The Role of Sarcoplasmic Reticulum in the Protective Effect of Class III Drugs Against Ca^{2+} Overload

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Abstract. Various studies on humans and experimental mammals showed that d-sotalol and tedisamil (class III antiarrhythmic drugs with positive inotropic effect) facilitate spontaneous ventricular defibrillation Following our previous results, we summarized that spontaneous ventricular defibrillation requires high level of intercellular coupling and synchronization, both of which depends on intracellular free Ca^{2+} concentration We hypothesized that any antiarrhythmic compound that facilitates spontaneous defibrillation, including d-sotalol and tedisamil, should prevent intracellular free Ca^{2+} overload most likely by elevating cAMP level and enhancing cAMP -related Ca^{2+} uptake of the sarcoplasmic reticulum (SR) The aim of the present study was to examine the role of the SR uptake function in their effect against Ca^{2+} overload Methods The effect of d-sotalol, tedisamil and dbcAMP on increased intracellular Ca^{2+} level were examined in cultured rat cardiomyocytes during blockade of SR Ca^{2+} uptake by administration of thapsigargin (TG), a selective inhibitor of Ca^{2+}-ATPase Results Administration of 3×10^{-6} mol/l TG, prior to d-sotalol, tedisamil and dbcAMP, significantly increased intracellular free Ca^{2+} concentration and prevented the effect of d-sotalol, tedisamil or dbcAMP to decrease intracellular Ca^{2+} level to its beseline, while 10^{-6} mol/l TG prevented it only partially Administration of either d-sotalol or tedisamil (at concentration of 10^{-5} mol/l) before the administration of 10^{-6} mol/l TG prevent the TG induced elevation of $[Ca^{2+}]_i$ Conclusion These results support our hypothesis that d-sotalol and tedisamil prevent Ca^{2+} overload by the cAMP dependent SR Ca^{2+} uptake

Key words: Calcium overload — d-Sotalol — Tedisamil — cAMP — Thapsigargin — Class III antiarrhythmic compounds — Spontaneous defibrillation

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Introduction

Class III antiarrhythmic drugs (with positive inotropic effects) were found to facilitate self ventricular defibrillation in human (Dorian and Newman 1993) and mammals (Belokopitov 1995) Studies carried out on various experimental animals showed that self ventricular defibrillation requires high level of intercellular coupling, and synchronization (Manoach *et al* 1987) that depends on intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), a key factor involved in regulation of gap junction channel and intercellular coupling (DeMello 1975) Since abnormaly elevated $[Ca^{2+}]_i$ may cause cell-to-cell uncoupling and inhibition of intercellular communication (DeMello 1975, Burt 1987), we assumed that the defibrillating action of d-sotalol and tedisamil might be associated with prevention of undesirable $[Ca^{2+}]_i$ accumulation (Belokopitov 1995, Manoach and Watanabe 1995, Tribulova *et al* 1998) To proove this assumption, we examined the effect of d-sotalol and tedisamil in cultured rat cardiac myocytes with normal and increased $[Ca^{2+}]_i$ (Manoach *et al* 1997) The results showed that d-sotalol and tedisamil, in concentrations of 10^{-7}–10^{-5} mol/l, did not change $[Ca^{2+}]_i$ in normal conditions, but they were capable to decrease the excessive $[Ca^{2+}]_i$ to its basal level (Manoach *et al* 1997) Pretreatment with d-sotalol or tedisamil prevented Ca^{2+} overload and Ca^{2+} overload injury (Manoach and Watanabe 1995, Tribulova *et al* 1998) Basing on results obtained with cats and guinea pigs hearts (Parmley *et al* 1972, Tribulova *et al* 1999), in which d-sotalol and tedisamil induced an increase of intracellular cAMP level, we hypothesized that these compounds prevent an increase in $[Ca^{2+}]_i$ by supporting the cAMP-related Ca^{2+}-uptake of the sarcoplasmic reticulum (SR) (Manoach *et al* 1993, Miyachi *et al* 1995, Tribulova *et al* 1998) The aim of the present study was to examine whether d-sotalol, tedisamil and dbcAMP will be capable to stimulate the SR Ca^{2+} uptake in cultured rat cardiomyocytes if their SR Ca^{2+} uptake was inhibited by thapsigargin

Materials and Methods

The experiments were carried out on 3–4 days old cultured cardiomyocytes obtained from newborn rats $[Ca^{2+}]_i$ was measured by Indo1 method (Grynkevicz *et al* 1985) The cells were submitted to a normal Tyrode solution, before and following administration of 1 or 3×10^{-6} mol/l thapsigargin (TG) TG was used as specific inhibitor of the activity of SR Ca^{2+}-ATPase (Thastrupet *et al* 1990) D-sotalol, tedisamil and dbcAMP were administered in two concentrations, 10^{-6} mol/l (used in our experiments of self ventricular defibrillation, (Manoach et al 1987) and 10^{-4} mol/l (as examination of overdosage) D-sotalol, tedisamil and dbcAMP were administered either before or after the administration of TG

Results

Administration of 1 or 3×10^{-6} mol/l TG induced a significant increase in the $[Ca^{2+}]_i$ from 100 nmol/l to 700 and 1000 nmol/l ($p < 0\ 05$), respectively and abolished $[Ca^{2+}]_i$ transients (Fig 1 A,C,E and 2 A,C) In cells exposed to 10^{-6} mol/l TG, administration of 10^{-6} mol/l either d-sotalol ($n = 4$), tedisamil ($n = 3$) or dbcAMP ($n = 6$) slightly decreased $[Ca^{2+}]_i$ (Fig 1 B,D,F) However, in cells exposed to higher concentration of TG, 3×10^{-6} mol/l, administration of 10^{-5}mol/l of d-sotalol or dbcAMP failed to decrease the TG-induced $[Ca^{2+}]_i$ accumulation (Fig 2 B,D) Administration of 10^{-5}mol/l of d-sotalol or 10^{-5} mol/l tedisamil prior to the administration of 10^{-6} mol/l TG, reduced and/or

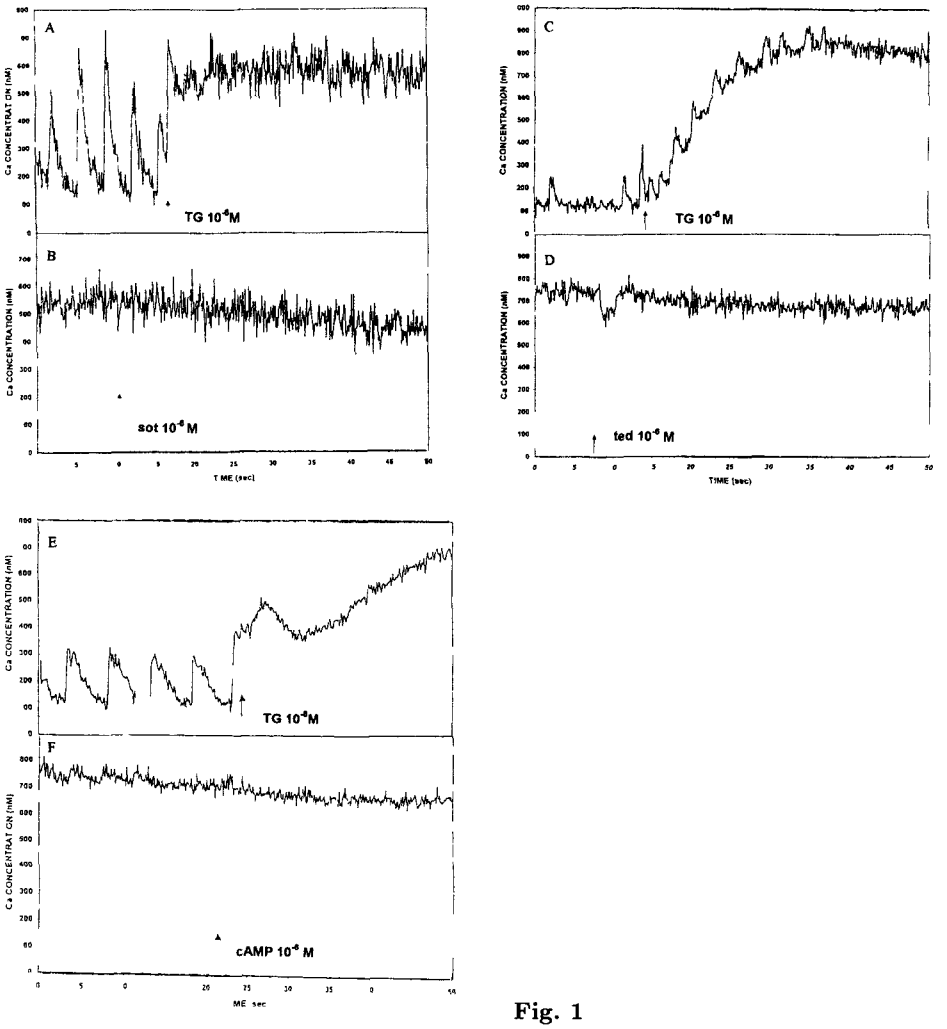

Fig. 1

slowed down the TG-induced effect (Fig 3 A,B), but they were not capable to conteract the effect of 3×10^{-6} mol/l TG (Fig 4 A,B)

Discussion

Calcium homeostasis in cardiac myocytes results from integrated function of transsarcolemmal Ca^{2+} influx and efflux pathway It is modulated by membrane potential and intracellular Ca^{2+} uptake and release, caused predominatly by the sarcoplasmic reticulum function SR is the most important system regulating free Ca^{2+}, since Ca^{2+} influx and removal by the sarcolemmal transport system are too small to account for total rise and fall of $[Ca^{2+}]_i$ (Dhalla et al 1996)

It is commonly accepted that elevation of $[Ca^{2+}]_i$ (Clusin *et al* 1983, Dahl and

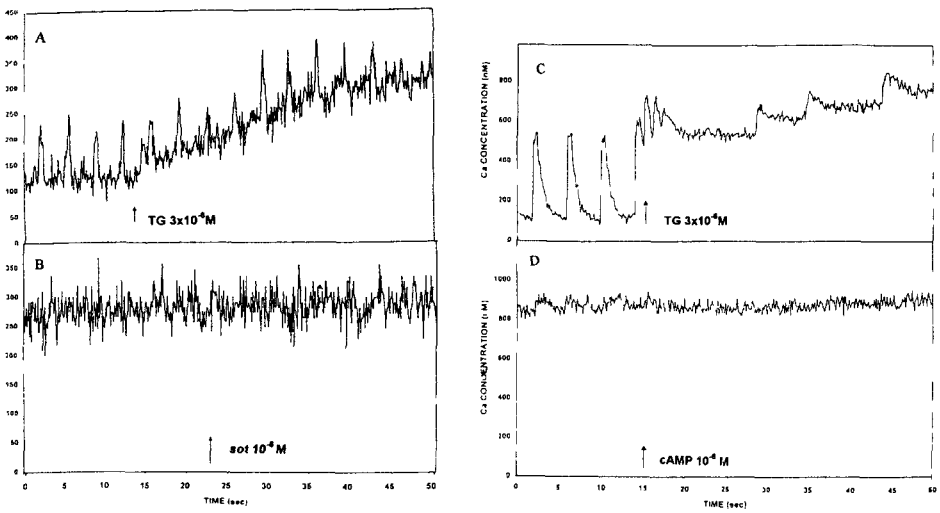

Fig. 2

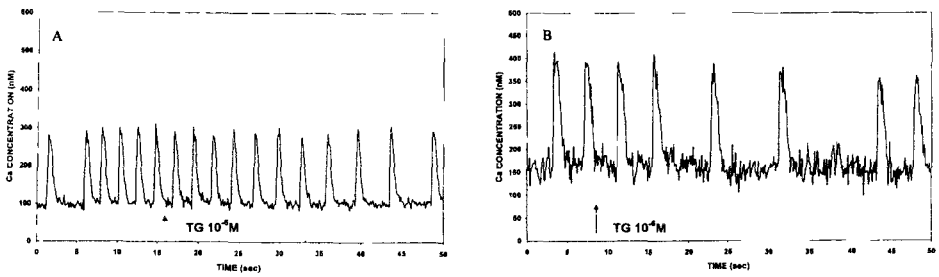

Fig. 3

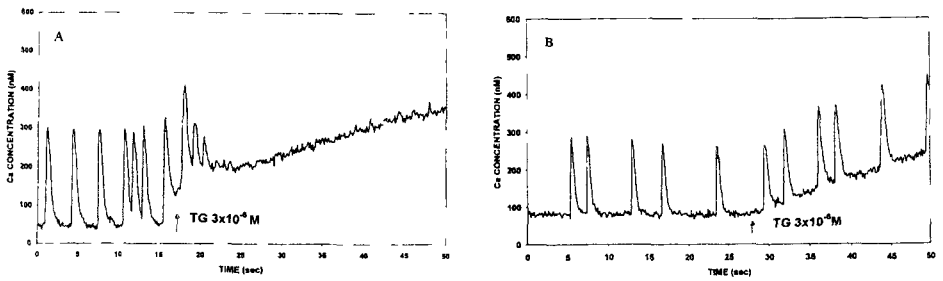

Fig. 4

Isenberg 1980, Thandroyed *et al.* 1991), $[Ca^{2+}]i$ oscillations (Kusooka *et al.* 1988, Kihara and Morgan 1991) and early or late afterdepolarizations (Marban *et al.* 1986, Priori *et al.*

1990), that are accompanying frequently the prolongation of myocardial action potentials, can initiate and/or support cardiac arrhythmias Significant increase in $[Ca^{2+}]_i$ lead to uncoupling of intercellular gap junctions (Manoach et al 1997) that may favore reentrant excitation resulting in VF (Merillat and Lakatta 1990) It was also well documented that hypertrophy or diabetic cardiomyopathy as well as age-related myocardial alterations are accompanied with altered Ca^{2+} handling, increase in $[Ca^{2+}]_i$ and suppresion of Ca^{2+} homeostatic systems (Kaufman *et al* 1990, Dhalla *et al* 1996) Furthermore, various conditions like acute myocardial ischemia (Steenbergen *et al*), increase in $[Ca^{2+}]_i$ (Reuter 1967, Thandroyen *et al* 1991), and even ventricular tachycardia and VF (Bredikis *et al* 1981,Thandroyen *et al* 1991) lead to accumulation of $[Ca^{2+}]_i$

cAMP-mediated signal is known to be implicated in modulation of contraction-relaxation processes by catecholamines Whereby, increased sarcolemmal Ca^{2+} influx followed by Ca^{2+}-induced Ca^{2+} release from SR is followed by enhanced Ca^{2+} uptake to ensure maintenance of diastolic resting state and to avoid increase of $[Ca^{2+}]_i$

Following our findings that dbcAMP attenuates elevated $[Ca^{2+}]_i$ like d-sotalol and tedisamil (Manoach *et al* 1997), the fact that d-sotalol and tedisamil increase cAMP concentration, in cats and guinea pigs, (Parmley *et al* 1972, Tribulova *et al* 1999) and the De Mello's finding that increase in cAMP enhances the reuptake of Ca^{2+} into the sarcoplasmic reticulum (DeMello 1986) brought us to hypothesize that the effect of these compounds on $[Ca^{2+}]_i$ is mediated by cAMP-enhanced Ca^{2+}-uptake by SR (Manoach et al 1987, Manoach *et al* 1993, Manoach and Watanabe 1995, Miyachi *et al* 1995)

To evaluate this hypothesis, we examined the effect of d-sotalol, tedisamil and db-cAMP on the enhanced $[Ca^{2+}]_i$ in the presence of TG Thapsigargin is known to inhibit the activity of SR Ca^{2+} ATPase and thus to cause a dysfunction of Ca^{2+} pump and blockade of Ca^{2+} uptake by the SR (Thastrup *et al* 1990) This is followed by a significant increase of $[Ca^{2+}]_i$ in self contracting myocytes, and by abolishment of Ca^{2+} transients and contractions The fact that neither tedisamil, d-sotalol nor dbcAMP in 3×10^{-6} mol/l TG pretreated heart cells were capable to restore steady state conditions of cytoplasmic free Ca^{2+} indicates that their effect against Ca^{2+} overload depends on the functioning of SR-Ca^{2+}-uptake

The fact that d-sotalol and tedisamil like dbcAMP in 10^{-6} mol/l attenuate the Ca^{2+} overload, caused by TG (in lower concentration, 10^{-6} mol/l, corresponding to lower inhibition of Ca^{2+} ATPase) can explain the mechanisms involved in the d-sotalol reestablishment of the intercellular electrical coupling, during partial dysfunction of SR, caused by short ischemia (Manoach *et al* 1996) The mechanisms involved in their preventive effects remain to be elucidated We can only speculate that pretreatment by examined drugs may in some way precondition SR to manage more efficiently TG-induced elevation in $[Ca^{2+}]_i$

The present results strongly point out a new possible mechanism of their action, responsible for d-sotalol and tedisamil antiarrhythmic-defibrillating ability

Conclusion Results presented in this paper confirm our hypothesis that class III agents with positive inotropic effect (like d-sotalol and tedisamil) may prevent Ca^{2+} overload probably by increase of intracellular cAMP level and the related Ca^{2+}-uptake by SR This cardioprotective effect of d-sotalol and tedisamil against Ca^{2+} overload may also explain their antiarrhythmic defibrillating ability and scans not to be related to their class III effect, prolongation of action potential duration

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Cerebral Angiogenesis Shows Nestin Expression in Endothelial Cells

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Abstract. The class VI intermediate filament protein nestin has been generally considered as a specific marker for neural precursor cells or developing muscles In the prenatal developing rat central nervous system (CNS), we localized immunoreactivity for the nestin in blood vessels Although the widespread nestin expression in cerebral blood vessels persisted in early postnatal periods, it was down-regulated in the adulthood However, when the adult rat brains were subjected to procedures that trigger neovascularization, e g grafting fetal nervous tissue or C6 glioma, the abundant immunoreactivity was detected in all newly formed vessels and adjacent host vasculature Our results demonstrate that nestin expression in endothelial cells lining cerebral vessels accompanies the process of angiogenesis

Key words: Angiogenesis — Endothelium — Nestin — Immunohistochemistry — Rat

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