkinje fibers, with a more pronounced reverse frequency dependence observed in Purkinje fibers. In papillary muscle,

Table 1
Effect of terikalant on the action potential parameters in canine ventricular muscle and Purkinje fiber

| | Papillary muscle | | | Purkinje fiber | | |
|---|---|---|---|---|---|---|
| | Control | Terikalant 2.5 μM ($n$=6) | Control | Terikalant 10 μM ($n$=6) | Control | Terikalant 2.5 μM ($n$=6) |
| Maximum diastolic potential (mV) | −85.1±1.1 | −84.1±0.9 | −81.1±1.3 | −82±1.2 | −86.1±0.8 | −86.4±0.6 |
| Action potential amplitude (mV) | 112.5±1.3 | 116.3±1.8 | 108.7±2.4 | 105.7±2.3 | 132.6±2.4 | 127.6±3.4[a] |
| Action potential duration at 50% repolarization (ms) | 224.7±6.9 | 259.8±9.6 | 259.8±9.6 | 259.8±9.6 | 259.8±9.6 | 259.8±9.6 |
| Action potential duration at 90% repolarization (ms) | 224.7±6.9 | 259.8±9.6[a] | 253.9±7.7 | 288.9±7[a] | 241±13.2 | 320.8±16.2[a] |
| Maximum upstroke velocity of the action potential ($V_{max}$) (V/s) | 228.2±12.3 | 255±18.2 | 211±14 | 183±14[a] | 747.4±68.6 | 586.3±79.2[a] |

Values are mean±S.E.M. Action potentials were recorded at a cycle length of 1000 ms.
[a] Significantly different ($p$<0.05) from the corresponding control value.

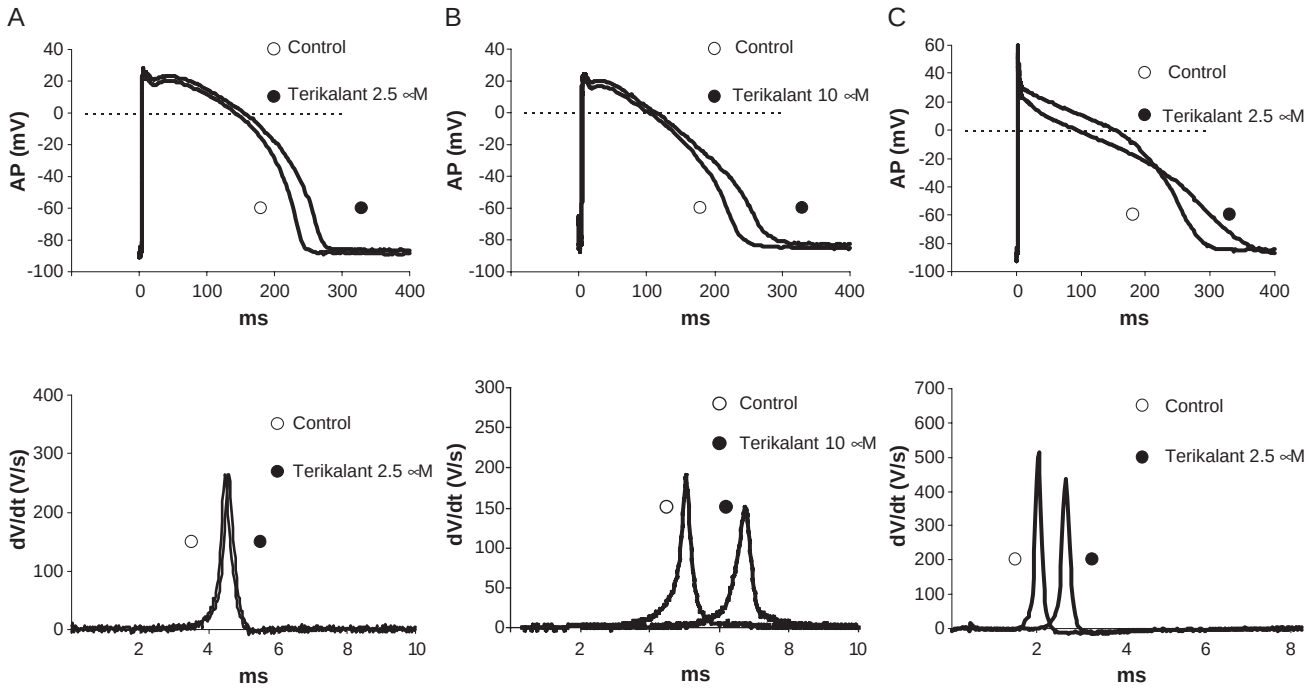


Fig. 2. Representative traces of the transmembrane action potential (top) and the first derivative of the action potential upstroke, d$V$/d$t$ (bottom) recorded from right ventricular papillary muscle (A and B) and from Purkinje fiber (C) in control conditions (○) and in the presence of 2.5 μM (A and C) and 10 μM (B) terikalant (●). Traces were recorded at a cycle length of 1000 ms.

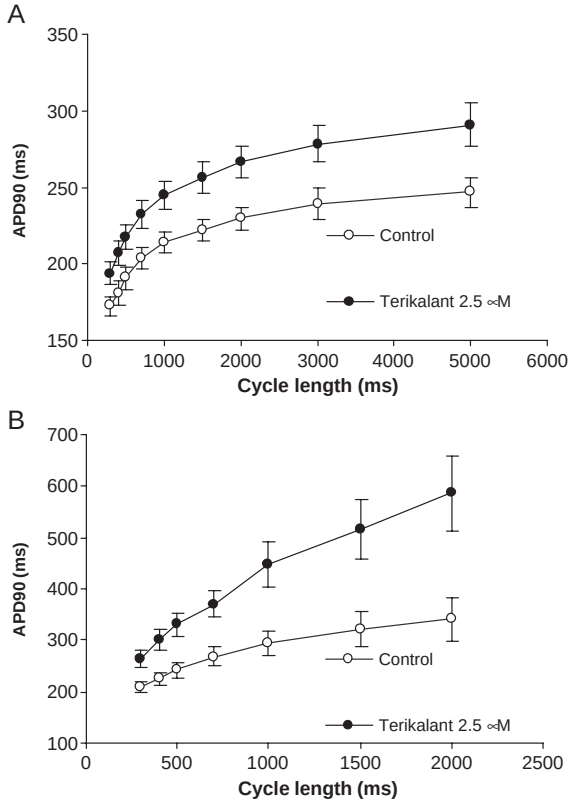


Fig. 3. Cycle length-dependent effect of terikalant (2.5 μM; ●) on the action potential duration measured at 90% of repolarization in right ventricular papillary muscle (A) and Purkinje fiber (B; $n$=6).

frequency-dependent effect of terikalant (2.5 μM and 10 μM) on the maximum upstroke velocity of the action potential ($V_{max}$) was also investigated. $V_{max}$ was unaffected by the drug given in the lower (2.5 μM) concentration at any given stimulation rate (Fig. 4A). However, as shown in Fig. 4B, 10 μM terikalant caused a rate-dependent depression in the maximal upstroke velocity of the action potential in this preparations, i.e. decreasing $V_{max}$ more at high than at slow stimulation rate.

### 3.3. Offset and onset kinetics of $V_{max}$ block

Fig. 5B shows the offset kinetics of $V_{max}$ block induced by 10 μM terikalant in papillary muscle preparations. The $V_{max}$ values of premature beats elicited once after every 10th basic beat (at a cycle length of 1000 ms) are plotted as a function of diastolic interval (interval between the 90% repolarization of the basic beat and the upstroke of the premature beat). The recovery curves illustrated in Fig. 4B show that the drug, in addition to the fast recovery (<50 ms), which presumably reflects the drug-free sodium channels, induced a slow phase of recovery of $V_{max}$ with a time constant (τ) of 2956±696 ms ($n$=6).

The onset kinetics of $V_{max}$ block induced by 10 μM terikalant was also studied in dog right ventricular papillary muscle (Fig. 5A). Preparations were continuously stimulated at a cycle length of 1000 ms. The stimulation was interrupted for 1 min, and then a train of

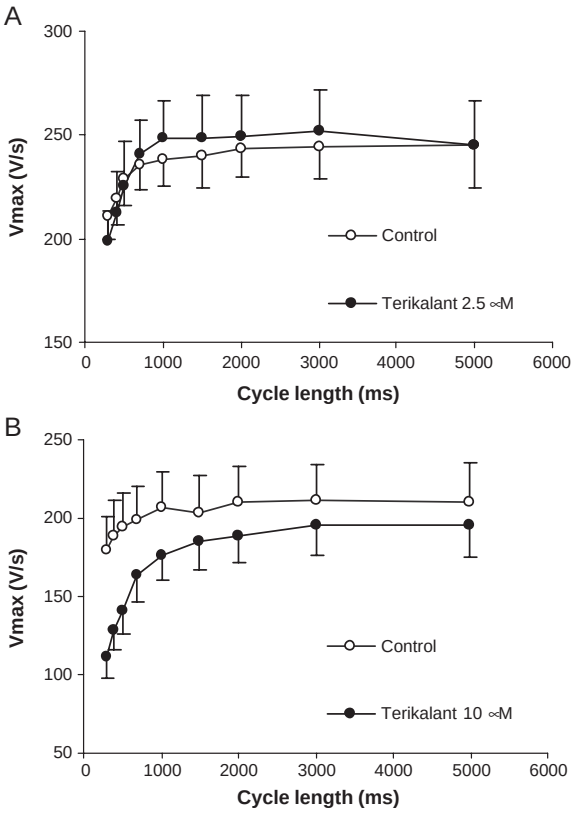


Fig. 4. Cycle length-dependent effect of terikalant (●) on the maximum upstroke velocity of the action potential ($V_{max}$) in right ventricular papillary muscle at the concentration of 2.5 μM (A) and 10 μM (B; $n$=6).

40 stimuli was applied with a cycle length of 500 ms (Fig. 5A). In control conditions, there was only a minor change between the first and last $V_{max}$ values in the train. In the presence of 10 μM terikalant, however, a marked use-dependent $V_{max}$ block developed with an onset kinetic rate constant of 0.6±0.1 beat$^{-1}$ ($n$=6).

## 4. Discussion

In the present study, we investigated the cellular electrophysiological action of terikalant in canine ventricular papillary muscle and Purkinje fiber preparations. Although the therapeutic free plasma level of terikalant is not fully established as yet, it lies in the micromolar range (Jurkiewicz et al., 1996). Therefore, the concentrations applied in our experiments reflect therapeutically meaningful concentrations. Our findings showed that the main effect of terikalant is to lengthen the duration of the action potential measured at 50% and 90% of repolarization (a Class III antiarrhythmic action) in low concentrations (1 μM and 2.5 μM) which is combined with $V_{max}$ block (Class I+Class III action) in higher concentrations (10 and 20 μM). RP 58866 and its active enantiomer, terikalant were first considered as selective blockers of the inward rectifier potassium current ($I_{K1}$; Escande et al., 1992). However, the selectivity of terikalant on $I_{K1}$ was later questioned (Yang et al., 1999), especially at high concentration. Accordingly, it was found that terikalant also inhibited the rapidly activating delayed-rectifier $K^+$ current. Later, Williams et al. (1999), consistent with the earlier results of Escande et al. (1992), but in contrast to the results of Jurkiewicz et al. (1996), found that only $I_{K1}$ was significantly affected by 10 μM terikalant, further increasing the uncertainty of the cellular mechanism of terikalant (Yang et al., 1999).

Terikalant, like other Class III antiarrhythmic drugs (sotalol, bretylium) elicits reverse rate-dependent effect on the repolarization both in papillary muscle and Purkinje fibers and increase the dispersion of repolarization between Purkinje fibres and ventricular muscle (Varro et al., 1986). In Purkinje fibers during bradycardia early afterdepolarizations (EADs) might appear which can trigger the torsade de pointes ventricular tachycardia. This serious proarrhythmic effect could be avoided by amiodarone because chronic amiodarone treatment decreases the dispersion of repolarization between Purkinje fibers and ventricular muscle (Papp et al., 1996).

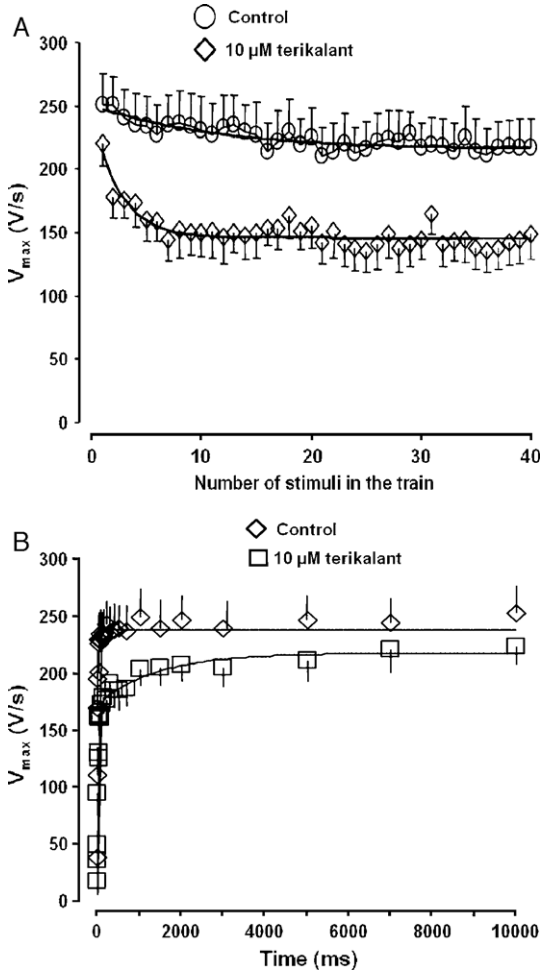


Fig. 5. Effect of 10 μM terikalant on the onset kinetics of $V_{max}$ block (A) and on the recovery of $V_{max}$ from inactivation (B) in canine right ventricular papillary muscle ($n$=6).

Also, as a new finding in our experiments, terikalant in addition to prolonging the action potential, depressed the maximal upstroke velocity of the action potential ($V_{max}$) in a frequency-dependent manner. The offset kinetic time constant of $V_{max}$ block produced by the drug was calculated about 3 s, which value appears to be typical for Class I/A antiarrhythmic drugs. The onset kinetics of $V_{max}$ block induced by terikalant in this study was found to be rapid, resembling that of Class I/B antiarrhythmic drugs. Since the present study is the first to demonstrate the effect of terikalant on $V_{max}$ and on its offset and onset kinetics, our results may serve as a basis for its classification. It can thus be concluded that terikalant is a drug with combined Class III and I A antiarrhythmic actions with fairly rapid onset kinetics. The action potential lengthening effect of terikalant can be best explained by its well-documented depressing effect on the cardiac transmembrane potassium currents (Jurkiewicz et al., 1996). The use-dependent block of $V_{max}$ is a new finding and reflects a diminution of the inward sodium current in accordance with the modulated receptor hypothesis (Hondeghem and Katzung, 1977).

In addition to the depression of the maximal upstroke velocity of the action potential ($V_{max}$) which is due to the sodium channel inhibition, a possible proarrhythmic effect could also appear via increasing the dispersion of repolarization. In fact, since the Cardiac Arrhythmia Suppression Trial (CAST, 1989), the development of potential antiarrhythmic drugs has been cancelled in cases of those novel compounds that turned out to have sodium channel blocking properties, especially the I/C subgroup. According to our results, besides its well-established antiarrhythmic effects, terikalant may increase the proarrhythmic risk as well.

As a conclusion, our study reveals that the investigational drug terikalant, in addition to its potassium channel-inhibiting effect, also blocks the fast sodium channels in a rate-dependent manner and it possesses combined electrophysiological mode of action which most likely contributes to its established antiarrhythmic efficacy.

## Acknowledgements

This work was supported by grants from the Hungarian National Research Foundation (OTKA T-035018 and T-037520), Hungarian Ministry of Health (188/2003), Hungarian Ministry of Education (Bio-37 KPI), National Research and Development Programmes (NKFP 1A/0011/2002), by the Hungarian Academy of Sciences, and by János Bolyai Research Scholarship (for L.V.).

## References

Antzelevitch, C., Sicouri, S., 1994. Clinical relevance of arrhythmias generated by afterdepolarizations: the role of M cells in the generation of U waves, triggered activity and torsade de pointes. J. Am. Coll. Cardiol. 23, 259–277.

The CAST Investigators, 1989. Preliminary report: effect of encainide and flecainide on mortality in randomized trial arrythmia supression after myocardial infarction. N. Engl. J. Med. 321, 406–412.

Escande, D., Mesre, M., Cavero, I., Brugada, J., Kirchhof, C., 1992. RP 58866 and its active enantiomer RP 62719 (terikalant) blockers of the inward rectifier $K^+$ current acting as pure class III antiarrhythmic agents. J. Cardiovasc. Pharmacol. 20 (Suppl. 2), S106–S113.

Farkas, A., Coker, J.S., 2002. Limited induction of torsade de pointes by terikalant and erythromycin in an in vivo model. Eur. J. Pharmacol. 449, 143–153.

Hondeghem, L.M., Katzung, B.G., 1977. Time and voltage dependent interactions of antiarrhythmic drugs with cardiac sodium channels. Biochim. Biophys. Acta 472, 373–398.

Hondeghem, L.M., Snyders, D.J., 1990. Class III antiarrhythmic agents have a lot of potential but have a long way to go: reduced effectiveness and dangers of reverse used dependence. Circulation 81, 689–690.

Jurkiewicz, N.K., Wang, J., Fermini, B., Sanguinetti, M.C., Salata, J.J., 1996. Mechanism of action potential prolongation by RP 58866 and its active enantiomer terikalant. Circulation 94, 2938–2946.

Link, M.S., Homoud, M., Foote, C.B., Wang, P.J., Estes III, N.A., 1996. Antiarrhythmic drug therapy for ventricular arrhythmias: current perspectives. J. Cardiovasc. Electrophysiol. 7, 653–670.

Nattel, S., Talajic, M., 1988. Recent advances in understanding the pharmacology of amiodarone. Drugs 36, 121–131.

Papp, J.Gy., Nemeth, M., Krassoi, I., Mester, L., Hala, O., Varro, A., 1996. Differential electrophysiologic effects of chronically administered amiodarone on canine Purkinje fibers versus ventricular muscle. J. Cardiovasc. Pharmacol. Ther. 1, 287–296.

Podrid, P.J., 1995. Amiodarone. Reevaluation of an old drug. Ann. Intern. Med. 122, 689–700.

Rees, S.A, Curtis, M.J., 1994. $I_{K1}$ blockade is a potentially useful antiarrhythmic mechanism. Cardiovasc. Res. 28, 421.

Singh, B.N., Vaughan Williams, E.M., 1970. A third class of antiarrhythmic action. Effect on atrial and ventricular intracellular potentials, and other pharmacological actions on cardiac muscle, of MJ 1999 and AH 3474. Br. J. Pharmacol. 39, 675–687.

Task Force of the Working Group on Arrhythmias of the European Society of Cardiology, 1991. The Sicilian gambit: a new approach to the classification of antiarrhythmic mechanism. Circulation 84, 1831–1851.

Varro, A., Nakaya, Y., Elharar, V., Surawicz, B., 1986. Effect of antiarrhythmic drugs on the cycle length dependent action potential duration in dog Purkinje and ventricular muscle fibers. J. Cardiovasc. Pharmacol. 8, 178–185.

Williams, A.B., Dickenson, R.D., Beatch, N.G., 1999. Kinetics of rate-dependent shortening of action potential duration in guinea-pig ventricle; effect of $I_{K1}$ and $I_{Kr}$ blockade. Br. J. Pharmacol. 126, 1426–1436.

Yang, F.B., Li, G.R., Xu, C.Q., Nattel, S., 1999. Effect of RP58866 on transmembrane $K^+$ currents in mammalian ventricular myocytes. Zhongguo Yaoli Xuebao 20, 961–969.