Role of gap junction channel in the development of beat-to-beat action potential repolarization variability and arrhythmias

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Running title:

 Gap junctions and beat-to-beat variability of action potential repolarization

Abstract

The short-term beat-to-beat variability of cardiac action potential duration (SBVR) occurs as a random alteration of the ventricular repolarization duration. SBVR has been suggested to be more predictive of the development of lethal arrhythmias than the action potential prolongation or QT prolongation of ECG alone. The mechanism underlying SBVR is not completely understood but it is known that SBVR depends on stochastic ion channel gating, intracellular calcium handling and intercellular coupling.

Coupling of single cardiomyocytes significantly decreases the beat-to-beat changes in action potential duration (APD) due to the electrotonic current flow between neighboring cells. The magnitude of this electrotonic current depends on the intercellular gap junction resistance. Reduced gap junction resistance causes greater electrotonic current flow between cells, and reduces SBVR.

Myocardial ischaemia (MI) is known to affect gap junction channel protein expression and function. MI increases gap junction resistance that leads to slow conduction, APD and refractory period dispersion, and an increase in SBVR. Ultimately, development of reentry arrhythmias and fibrillation are associated post-MI. Antiarrhythmic drugs have proarrhythmic side effects requiring alternative approaches. A novel idea is to target gap junction channels. Specifically, the use of gap junction channel enhancers and inhibitors may help to reveal the precise role of gap junctions in the development of arrhythmias. Since cell-to-cell coupling is represented in SBVR, this parameter can be used to monitor the degree of coupling of myocardium.

Keywords: heart, gap junction, beat-to-beat variability, arrhythmia

1. Introduction

It has been estimated that about half of the patients suffering from heart failure die from an arrhythmia, accounting for >500.000 deaths per year worldwide. Research is focused biomarkers as predictors of arrhythmia development. The prolongation of QT interval is a risk factor and an important predictor for the development of arrhythmias, in particular Torsades de Pointes arrhythmia (TdP) [1]. Clinical observations, as well as canine and rabbit model experiments suggest that the short term beat-to-beat variability of cardiac action potential

duration (SBVR) is a better predictor of drug-induced TdP arrhythmias than the measurement of repolarization prolongation alone [2-6]. It is likely that the development of SBVR is multifactorial and the electrotonic interactions among cardiac cells, namely the gap junction channels limit the temporal dispersion of repolarization. Changes in function, quantity, and location of gap junction channels alter cardiac impulse conduction, membrane refractoriness, leading to the development of arrhythmias [7, 8]. Therefore the alteration of gap junction channel function may represent a putative antiarrhythmic therapeutic target.

In this review we summarize the possible role of gap junction channels in the development of SBVR, arrhythmias, and important gap junction enhancer and inhibitor molecules.

2. The short term beat-to-beat variability of cardiac action potential duration (SBVR)

The SBVR or the beat-to-beat variability of the QT interval occurs as random variations of the ventricular repolarization duration or QT intervals in consecutive heart beats at stable rates [9-11]. The exact mechanism underlying SBVR is not completely understood. To the best of our knowledge the mechanism of SBVR is multifactorial including: stochastic ion-channel gating [12-14], pharmacological interventions influencing ion channels that operate during the action potential plateau and repolarization [2], periodic calcium release of sarcoplasmic reticulum [15], and electrotonic interactions among the cardiac cells can influence it [10, 12].

A number of different ion channels contribute to the action potential configuration. The membrane resistance varies during the different phases of the action potential [16]. Stochastic ion channel gating contributes to variable action potential morphologies on a beat by beat basis. Lemay *et al.* published that stochastic gating of certain ion channels (ion channels responsible for L-type calcium current, late sodium current, slow component of the delayed rectifier potassium current) contribute primarily to the action potential repolarization variability of single cardiac cells [13]. Our results underline the importance of stochastic channel gating in the development of SBVR, too. We have previously described that the size of early repolarization phase of canine ventricular action potential influences the gating of L-type calcium channels [17], namely large early repolarization causes the reopening of calcium channels during the action potential plateau. That means that a small change in the transient outward potassium current (I_{to}) modifies the process of early repolarization, the calcium current, and consequently the duration of action potential repolarization.

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The action potential duration (APD) and the SBVR is also modulated by intracellular calcium handling. Johnson e*t al.* reported that in dog heart spontaneous calcium release leads to APD prolongation via increased I_{Ca-L}, which in turn increased SBVR [15]. In agreement with these data buffering of intracellular calcium suppressed SBVR [2]. In a rabbit LQT2 model abnormal calcium handling preceded fluctuations in membrane potential [18].

It is well known among cardiac electrophysiologists that SBVR is smaller for multicellular heart preparations than for isolated cardiac cells [10], implicating the involvement of gap junction channel function to SBVR.

In canine and rabbit models inhibition of the rapid and slow components of the delayed rectifier potassium current (I_{Kr} and I_{Ks}), and augmentation of late I_{Na} significantly increased SBVR and prolonged the duration of repolarization. In these experiments the increased SBVR better predicted the development of TdP arrhythmia than repolarization prolongation alone [3, 4, 19, 20]. Hinterseer *et al.* published similar findings in selected human patients [21] and it is also known that the successful antiarrhythmic treatment does not need to be accompanied by QT interval shortening [22, 23]. These results question the association between QT prolongation and the arrhythmia development. Thus, quantitative assessment of SBVR could be a reliable parameter to predict proarrhythmic conditions [3, 9, 11].

3. The gap junction

3.1 The structure of gap junction

Gap junction channels are involved in physiological and pathological processes such as embryonic development, cell differentiation, growth [24-26], pathogenesis of neuropathies [27], epilepsy [28], cardiovascular diseases [29], and arrhythmias [30, 31]. Numerous densely packed gap junction channels form clusters that directly connect the cytoplasmic compartment of cells [32]. The structure of these channels has been described by Unwin & Zampighi in 1980 [33]. The gap junction channel is composed of two hemichannels (connexons), provided by each of two neighboring cells. The connexon consists of six connexin proteins. Each connexin has four transmembrane domains, two extracellular loops, one intracellular loop, and cytoplasmic-localized amino- and carboxy-termini [34, 35]. The amino acid sequence of the transmembrane domains and extracellular loops are highly conserved among the different isoforms; however, the length of the connexin C-terminus is variable [36]. The C-terminus contains phosphorylation sites providing putative substrate sites to regulate the gap junction channel function [36].

Until now more than twenty different connexin encoding genes have been described in mouse and in human [37]. The variable C-termini length among isoforms contributes to differing molecular weight of connexins, thus giving rise to the classification of these proteins [38].

Gap junction channels are often composed of the same type of connexin, but it is also possible that the functional gap junction channel is composed of different connexin isoforms. For example, in atrium Cx40 and Cx43 isoforms are co-localized suggesting heterotypic connexons [39]. Although the structure of various connexin isoforms is very similar, the permeability, the pH sensitivity, and the voltage gating of the channels formed by different connexin isoforms may differ [34, 35, 40, 41].

3.2 The distribution of gap junctions

In the mammalian heart expresses Cx37, Cx40, Cx43, Cx45 and Cx50 isoforms. Cx37 is detected in the endocardial endothelium [42], while Cx50 was described in rat atrioventricular valves [43]. Cx40, Cx43 and Cx45 are the most abundant connexin isoforms of the working mammalian myocardial cells and the conduction system. Cx40 is mainly expressed in the atrium and in the conduction system [44-49], while Cx43 can be found both in atrial and ventricular cells but not in sinoatrial and atrioventricular nodes [46, 48-55]. Cx45 is preferentially expressed in sinoatrial and atrioventricular nodes, His-bundle and bundle branches [56-58]. More recently, Cx30.2 expression was described in mouse sinus node and in the conduction system, but the human ortholog Cx31.9 is not expressed in the human heart [59-61].

The biophysical properties of these gap junction channel isoforms are different (reviewed by Dhein [36]). The single channel conductance is about 200 pS, 80 pS and 20 pS, for Cx40, Cx43 and Cx45 channel, respectively [42, 62, 63]. The conductance values can be influenced by many factors such as intracellular calcium, magnesium, sodium ion concentration, pH, hypoxia and the phosphorylation state of connexins [34, 36]. If we take into account the different conductances of the gap junction channels and the non-uniform expression of the connexin isoforms, then gap junctions seem to form different functional compartments within the heart. This provides the basis of the impulse propagation from the sinoatrial node toward the working ventricular cells.

In addition to this functional compartmentalization, the gap junction channels are unevenly distributed in the cell membrane. The large portion of the densely packed gap junction channels are localized at the poles of cardiomyocytes [64], while the smaller portion

can be found on the lateral side of these cells. These gap junctions conduct the electrical impulses both in longitudinal and transverse direction. The uneven distribution of gap junction channels around the cell causes an anisotropic conduction, namely the longitudinal conduction velocity is nearly twice as much as the transversal one. In rabbit ventricle cells these values were obtained to 56±10 cm/s and 26±7 cm/s, respectively [65].

3.3 Modulation of gap junction channel function

Gap junction channel function can be regulated by the modification of connexin expression, rate of degradation, by phosphorylation, by localization, and by ionic and metabolic changes.

The transcription of Cx43 is controlled by homebox factors including Nxk2.5 and Irx3 [66, 67]. Posttranslational modification regulates channel assembly, trafficking, gating, internalization, and protein degradation. Phosphorylation of different residues by protein kinase C (PKC) isoforms and the multiple phosphorylation of Cx43 may lead to either enhanced or reduced cell-to-cell communication [68-72]. Dephosphorylation of connexins by protein phosphatases seems also to regulate gap junction function [73, 74]. In ischaemia, dephosphorylation of Ser^{297} and Ser^{368} causes gap junction uncoupling which can be rescued by suppression of Cx43 dephosphorylation [75, 76]. Phosphorylation of Ser^{297} and Ser^{330} induces the internalization of gap junction [75], while dephosphorylation of Ser^{356} makes the gap junction less sensitive to acidosis and increased intracellular calcium concentration [77, 78]. These data suggest that Cx43 function cannot be simply evaluated by the altered balance between phosphorylation and dephosphorylation. A reduced rate of Cx43 expression, and/or increased rate of internalization, and protein degradation can contribute to reduced cell-to-cell coupling.

Most gap junction channels are located at the cell poles; however, in ischaemia a lateral redistribution occurs. Cx43 redistributes from the cell poles to the lateral side of the cardiomyocytes [79-81]. Reduced intracellular pH promotes unhooking of Cx43 from scaffolding proteins and movement of Cx43 from intercalated disk to lateral membrane. Therefore not only the total number of gap junction channel is important for the physiological impulse propagation but also the channel distribution within the cell membrane.

Increasing concentration of intracellular calcium ion, reduced pH, and the loss of ATP is considered to be the main stimuli to cause acute reduction in gap junction conductance during ischaemia [36, 82]. After the onset of ischaemia a progressive increase in intracellular calcium concentration and decrease in pH can be observed. Gap junction conductance

decreases if the intracellular calcium concentration exceeds 320-560 nmol/l [83]. Poor coupling may lead to the development of unidirectional conduction block. Such a reduced gap junction conductance slows the impulse propagation and contributes to reentry arrhythmias.

4. The effect of gap junction on short term beat-to-beat variability

It is well known that the APD of single ventricular cells shows temporal variability. For example, guinea pig isolated ventricular cells with 20-40 ms APD changes were reported [10], while we observed 15-25 ms beat-to-beat APD changes on canine ventricular cells [unpublished data]. Action potentials recorded on multicellular ventricular preparations do not show this kind of beat-to-beat APD changes. Electrical coupling of isolated ventricular cells also reduces the alteration in the duration of consecutive action potentials [10, 13, 14].

Coupling cardiomyocytes with different intrinsic APD resulted in a common APD for both cells. When both coupled cells are excited, electrotonic current flows to delay the repolarization of the cell with short intrinsic APD, and simultaneously the loss of current accelerates the repolarization of the cell with intrinsically long APD [84]. The changes in APD are asymmetrical. Namely the cardiomyocyte with the relatively short action potential prolonged much less than the corresponding shortening in the long APD cardiomyocyte. This can be related to the changes in the membrane resistance during the action potential. The membrane resistance is larger in the course of plateau than during the action potential repolarization [10]. Therefore the same charge movement causes larger membrane potential change of plateau than along the process of repolarization due to Ohm's law. Coupling two cardiac cells with identical intrinsic action potential duration does not change APD but it reduces SBVR. The extent of SBVR reduction is larger in cases of asymmetrical cell pairs than that of symmetrical pairs [12].

In guinea pig single ventricular cells I_{Ks} blockade causes action potential prolongation, increases SBVR, and induces early afterdepolarizations (EAD). Coupling of a cell with I_{Ks} blockade to another cardiomyocyte with normal action potential repolarization eliminates the EAD readily and completely [10]. In these experiments SBVR reduced as well. This result suggests that as long as the gap junction resistance is low, a relatively high degree of junctional intercellular coupling can suppress the development and spreading of EAD [12].

The magnitude of electrotonic current flow between neighboring cells depends on the coupling resistance. Lesh *et al.* published that dispersion of action potential duration was reduced because low-resistance cellular coupling masked intercellular variability [84][10, 13]. Increases in coupling resistance or in gap junction channel resistance yields unmasking of

differences in action potential duration [12, 84]. In guinea pig ventricular cells the gap junction resistance has to be larger than 10 GOhm to achieve a complete uncoupling and to restore the intrinsic APD of the cardiomyocytes [10]. Similar results were obtained by computer simulation models. Gap junction resistance over the range of successful impulse propagation influenced neither APD nor SBVR in a one-dimensional strand [12, 85].

Under pathological conditions such as ischaemia, increased coupling resistance leads to slowed conduction and reduced magnitude of electrotonic current flow between the cells. Consequentially, reduced APD masking resulted in dispersion of APD and refractory period, thus increasing SBVR [12, 86]. Thus SBVR indicates the degree of myocardial coupling, as well [12]. APD dispersion is pronounced throughout the ischaemic area especially in the border zone [87, 88]. The large APD variability and refractory period dispersion may result in a meandering activation pathway, conduction block, reentry arrhythmias [10, 84, 89], ventricular fibrillation, and sudden cardiac death [90-93].

5. *Gap junction enhancers and inhibitors*

A number of papers support the contention that reduced function of gap junction channels often associate with ischaemia and arrhythmias [75, 94-97]. Therefore the modification of gap junction channel function can be a target of arrhythmia treatment. The important known gap junction channel enhancer (Fig 1.) and inhibitor (Fig 2.) molecules are summarized next.

5.1 Gap junction channel enhancers (Fig 1.)

Antiarrhythmic peptides (AAP)

The common antiarrhythmic agents (Class I-IV) are targeted to transmembrane ion channels or cardiac receptors [98] but during arrhythmogenesis and action potential propagation, the conductance of gap junctions can be an important factor [99]. The first reported paper of these peptides was in 1980, when Aonuma *et al*. found a natural antiarrhythmic peptide (hexapeptide, H-Gly-Pro-Hyp-Gly-Ala-Gly) in bovine atrium, which improved the rhythmicity of cultured myocardial cell clusters [100].

AAP10

AAP10 (H-Gly-Ala-Gly-Hyp-Pro-Tyr-$CONH_2$) was one of the first studied antiarrhythmic coupling peptides. AAP10 has a horseshoe-like spatial structure [101]. The electron density in

the Tyr-benzene ring, the van der Waals bonds, and intramolecular H-bonds are important to achieve the biologically active conformation, as well as proline and hydroxyproline groups. AAP10 improved both electrical and metabolic coupling on transfected HeLa cells, rat and guinea pig cardiomyocytes [98, 102, 103] in a concentration range of 50 nM – 1 μM. It was also shown that the incidence of sustained type Ib ventricular fibrillation was reduced by AAP10, with a reduction in dispersion [101]. AAP10-like rotigaptide, prevents Cx43 dephosphorylation [104] and the activity of this drug depends on activation of PKCα. The specific PKCα-inhibitor CGP54345 completely prevented its action [102]. In ischaemia the duration of the action potentials is shortened, dispersion (inhomogeneity) is increased, and homogeneity is decreased. Jozwiak and Dhein showed that ischaemia-related slowing of the activation wave propagation and increased repolarization inhomogeneities, were antagonized by AAP10 in the border zone [105].

Rotigaptide (ZP123, GAP-486)

The antiarrhythmic peptide rotigaptide (molecular formula $C_{28}H_{39}N_7O_9$, H$_2$N-Gly-D-Ala-Gly-D-4Hyp-D-Pro-D-Tyr-Ac) or formerly ZP123 (developed by Zealand Pharmaceuticals, Glostrup, Denmark) can selectively increase gap junctional conductance without affecting other ion channels. The hexapeptide rotigaptide is constructed using a retro-all-D-amino acid design of AAP10 template. The L-amino acids substituted with D-isomers are expected to protect against enzymatic degradation. Rotigaptide plasma half-life was more than 10 days compared with less than 15 min for AAP10 [106]. The same research group described that rotigaptide and AAP10 have no effect on average APD, but rotigaptide prevented the increased APD dispersion caused by hypokalemic ischaemia. Rotigaptide prevents Cx43 dephosphorylation (of Ser^{297} and Ser^{368}) in a model of global ischaemia [104]. This is important because in normal myocardium Cx43 is phosphorylated and becomes dephosphorylated during ischaemia [75]. The beat-to-beat variability of the epicardial activation pattern was stabilized by AAP10 and rotigaptide. Both peptides enhanced the homogeneity of sub-epicardial action potential duration by significantly reducing sub-epicardial dispersion [107]. The commonly used concentration of rotigaptide is 50 – 250 nM.

Danegaptide (GAP134, ZP1609)

Danegaptide (molecular formula $C_{14}H_{17}N_3O_4$, (2S,4R)-1-(2-aminoacetyl)-4-benzamidopyrrolidine-2-carboxylic acid) is an orally administered modified dipeptide that

mimicking the localization and the functional groups of AAP10 and rotigaptide at an average plasma concentration of 250 nM. Danegaptide reduces atrial fibrillation in a dog model and prevents conduction slowing in rat atrial strips [108]. Hennan *et al.* showed a robust cardioprotective effect that limited infarct size [109]. In contrast with AAP10, danegaptide decreased the dye uptake in C6 cells stably transfected with Cx43 [108]. Hence this compound may display favorable effects both at the level of gap junctions, as well as at the level of hemichannels [110].

HP-5
HP-5 (N-3-(4-hydroxyphenyl)propionyl, Pro-Hyp-Gly-Ala-Gly-OH) is a modified antiarrhythmic pentapeptide. HP-5 is a synthetic analogue that has been synthesized by altering the amino-acid sequence of AAP to produce a propionyl derivative [111]. In a rabbit ischaemia/reperfusion model the elevated dispersion of APD was reduced after HP-5 treatment and stayed unaltered during late ischaemia [112]. HP-5 reduced the dispersion of APD without altering APD and the shape of action potential, the effective refractory period, heart rate, and contractility.

5.2 Gap junction channel inhibitors (Fig 2.)
Glycyrrhetinic acid (GA)
Terpenes are a class of compounds composed of repeating 5-carbon units of hemiterpenes. Triterpenes are terpenes consisting of six isoprene units and have the molecular formula $C_{30}H_{48}$. The pentacyclic triterpenes are five-ring derivatives of dammarane having a chair-chair-chair-boat configuration and can be classified into lupane, oleanane, ursane or glycyrrhizic acid groups, and are one group of promising secondary plant metabolites [113]. GA is an oleic acid from the Liquorice of the Glycyrrhiza glabra L. Glycyrrhiza contains a saponin glycoside called glycyrrhizin, which is the calcium and potassium salt of GA. While the GA is 50 times sweeter than sucrose, upon hydrolysis, the glycoside loses its sweet taste and is converted to the aglycone GA (molecular formula $C_{30}H_{46}O_4$) plus two molecules of glucuronic acid. These agents inhibit intercellular transfer of metabolites, and this has been attributed to the inhibition of gap junctions [114]. GA has two isoforms, the 18-α-glycyrrhetinic acid (18-α-GA) and its diastereomer, the 18-β-glycyrrhetinic acid (18-β-GA) and these block gap junctions in concentrations of about 50 µM [115] and 5 µM [116], respectively. Shi *et al.* demonstrated that β$_2$-AR-mediated signal transduction is enhanced after GA treatment by changing the location of Gαs in lipid rafts [117]. Du *et al.* reported that

18-β-GA preferentially blocked the late sodium current without affecting HERG and Kv1.5 channels [118].

Carbenoxolone (CBX)

CBX (3β-hydroxy-11-oxoolean-12-en-30-oic acid 3-hemisuccinate, molecular formula $C_{34}H_{50}O_7$) is a synthetic, hemisuccinate derivative of 18-β-GA with a steroid-like structure [119]. CBX is shown to reduce fat mass, plasma triglyceride and cholesterol levels in obese rodent models acting as a non-selective inhibitor of the 11-β-hydroxysteroid dehydrogenase 1 (11-β-HSD1) [120]. It also blocks L-type calcium channels [121], pannexin hemichannels [122], and gap junctions [123]. Thus CBX is not selective to gap junctions [124]. In some experiments CBX did not alter connexin 43 hemichannel (Cx43Hc) conductance [125]. In another study CBX has been proven to disrupt hemichannel packing and aggregation *in vivo* [126], and CBX acts indirectly by intercalating into the cell membrane, altering the local lipid environment to hinder plaque formation [127].

Tetradecanoylphorbol acetate (TPA)

Phorbol esters are natural products derived from Croton tiglium, the source of croton oil, and from other plants of the family Euphorbiaceae [128]. TPA (12-Tetradecanoylphorbol 13-acetate, tumor-promoting phorbol ester, molecular formula: $C_{36}H_{56}O_8$) is the biologically most active phorbol ester, the compound had extraordinarily high potency, being active at nanomolar concentrations (10-300 nM). The myristate side chain makes the molecule highly lipophilic, and specific binding is therefore obscured by very high nonspecific uptake. Small structural changes in the molecule can markedly alter its activity. The elimination of the hydroxyl group at position 20 of phorbol led to complete loss of activity, and methylation of the hydroxyl group at position 4 led to a several hundred-fold loss in potency [129]. TPA is a specific regulator of PKC activity, therefore it could influence a wide variety of cellular processes related to gap junctions [130]. The connexin protein Cx43 is phosphorylated at Ser[368] in response to TPA-stimulated PKC activation, which could be prevented by PKC inhibitors. TPA induced internalization and degradation of Cx43 in human lens epithelial cells [131] and completely inhibited the assembly of functional gap junctions [132].

GAP26/GAP27

Warner *et al*. developed motifs that included short sequence motifs, SRPTEK in extracellular loop 1 and SHVR in extracellular loop 2, as likely potent peptides for use in disrupting cell-

cell communication. Mimetic peptides, called gap junction peptides (GAP26 and GAP27) contained amino acid sequence VCYDKSFPISHVR and SRPTEKTIFII, respectively [133, 134]. To test the efficacy of mimetic peptides, reporter dyes were designed, by which the uptake of the reporter dyes across Cx43Hc became a reliable and routine method to demonstrate open or leaky Cx43 channels [135]. GAP26 and GAP27 (molecular formula: $C_{70}H_{107}N_{19}O_{19}S$ and $C_{60}H_{101}N_{15}O_{17}$, respectively) are powerful inhibitors of these hydrophilic transmembrane pathways with little or no immediate effects on gap junctions [136]. They attenuate ACh-induced arterial relaxation and reduce potassium-mediated smooth muscle repolarization in endothelium-intact vessels *in vitro*. Wright *et al*. described a beneficial effect of GAP27 on scratch wound closure rates that correlated with decreased gap junctional intercellular communication in cultured human keratinocytes and fibroblasts [137]. The same research group showed altered susceptibility of diabetic versus non-diabetic cells to GAP27 treatment. They found an up-regulation of Ser^{368}-phosphorylation by GAP27 in diabetic cells compared to non-diabetic cells [138]. Different studies used mainly protein concentrations of 100-250 μM. Ko *et al*. demonstrated that the formation of functional gap junctions is temperature dependent, and that the truncated version of GAP27 (amino acid sequence SRPTEKTIF) that lacks the two required isoleucine residues could not inhibit dye transfer into the cell [139].

6. The possible antiarrhythmic effects of gap junction modulators

Antiarrhythmic drugs exert their effects on the ion channels responsible for cardiac action potential generation thus modifying the properties of membrane repolarization and impulse conduction. Besides their beneficial effects, antiarrhythmic drugs have proarrhythmic side effects as well. Certain diseases are known to affect gap junction channel protein expression and function causing arrhythmias [140-142]. Therefore, a novel idea is to target gap junction channels in arrhythmia treatment. This novel strategy can be straightforward only in those cases where the arrhythmia is based on reduced intercellular coupling.

The development of a gap-junction-targeted antiarrhythmic theory requires the better understanding of the contribution of these channels to the process of arrhythmogenesis. It is known that arrhythmia occurs in different phases of ischaemia [143, 144] and in these phases different processes may lead to reduced intercellular coupling. During the early phases of ischaemia closure of existing gap junction channels increase the gap junction resistance. Later the increased intercellular resistance is due to structural changes, the altered connexin/connexon synthesis/degradation and lateralization. Aside from these temporal

changes of hypoxia, spatial changes have to be taken into account as well. Ischaemia divides the heart into non-ischemic and ischemic zones. These zones can be characterized by normal, reduced, or complete uncoupling. The spatial dispersion of repolarization with slow conduction increases the susceptibility to reentry arrhythmias.

The onset of ischaemia changes intracellular calcium concentration, causes acidification, loss of ATP, and altered phosphorylation of Cx43 increase gap junction resistance. These events are associated with slow conduction, APD and refractory period anisotropy, increased SBVR, reentry, and fibrillation. APD and refractory period dispersion are pronounced in the border zone of ischaemia [145]. In the border zone, the ischemic inexcitable cells and the viable cells are not completely uncoupled. Therefore the ischemic cells electronically depress the viable cells and may form an arrhythmogenic substrate [146, 147]. At this stage of ischaemia, administration of gap junction channel enhancers seems to be beneficial. The acidosis-induced increase in APD dispersion was prevented by ZP123 administration in Langendorff-pefused guinea pig hearts. ZP123 diminished conduction velocity slowing and heterogeneous repolarization [148]. AAP10 enhanced gap junction conduction in guinea pig cardiomyocytes [149]. In a canine long QT model AAP10 altered the phosphorylation state of Cx43. AAP10 prevented the increase of transmural dispersion of repolarization, and suppressed the development of TdP arrhythmias [150].

In certain cases enhanced coupling can be a disadvantage. Under physiological circumstances the region of excited cells supplies sufficient amount of electrical charge (source) for the cells of neighboring region to depolarize them (sink). In those cases when the source to sink ratio decreases, reduction in gap junction resistance may cause conduction delays and anterograde conduction block.[151].

In ischaemia the reduced intercellular coupling has advantages, as well. Ischaemia is associated with reduced sodium current (I_{Na}) that may cause conduction block [148] whereas the structural inhomogeneity and the increased gap junction resistance restore conduction [151, 152]. On the other hand reduced intercellular coupling can limit the spread of mediators of cell death and this way it may reduce the size of infarction [153]. Administration of gap junction channel inhibitors like carbenoxolone can partly close gap junctions, preventing these channels from further uncoupling during the prolonged ischaemia to provide antiarrhythmic protection similar to preconditioning [97]. Partial uncoupling of gap junctions prior to ischaemia by ischemic-preconditioning preserves the electrical coupling of cells during a subsequent ischemic insult, indicating that a partial closure of gap junctions may play a trigger role in the protection [94, 96, 154, 155]. GAP26 and GAP27 blocks calcium-

triggered ATP release mediated by Cx43 hemichannels [156-159]. Hemichannels are not engaged to gap junctions, and they are open under several physiological and pathophysiological conditions [160]. In ischaemia uncontrolled ATP release through hemichannels may result in cellular ATP depletion. Administration of GAP26 before or after the ischaemia protected heart cells against hypoxia and reperfusion and decreased infarct size [161]. Increased hemichannel function could be a side effect of gap junction channel enhancer treatment, via greater loss of ATP [162]. GAP134 has beneficial effects. Namely GAP134 promotes intercellular coupling, enhances the conduction velocity, and simultaneously limits the cellular ATP release through hemichannels [110].

During the late phase of ischaemia structural changes can occur. Decreased quantity of Cx43, lateralization and fibrosis can be responsible for increased gap junction resistance and local slowing of conduction. The process of Cx43 synthesis, trafficking, and reduced degradation should be targeted to restore gap junction resistance. ZP123 had beneficial effects on acidosis-induced electrical uncoupling, and ZP123 increased Cx43 protein level in cultured neonatal rat ventricular myocytes after 24h [163]. This effect was due to the increased rate of Cx43 synthesis, decreased rate of degradation and phosphorylation [98, 164]. However, the computer model of Tveito *et al.* predicts that in case of a significant load of fibroblasts on the cardiomyocyte, the administration of gap junction channel enhancer ZP123 reduces conduction velocity, prone to reentrant arrhythmias and conduction block [165].

7. Conclusion

In this review we summarized the role of the gap junction channel in the development of SBVR and arrhythmias. We also reviewed the utility of gap junction enhancers and inhibitors.

Modulation of gap junction channels may be an interesting and novel strategy to treat certain types of arrhythmias such as ischaemia-induced arrhythmias. Many agents can affect gap junction resistance and various strategies should be useful to prevent arrhythmia development short and long term after the onset of ischaemia. Using known and new gap junction channel enhancers and inhibitors may also improve our understanding of the fundamental basis underpinning the pathophysiology of arrhythmogenesis.

Abbreviations
AAP, Antiarrhythmic peptides

APD, action potential duration;
CBX, Carbenoxolone
EAD, early afterdepolarization
GA, Glycyrrhetinic acid (GA)
I_{Ca-L}, L-type inward calcium current;
I_{Kr}, rapid delayed rectifier potassium current;
I_{Ks}, slow delayed rectifier potassium current;
I_{Na}, inward sodium current;
I_{to}, transient outward potassium current;
MI, Myocardial ischaemia
PKC protein kinase C
SBVR short term beat-to-beat variability of cardiac action potential duration
TdP, Torsades de Pointes
TPA, Tetradecanoylphorbol acetate

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Figures
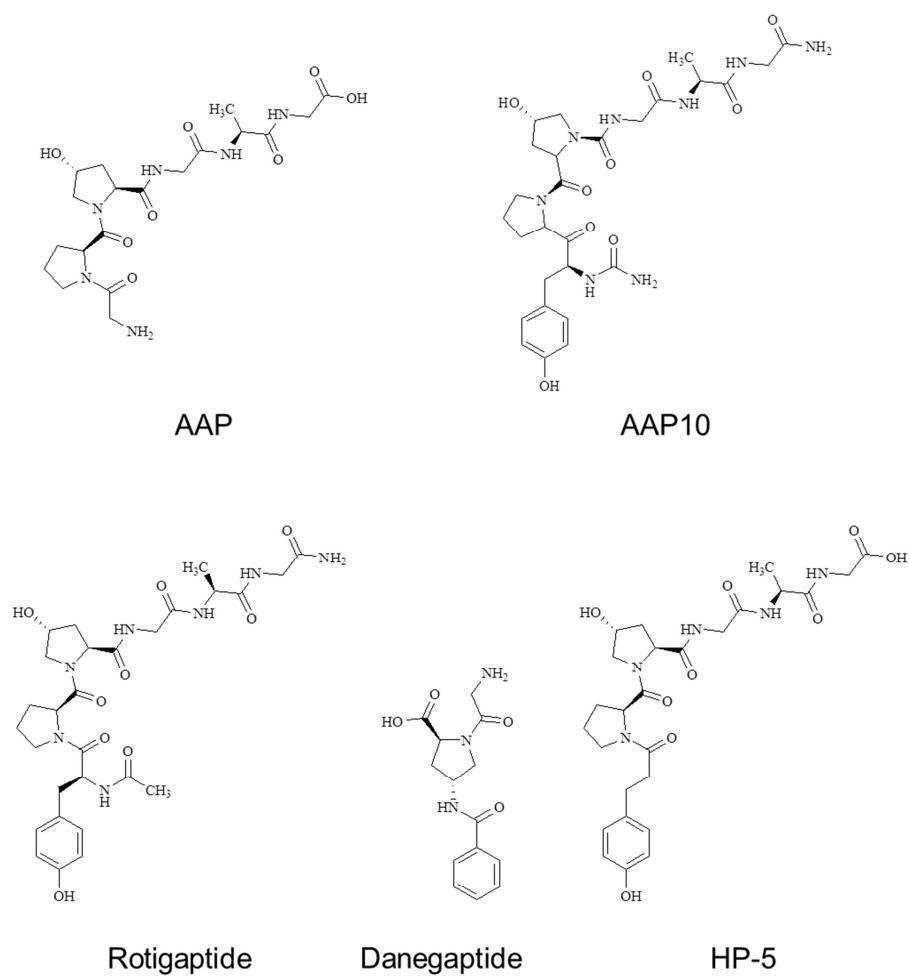

AAP AAP10

Rotigaptide Danegaptide HP-5

Fig. (1). Gap junction enhancers
Chemical structure of AAP, AAP10, Rotigaptide (ZP123), Danegaptide (GAP134) and HP-5
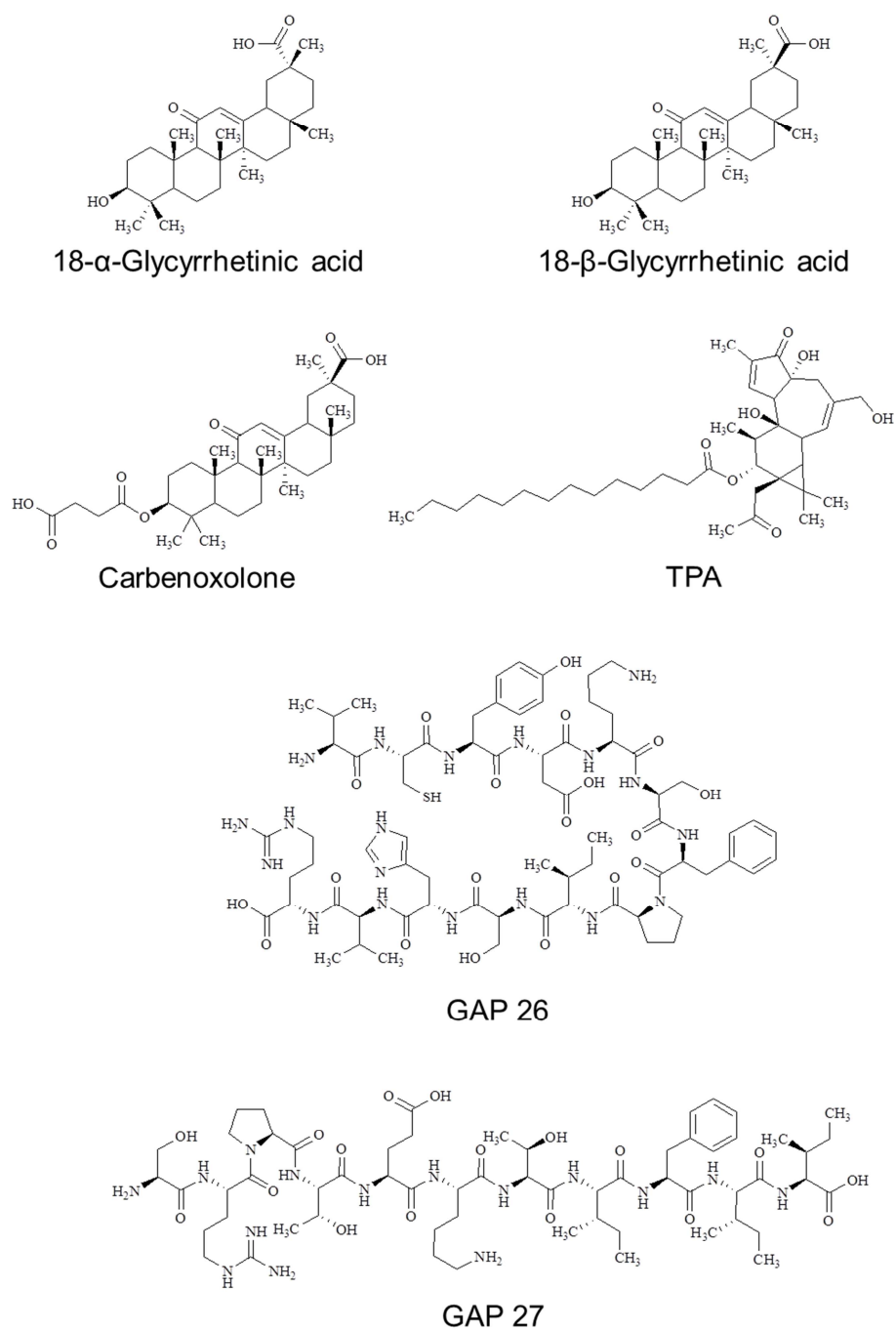(N-3-(4-hydroxyphenyl)propionyl) molecules.

Fig. (2). Gap junction inhibitors
Chemical structure of 18-α-Glycyrrhetinic acid and 18-β-Glycyrrhetinic acid, Carbenoxolone, Tetradecanoylphorbol acetate (TPA), GAP26 and GAP27 molecules.