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Importance of extracardiac α_1-adrenoceptor stimulation in assisting dofetilide to induce torsade de pointes in rabbit hearts

Attila S. Farkas [a], Károly Acsai [b], András Tóth [b], László Dézsi [c], Szabolcs Orosz [c], Tamás Forster [a], Miklós Csanády [a], Julius Gy. Papp [b,d], András Varró [b], András Farkas [a,*]

[a] 2nd Department of Internal Medicine and Cardiology Centre, Faculty of Medicine, University of Szeged, Korányi fasor 6., Szeged, H-6701, Hungary
[b] Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary
[c] Gedeon Richter Ltd. Budapest, Hungary
[d] Division of Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged, Hungary

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Abstract

In anaesthetized rabbits, α_1-adrenoceptor stimulation increases the propensity of repolarization-prolonging drugs to induce torsade de pointes ventricular tachycardia. However, it is not known whether the stimulation of intracardiac α_1-adrenoceptors, or the increased ventricular stretch caused by extracardiac α_1-adrenoceptor-mediated peripheral vasoconstriction and increased resistance, are the sensitizing factors. Accordingly, this study investigated whether a sustained load-induced left ventricular stretch or stimulation of the intracardiac α_1-adrenoceptors with 100 nM methoxamine, or the co-application of these two, can assist dofetilide (100 nM) to elicit torsade de pointes in isolated Langendorff-perfused, rabbit hearts. In the stretched hearts, a constant high level of stretch was produced by a water-filled left ventricular balloon inflated to a volume of 1.4 ml, whereby the systolic and end-diastolic pressures virtually did not exceed the physiological range ($\leq 157 \pm 11$ mm Hg and $\leq 9 \pm 2$ mm Hg, respectively; mean ± S.E.M.). Perfusion with dofetilide prolonged the QT interval significantly and indifferently in all hearts. Neither this stretch nor methoxamine nor the in combination affected the QT interval, the heart rate or the coronary flow. Interestingly, neither the stretch ('dofetilide + stretch' group, $n=8$ hearts), nor methoxamine ('dofetilide + methoxamine' group, $n=8$ hearts), nor the in combination ('dofetilide + stretch + methoxamine' group, $n=8$ hearts) elevated the incidence of torsade de pointes as compared with the 'dofetilide alone' group ($n=9$ hearts) (0%, 25%, 0%, versus 44%, respectively). In conclusion, neither a sustained load-induced stretch nor α_1-adrenoceptor stimulation nor the in combination assisted dofetilide to induce torsade de pointes in isolated rabbit hearts, suggesting the importance of extracardiac α_1-adrenoceptor stimulation in this phenomenon.

Keywords: Ventricular stretch; α_1-Adrenergic receptor; Repolarization prolongation; Torsade de pointes; Proarrhythmia; QT interval

1. Introduction

Most of the Class III antiarrhythmic agents, such as dofetilide, block the rapidly activating delayed rectifier K^+ current (I_{Kr}), and therefore lengthen the repolarization phase of the action potential of the cardiomyocytes, represented as a prolongation of the QT interval in the surface electrocardiogram (ECG) (Belardinelli et al., 2003). However, prolongation of the repolarization and the QT interval is a risk factor for the development of malignant tachyarrhythmias such as torsade de pointes (Belardinelli et al., 2003).

Several hypotheses have been proposed as concerns the generation of torsade de pointes, but the exact mechanism of this arrhythmia is still not clear. In the most widely accepted theory, the intrinsic transmural dispersion of repolarization is amplified by any effect that reduces net repolarizing currents (e.g. drugs such as dofetilide, certain ion channel mutations, or remodelling of the ventricular wall) by preferential prolongation of the repolarization of the M cells, which sets the stage for circus movement reentrant arrhythmias (Belardinelli et al., 2003). Furthermore, the reduction in net repolarizing currents

* Corresponding author. Tel.: +36 62 545 220; fax: +36 62 544 568.
E-mail address: farkasa@in2nd.szote.u-szeged.hu (A. Farkas).

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also predisposes to the development of early afterdepolarization-induced ventricular extrasystoles, which can provide the initiating beat that triggers reentrant tachyarrhythmias (Belardinelli et al., 2003). Thus, the mechanism of torsade de pointes initiation involves the early afterdepolarization-induced ectopic beat, while the mechanism of the arrhythmia maintenance involves the reentry.

The in vivo experimental model of the acquired long QT syndrome developed by Carlsson et al. (1990) utilizes anaesthetized rabbits. The ability of a test agent to evoke torsade de pointes is evaluated during co-administration of a 'priming' substance, the selective α_1-adrenoceptor agonist methoxamine (Carlsson et al., 1990) or phenylephrine (Farkas et al., 1998, 2002). Although this model has been widely used to examine the proarrhythmic activity of novel antiarrhythmics and other non-cardiac agents, the mechanism by which the priming agent facilitates torsade de pointes in rabbits is unknown. Although Carlsson et al. (1990) hypothesized that direct stimulation of the cardiac α_1-adrenoceptors is the facilitating factor in their model, this hypothesis has as yet not been proven. On the other hand, it may also be hypothesized that reflex bradycardia and/or an increased ventricular stretch, which develop as a consequence of α_1-adrenoceptor-mediated peripheral vasoconstriction and increased resistance, are the critical sensitizing factors in the rabbit model of acquired long QT syndrome developed by Carlsson et al. However, this latter hypothesis has not yet been proven either.

Acute mechanical stretch induces complex changes in the heart muscle. An acute stretch during diastole may induce membrane depolarizations resembling delayed afterdepolarizations, whereas an acute stretch during systole usually shortens the action potential and the refractory period of the myocytes in a heterogeneous manner; however, action potential prolongation and the induction of early afterdepolarizations have also been reported (Janse et al., 2003). Hence, an acute stretch can be arrhythmogenic by (i) inducing triggered arrhythmias, e.g. extrasystoles, when stretch-induced early or delayed afterdepolarizations reach the threshold, or (ii) facilitating reentrant arrhythmias by the heterogeneous shortening of the action potential and refractory period of the cardiomyocytes.

Despite intensive examinations of the arrhythmia genesis in the long QT syndrome, so far no data have been published on the contribution of a ventricular stretch to the development of torsade de pointes. Similarly, despite the frequent use of the anaesthetized rabbit model of the acquired long QT syndrome (Carlsson et al., 1990), hardly any experimental data have been published on the role of α_1-adrenoceptor stimulation in the model. Since a ventricular stretch, α_1-adrenoceptor stimulation and bradycardia can be separated from each other in isolated Langendorff-perfused rabbit hearts, this model is suitable for examining the effects of these factors separately and in combination on the genesis of torsade de pointes. Accordingly, the present study investigated whether triggered arrhythmias induced by a constant high level of left ventricular stretch are able to initiate torsade de pointes in the presence of functional reentries caused by the repolarization-lengthening dofetilide in the presence or the absence of α_1-adrenoceptor stimulation in isolated Langendorff-perfused rabbit hearts.

2. Methods

2.1. Animals and general methods

Female New Zealand white rabbits weighing 2.3–2.9 kg were used for the experiments, as female gender may increase the susceptibility to the generation of drug-induced torsade de pointes in rabbits, similarly as in humans (Liu et al., 1999; Lu et al., 2001). The animals were handled in accordance with the European Community guidelines for the use of experimental animals, and the protocol was reviewed and approved by the Ethical Committee for the Protection of Animals in Research at the University of Szeged, Hungary.

The animals were anticoagulated with sodium heparin (1000 international units) injected into the marginal ear vein and stunned by a blow to the neck. The heart was rapidly removed via thoracotomy and rinsed in ice-cold modified Krebs–Henseleit buffer solution containing (in mM): NaCl 118.5, $CaCl_2$ 2.5, glucose 11.1, $MgSO_4$ 0.5, NaH_2PO_4 1.2, $NaHCO_3$ 25, and KCl 3. The K^+ concentration of 3 mM was chosen for the buffer, as the proarrhythmic action of dofetilide tended to be exacerbated by perfusion with 3 mM K^+ versus 4 mM K^+ in isolated rabbit hearts (Barrett et al., 2001). The aorta was cannulated and hung on a Langendorff apparatus. The hearts were retrogradely perfused at a constant temperature of 37 °C with the modified Krebs–Henseleit buffer solution described above. A mixture of 95% O_2 and 5% CO_2 was bubbled through the buffer, which was equilibrated to pH 7.4. All solutions were filtered (10 μm pore size filter) before use. The perfusion pressure was maintained constant at 70 mm Hg. Volume-conducted ECG and left ventricular pressure (described later) were recorded by using National Instruments data acquisition hardware (PC card, National Instruments, Austin, TX, USA) and SPEL Advanced Haemosys software (version 2.45, Experimetria Ltd. and Logirex Software Laboratory, Budapest, Hungary). Coronary flow was measured with a glass flowmeter (Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA) positioned immediately above the retrogradely perfused aorta.

2.2. Experimental protocol

In the first set of experiments, to find the appropriate dofetilide concentration in terms of the proarrhythmic potential, three groups of hearts [dofetilide 50 nM ($n=8$), dofetilide 100 nM ($n=9$), and control (solvent of dofetilide, $n=10$)] were compared. In each heart, the drug perfusion was started after 15 min of initial perfusion with modified Krebs–Henseleit solution, and lasted for 40 min. The choice of drug solution was made by reference to a randomization table. Randomization was achieved by coding each group with a letter whose meaning was unknown to the operator. Blinded analysis was achieved by using stock solutions prepared by a second operator, who did not participate in the heart perfusion or data analysis.

Table 1
Incidence of arrhythmias

	n	Incidence of arrhythmias (%)					
		VPB	BG	Salvo	Block	VT	TdP
Control	10	70	10	0	10	0	0
50 nM dofetilide	8	50	0	25	25	0	0
100 nM dofetilide	9	89	56	56	89[a]	44	44
Dof+stretch	8	100	63	88[a]	63	63[a]	0
Dof+Methox	8	75	25	38	75	25	25
Dof+Methox+stretch	8	100	38	75[a]	38	63[a]	0

Values are percentage incidences of arrhythmias. Dof, 100 nM dofetilide; Methox, 100 nM methoxamine; VPB, ventricular premature beat; BG, bigeminy; Block, conduction block of any kind; VT, ventricular tachycardia different from torsade de pointes; TdP, torsade de pointes ventricular tachycardia. Group size is indicated by *n*.

[a] $P<0.05$ as compared with the Control group.

Torsade de pointes occurred only in the 100 nM dofetilide group (Table 1) and it did so with an incidence which offered scope for the examination of additional effects that can further increase the incidence of this arrhythmia. This concentration was therefore chosen and used in the remainder of the study.

In the second set of experiments, three groups of hearts ($n=8$ hearts in each group) were perfused with 100 nM dofetilide for 40 min after 15 min of initial perfusion with modified Krebs–Henseleit solution. In two of the three dofetilide-perfused groups of hearts, methoxamine at a concentration of 100 nM was added to the dofetilide-containing perfusion solution. This concentration of methoxamine was chosen since 10 μM and 1 μM methoxamine reduced the coronary flow in rabbit hearts by more than 50%, while 100 nM methoxamine did not alter the coronary flow appreciably in our pilot studies (data not shown). In each heart, a non-elastic balloon was inserted into the left ventricle via the mitral valve and was connected to a pressure transducer. The intraventricular balloon was filled with water at a constant volume of 1.4 ml throughout the whole experiment in the group of hearts perfused only with dofetilide and the vehicle of methoxamine ('dofetilide+stretch' group) and in one of the groups of hearts perfused with dofetilide and methoxamine ('dofetilide+methoxamine+stretch' group). This balloon volume was chosen since in preliminary studies it provided the approximate maximum left ventricular developed pressure (developed pressure=systolic pressure−end-diastolic pressure) without damaging the hearts (data not shown). The balloon was not inflated in the other group of hearts perfused with dofetilide and methoxamine ('dofetilide+methoxamine' group). The second set of experiments was randomized, too.

Individual measurements of coronary flow, left ventricle pressure and ECG variables were made every 5 min and 1 min before and 1 min after the introduction of drug perfusion. At the end of each experiment, the atria were removed from the heart and the ventricles were weighed.

2.3. Drugs and materials

Perfusion solutions were prepared fresh each day. In the first set of experiments, the 'vehicle stock' was a 2 ml solution containing 1.8 ml of plain water+0.2 ml of 1 M NaOH. The control solution contained 0.12 ml of this 'vehicle stock' in 3 l of modified Krebs–Henseleit solution. The 100 nM dofetilide solution was prepared from 2 ml of 'dofetilide stock' (consisting of 2.21 mg of dofetilide dissolved in 1.8 ml of water+0.2 ml of 1 M NaOH) such that 0.12 ml of this 'dofetilide stock' dissolved in 3 l of modified Krebs–Henseleit solution yielded a 100 nM dofetilide solution. The 50 nM dofetilide solution was prepared from a 2 ml stock solution containing 1 ml of 'dofetilide stock'+1 ml of 'vehicle stock', such that 0.12 ml of this stock dissolved in 3 l of modified Krebs–Henseleit solution furnished a 50 nM dofetilide solution. The dofetilide was a generous gift from Gedeon Richter Ltd. (Budapest, Hungary).

The 100 nM methoxamine solution was prepared from 10 ml of 'methoxamine stock' consisting of 6.19 mg of methoxamine (Sigma-Aldrich, Inc., St. Louis, MO, USA) dissolved in 10 ml water, such that 0.12 ml of this stock dissolved in 3 l of modified Krebs–Henseleit solution of the 'dofetilide+methoxamine' group and the 'dofetilide+methoxamine+stretch' group yielded a 100 nM methoxamine solution. The methoxamine 'vehicle stock' was 10 ml of water, and 3 l of the modified Krebs–Henseleit solution of the 'dofetilide+stretch' group contained 0.12 ml of this 'vehicle stock'.

$CaCl_2$ and $MgSO_4$ were purchased from Sigma-Aldrich, Inc., St. Louis, MO, USA. All other salts were purchased from Molar Chemical Kft., Budapest, Hungary. Water for the preparation of perfusion solution was obtained from a reverse osmosis system (Milli-Q RG, Millipore Ltd., Billerca, MA, USA) fed by distilled water, and had a specific resistivity of >18 MΩ.

2.4. Exclusion criteria

Any heart with a sinus rate <120/min or a coronary flow >15 ml/min/g or <3 ml/min/g 5 min before the start of the 40-min drug perfusion protocol, or not in a constant sinus rhythm before the start of the 40-min drug perfusion, was excluded.

2.5. Arrhythmia diagnosis and ECG analysis

Coronary flow and ECG intervals were measured at predetermined time points. After the completion of experiments, the data were replayed and the RR, PR, QRS and QT intervals were measured by manual positioning on screen markers. The QT interval was defined as the time between the first deviation from the isoelectric line during the PR interval until the end of the TU wave. Where the T or U wave overlapped the following P wave or the QRS complex of the subsequent sinus beat, the extrapolation method was used to measure the length of the QT (or QU) interval (Farkas et al., 2004). From the ECG, the incidence and the time to onset of arrhythmias were obtained. Ventricular premature beats, bigeminies, salvoes and ventricular fibrillation were defined according to the Lambeth Conventions (Walker et al., 1988). Torsade de pointes was defined as a polymorphic ventricular tachycardia with runs of 4 or more ventricular premature beats, where clear twisting of the QRS complexes around the isoelectric axis could be seen in at least one ECG lead. Runs

of 4 or more ventricular premature beats without the torsade-like twisting QRS morphology were differentiated from torsade de pointes and were defined as ventricular tachycardia. Blocks in the conduction system were also monitored. Conduction disturbances included atrioventricular blocks and intraventricular conduction defects (right or left bundle branch block).

2.6. Statistics

Continuous data were expressed as mean ± standard error of the mean (S.E.M.). All data from independent samples, except arrhythmia incidences, were compared with Kruskal–Wallis tests. The incidences of arrhythmias were compared by using Fisher's exact probability test with the Bonferoni correction, i.e. the P values of Fisher's exact probability test were multiplied by 6 (the number of groups) to allow multiple comparisons (Altman, 1991). $P<0.05$ was taken as indicative of a statistically significant difference between values.

3. Results

3.1. Arrhythmia incidences

In the first set of experiments, spontaneous torsade de pointes occurred only in the 100 nM dofetilide group (Fig. 1) and it did so with a relatively low incidence (Table 1), which offered scope for the examination of additional effects that can further increase the incidence of this arrhythmia. On the other hand, dofetilide at a concentration of 100 nM evoked ventricular conduction blocks in a majority of the hearts (Table 1).

In the second set of experiments, neither the sustained load-induced left ventricular stretch nor methoxamine nor the in combination increased the incidence of dofetilide-provoked torsade de pointes. However, the stretch was arrhythmogenic in terms of increasing the incidence of salvo and of ventricular tachycardia different from torsade de pointes as compared with the control group, and ventricular premature beats occurred in each heart only in those two groups in which the stretch was applied (Table 1).

3.2. ECG intervals

Dofetilide reduced the heart rate similarly in all groups of hearts as compared with the control (Fig. 2A). On the other hand, dofetilide caused a concentration-dependent lengthening of the QT interval and neither the stretch nor methoxamine significantly affected the dofetilide-induced QT prolongation (Fig. 2B). As there was no significant difference between the heart rates of the dofetilide-treated groups (Fig. 2A), the QT values were not corrected for heart rate. Dofetilide also caused a concentration-dependent lengthening of the QRS interval (Fig. 2C).

The mean baseline PQ interval ranged from 60 ± 1 to 74 ± 2 ms and remained stable throughout the experiments; there was no significant difference between the PQ values of the various groups at any time point of the experiments. Neither the stretch nor methoxamine influenced the length of the PQ interval (data not shown).

3.3. Coronary flow, left ventricular pressures

The mean coronary flow ranged from 4.0 ± 0.6 to 7.7 ± 0.4 ml/min/g, and there was no significant difference between the various groups at any time point of the experiments. Neither the systolic nor the end-diastolic left ventricular pressure differed significantly between the 'dofetilide + stretch' group and the 'dofetilide + methoxamine + stretch' group. The left ventricular systolic pressure ranged from 139 ± 4 to 157 ± 11 mm Hg

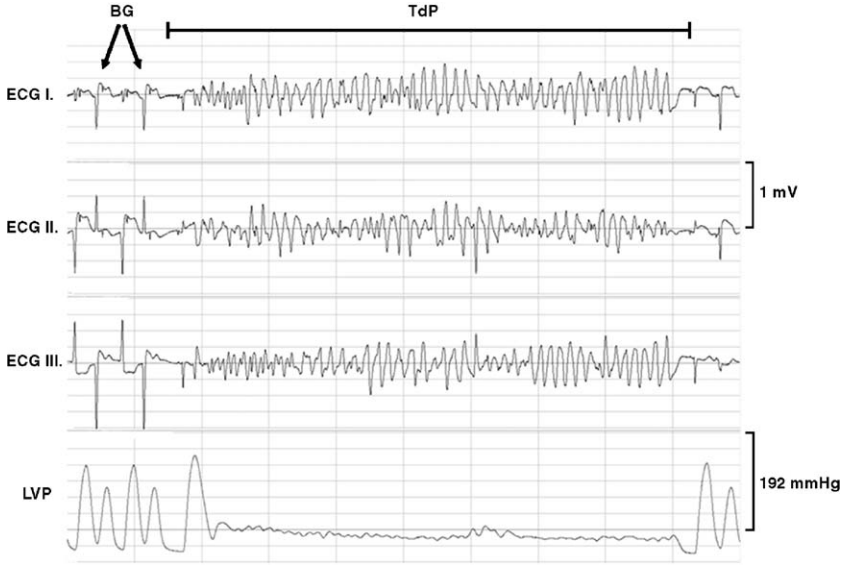

Fig. 1. Dofetilide-induced torsade de pointes ventricular tachycardia. ECG I–III, electrocardiographic leads similar to standard limb leads I, II and III; LVP, left ventricular pressure; BG, bigeminy; TdP, torsade de pointes.

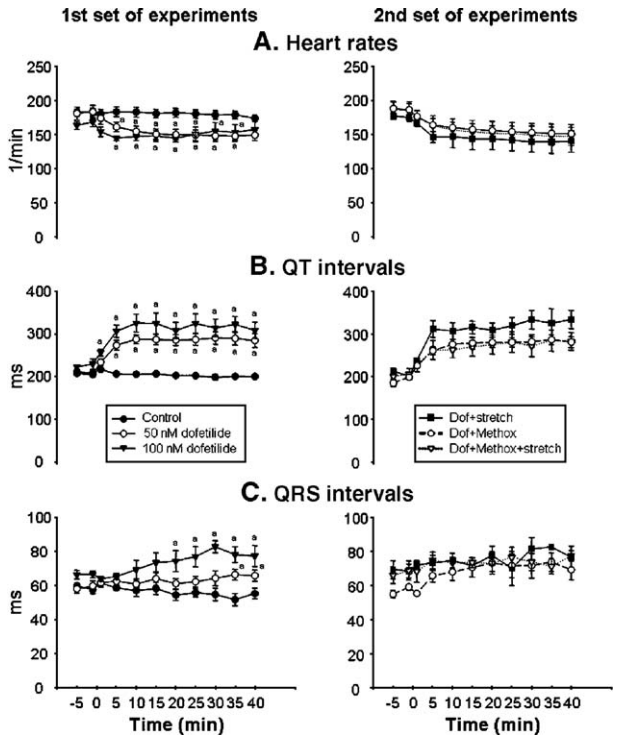

Fig. 2. Heart rates (A), QT intervals (B) and QRS intervals (C) in isolated rabbit hearts. In the 1st set of experiments (left), the hearts were perfused with 0 nM dofetilide (Control) or 50 nM or 100 nM dofetilide. In the 2nd set of experiments (right), each heart was perfused with 100 nM dofetilide, and in the 1st group of hearts only a left ventricular stretch was added (Dof+stretch), while in the 2nd group of hearts 100 nM methoxamine was added (Dof+Methox), and in the 3rd group of hearts 100 nM methoxamine and the stretch were added (Dof+Methox+stretch). Drug perfusion started at "0" min. Values are mean± S.E.M. $^{a}P<0.05$ as compared with the Control group.

and from 141±7 to 153±5 mm Hg, respectively. The mean end-diastolic pressure did not exceed 9±2 mm Hg in any of the groups at any time point of the experiments.

4. Discussion

The present results show that neither methoxamine, which sensitizes anaesthetized rabbits to the proarrhythmic effects of repolarization-prolonging drugs (Carlsson et al., 1990), nor a constant high level of left ventricular stretch, achieved by inflation of a left ventricular balloon, nor the combination of methoxamine and the stretch elevated the propensity of dofetilide to induce torsade de pointes in isolated rabbit hearts. This is in contrast with the previous speculation based on in vivo studies that these factors play a critical role in the development of torsade de pointes in the rabbit heart.

4.1. Sufficient dofetilide effect

It may be concluded that the lack of torsade de pointes in the present experiments was a result of the low concentration of dofetilide. In an earlier work on isolated guinea pig ventricular myocytes, 100 nM dofetilide prolonged the action potential duration and selectively inhibited the rapid component of the delayed rectifier K^+ current (I_{Kr}) with an IC_{50} value of 31.5 nM (Jurkiewicz and Sanguinetti, 1993). In another study (Lu et al., 2000), dofetilide lengthened the action potential duration markedly and induced frequent early afterdepolarizations in isolated rabbit Purkinje fibres at a concentration of 10 nM, i.e. one-tenth of the dofetilide concentration (100 nM) applied in the present study. In the hearts in the present investigation, 100 nM dofetilide markedly and significantly prolonged the QT interval and evoked torsade de pointes, which is clear evidence of the efficient repolarization prolongation in the rabbit (Carlsson et al., 1990). Furthermore, 100 nM dofetilide induced conduction blocks in most hearts and widened the QRS complex, which is direct and indirect evidence, respectively, of extremely prolonged repolarization and refractoriness in the conduction system of the rabbit, which occurs when high doses of repolarization-prolonging drugs are applied (Farkas et al., 2004). The presence of conduction blocks in the dofetilide-perfused hearts in this investigation suggests that the conditions were given for functional reentry circuits to develop as a result of a sufficient dofetilide effect. This supports the notion that the dofetilide concentration was high enough to set the stage for the development of torsade de pointes in this study.

Despite the sufficient effect of 100 nM dofetilide on repolarization and refractoriness, torsade de pointes occurred in fewer than half of the hearts in the first set of experiments. A zero incidence of this arrhythmia was earlier seen with 100 nM dofetilide in isolated Langendorff-perfused rabbit hearts (D'Alonzo et al., 1999), though that perfusion solution did not differ significantly from the one used in the present study. Extreme bradycardia was recently induced via a mechanical atrioventricular block in Langendorff-perfused rabbit hearts to achieve a higher incidence of torsade de pointes (71%) with a lower concentration of dofetilide (30 nM) (Barrett et al., 2001). Conversely, the induction of torsade de pointes with dofetilide alone in most hearts was not the aim of the present study since it would not have provided scope for the examination of additional effects that might increase the incidence of torsade de pointes.

4.2. Stretch did not initiate torsade de pointes

In the present experiments a constant high level of stretch did not increase the incidence of dofetilide-induced torsade de pointes, though the stretch was arrhythmogenic since it increased the incidence of salvo and of ventricular tachycardia different from torsade de pointes, and ventricular premature beats occurred in each heart only in those two groups, in which the stretch was applied. In theory, these stretch-induced arrhythmias could initiate torsade de pointes in the presence of dofetilide-produced functional reentry circuits. One possible explanation of the lack of a stretch effect on torsade de pointes initiation is that the absolute number of stretch arrhythmias was still low, and the statistical possibility of a stretch-induced arrhythmia falling in the vulnerable period of dofetilide-produced functional reentries was therefore low. Thus, further

elevation of the absolute number of stretch-induced arrhythmias might increase the incidence of dofetilide-induced torsade de pointes. In the present experiments, the left ventricular stretch applied was the highest that did not destroy the structure of the ventricle and provided physiological systolic and end-diastolic pressures. Elevation of the absolute number of stretch-induced arrhythmias would therefore be possible only by overstretching the left ventricle with non-physiological volumes (Eckardt et al., 2000) or by applying a dynamically changing stretch protocol with rapid volume pulses (Franz et al., 1992). However, the application of such procedures was not the aim of the study.

It is known that a stretch can shorten repolarization. In an earlier study, a sustained load produced by a filled left ventricular balloon shortened the duration of the monophasic action potential in isolated Langendorff-perfused rabbit hearts (Zabel et al., 1996). In guinea pig myocytes, a hypotonic-induced stretch shortened the action potential duration and counteracted the effect of the potent repolarization-prolonging agent E4031 (Groh et al., 1996). In the present study, the QT interval was not affected by the stretch, which implies that the stretch did not counteract the repolarization-prolonging effect of dofetilide.

A ventricular stretch produces complex electrophysiological effects by inducing afterdepolarizations, increasing the dispersion of repolarization, slowing impulse conduction, and either shortening or prolonging repolarization (Janse et al., 2003; Ravens, 2003), which may affect the genesis of torsade de pointes. However, no data have so far been published on the contribution of a ventricular stretch to the development of torsade de pointes. Accordingly, despite the fact that the present study did not reveal any effect of a sustained load-induced stretch on the occurrence of torsade de pointes in isolated rabbit hearts perfused with dofetilide, the roles of a ventricular stretch and stretch-induced complex electrophysiological changes in the generation of torsade de pointes necessitate further investigations in other experimental settings.

4.3. Methoxamine did not promote torsade de pointes

In the anaesthetized rabbit model of acquired long QT syndrome (Carlsson et al., 1990), the stimulation of α_1-adrenoceptors by either methoxamine (Carlsson et al., 1990) or phenylephrine (Farkas et al., 1998) sensitizes the animals to the proarrhythmic effects of repolarization-prolonging drugs. Stimulation of the cardiac α_1-adrenoceptors increases the free intracellular Ca^{2+} level via an increased turnover of phosphatidylinositols (Fedida et al., 1993), suppresses outward K^+ currents (Braun et al., 1990, 1992; Fedida et al., 1990, 1991; Li et al., 1996) and increases the amplitude of early afterdepolarizations (Ben-David and Zipes, 1990), all of which may contribute to the development of torsade de pointes in the setting of prolonged repolarization.

Interestingly, methoxamine did not promote torsade de pointes generation in rabbit hearts in the present study. In accordance with our pilot experiments, in order to avoid a direct effect of flow reduction on arrhythmia genesis, the maximum concentration of methoxamine was applied which did not reduce the coronary flow. A significant coronary flow reduction was considered undesirable for the following reason. Myocardial anoxia or hypoxia in anaesthetized rabbits is represented by an electrocardiographic ST segment elevation or depression (Farkas et al., 1998). Since none of the published in vivo rabbit proarrhythmia studies (e.g., Buchanan et al., 1993; Carlsson et al., 1990; Farkas and Coker, 2003) report the occurrence of an electrocardiographic ST segment elevation or depression in the event of a significant coronary artery constriction as a result of a high plasma concentration of the α_1-adrenoceptor agonist methoxamine or phenylephrine, it is highly unlikely that a coronary flow reduction plays a role in the generation of torsade de pointes in the anaesthetized rabbit model of acquired long QT syndrome.

Unfortunately, no data are available as concerns the blood and serum concentrations of methoxamine achieved by continuous infusion in the in vivo rabbit model of acquired long QT syndrome. The currently applied in vitro concentration of methoxamine (100 nM) may not match the in vivo serum concentration of the drug achieved by continuous venous infusion (usually at a rate of 15 μg/kg/min). However, the minimum concentrations of methoxamine that cause contraction of the pulmonary arteries (Haeusler, 1983), the ear arteries (Movahedi et al., 1995) and the thoracic aorta (Oshita et al., 1993) in the rabbit are 30 nM, 10–30 nM and 100 nM, respectively, which indicates that 100 nM methoxamine does exert pharmacological action in the rabbit. Thus, this concentration seems appropriate for testing the effects of α_1-adrenoceptor stimulation on torsade de pointes genesis in vitro.

Similarly to our results, it was earlier found that methoxamine at a concentration of 30 nM, which is approximately one-third of that used in the present experiments (100 nM), did not help dofetilide (100–700 nM) to elicit torsade de pointes consistently in isolated Langendorff-perfused rabbit hearts (D'Alonzo et al., 1999). In that work, repolarization-prolonging drug-induced torsade de pointes occurred frequently when acetylcholine (300 nM) was added to the methoxamine in the perfusion solution (D'Alonzo et al., 1999). Thus, it was concluded that the heart rate reduction achieved with acetylcholine and the increase in intracellular Ca^{2+} level caused by α_1-adrenoceptor stimulation were the factors promoting the high incidence of torsade de pointes attained with repolarization-prolonging drugs in isolated rabbit hearts (D'Alonzo et al., 1999). However, acetylcholine did not reduce the heart rate significantly in that study, and there were only a few hearts in each group perfused with a single drug or a drug combination (D'Alonzo et al., 1999), which raises questions concerning their conclusion of the need for methoxamine and acetylcholine to induce torsade de pointes in isolated rabbit hearts, since these small groups are inappropriate for statistical comparison. Their results and those of the present investigation rather suggest that the role of the stimulation of intracardiac α_1-adrenoceptors in the generation of torsade de pointes in the rabbit is questionable.

Furthermore, in the setting of prolonged repolarization, the lack of a sensitizing effect of α_1-adrenoceptor stimulation in isolated rabbit hearts and the presence of this effect in anaesthetized rabbits implies that the extracardiac effects of α_1-adrenoceptor stimulation are the crucial factors in the genesis of torsade de pointes.

The stimulation of extracardiac α_1-adrenoceptors causes peripheral vasoconstriction and elevates the resistance and arterial blood pressure, which reduces the heart rate via a vagus nerve-mediated autonomic reflex and increases the ventricular stretch. In a recent in vivo study (Coker and Farkas, 2004), bilateral vagotomy elevated the heart rate and prevented torsade de pointes in anaesthetized rabbits treated with simultaneous phenylephrine and clofilium infusions. This emphasizes the importance of extracardiac α_1-adrenoceptor stimulation-induced reflex bradycardia in the anaesthetized rabbit model of acquired long QT syndrome.

4.4. In vitro versus in vivo induction of torsade de pointes

In the present experiments, the heart rate, the QT interval and the systolic pressure were approximately the same in the α_1-adrenoceptor-stimulated, stretched hearts perfused with 100 nM dofetilide as those recorded immediately before torsade de pointes occurred in anaesthetized rabbits treated with phenylephrine and D-sotalol or almokalant (published and unpublished data of Farkas et al., 2002). Furthermore, similarly to the results of the present study, the end-diastolic pressure values remained virtually in the physiological range (<10 mm Hg) in anaesthetized rabbits sensitized by phenylephrine to the proarrhythmic effects of clofilium (unpublished data from the study by Farkas and Coker, 2002). Thus, although isolated rabbit hearts subjected to dofetilide, α_1-adrenoceptor stimulation and stretch exhibit nearly the same heart rate, QT interval and pressure as those measured in α_1-adrenoceptor-stimulated anaesthetized rabbits treated with repolarization-prolonging drugs, torsade de pointes occurs more frequently under in vivo conditions. This strongly suggests that the generation of torsade de pointes requires other additional factors, which are absent in the isolated heart, but present in vivo, e.g. hormonal and/or autonomic neuronal effects, loading of the right ventricle, a stretch produced by an increased afterload rather than an increased preload (which was used in the present study). The roles of these factors in the development of torsade de pointes demand further investigations.

5. Conclusion

The present results indicate that neither a sustained load-induced constant high level of left ventricular stretch nor α_1-adrenoceptor stimulation nor their co-application promote the generation of torsade de pointes in the setting of prolonged repolarization in isolated Langendorff-perfused rabbit hearts. The roles of intracardiac α_1-adrenoceptor stimulation and a sustained load-induced left ventricular stretch in the provocation of torsade de pointes in the rabbit are therefore questionable. In contrast, the present results suggest the importance of extracardiac α_1-adrenoceptor stimulation in torsade de pointes development in the rabbit heart.

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