Minireview

Myocardial gap junctions: targets for novel approaches in the prevention of life-threatening cardiac arrhythmias.

N. TRIBULOVÁ, V. KNEZL[1], Ľ. OKRUHLICOVÁ, J. SLEZÁK

Institute for Heart Research, Slovak Academy of Sciences, Bratislava

[1]Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava

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Address for correspondence:

Narcis Tribulová, PhD, DSc.

Institute for Heart Research, Slovak Academy of Sciences

840 05 Bratislava, Dubravska cesta 9, POBox 104

e-mail: narcisa.tribulova@savba.sk

Summary

Direct cell-to-cell communication in the heart is maintained via gap junction channels composed of proteins termed connexins. Connexin channels ensure molecular and electrical signals propagation and hence are crucial in myocardial synchronization and heart function. Disease-induced gap junctions remodeling and/or an impairment or even block of intercellular communication due to acute pathological conditions results in derangements of myocardial conduction and synchronization. This is critical in the development of both ventricular fibrillation, which is a major cause of sudden cardiac death and persistent atrial fibrillation, most common arrhythmia in clinical practice often resulting in stroke. Many studies suggest that alterations in topology (remodeling), expression, phosphorylation and particularly function of connexin channels due to age or disease are implicated in the development of these life-threatening arrhythmias. It seems therefore challenging to examine whether compounds that could prevent or attenuate gap junctions remodeling and connexin channels dysfunction can protect the heart against arrhythmias that cause sudden death in humans. This assumption is supported by very recent findings showing that an increase of gap junctional conductance by specific peptides can prevents atrial conduction slowing or re-entrant ventricular tachycardia in ischemic heart. Suppression of ischemia-induced dephosphorylation of connexin seems to be one of the mechanisms involved. Another approach for identifying novel treatments is based on the hypothesis that even non-antiarrhythmic drugs with antiarrhythmic ability can modulate gap junctional communication and hence attenuate arrhythmogenic substrates.

Key words:

atrial and ventricular fibrillation, gap junctions, connexin channel, therapeutic targets

Introduction

Sudden cardiac death due to ventricular fibrillation (VF) is a major health problem despite of current therapy based on implanted defibrillators (Zipes and Wellens 1998), while atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice and one of the major causes of stroke (Olson 2001). Both, AF and VF are considered to occur due to abnormal impulse formation and/or circuit movement re-entry (Gray et al. 1998, Witkowski et al. 1998, Allesie et al. 1984). Re-entry as underlying electrophysiological mechanism of cardiac fibrillation was defined as a persisting electrical impulse that reactivates an area of previously activated myocardial tissues that is no longer refractory, resulting in a circus movement of activation (Janse and Wit 1989). Nevertheless, the molecular and cellular mechanisms involved in the initiation and persistence of these re-entrant arrhythmias in humans are still not fully elucidated. Thus, further investigations are needed for managing of the arrhythmias.

As for AF, various animal models have been proposed to elucidate the factors involved in triggering and sustaining of this arrhythmia as well as to examine the efficacy of antiarrhythmic-defibrillating compounds (Wijffels et al. 1995, Gaspo et al. 1997). In principle, there are three complementary theories dealing with AF. The first one using computer model is based on multiple wavelets of reentrant impulses with short wavelength wandering trough the atria and continuously creating asynchronous electrical activity (Moe et. al., 1964). The second theory is known as "atrial fibrillation begets atrial fibrillation" (Wijffels et al. 1995). This model is based on the assumption that AF itself leads to tachycardiomyopathy and to electrical remodeling of the atria that results in shortening of wavelength, which facilitates sustaining of AF. The third approach is based on experimental and clinical studies suggesting that myocardial extensions around pulmonary veins are the most important sites for triggering of AF (Olsson 2001**),** while sustaining of this arrhythmia is

linked to a presence of proarrhythmic structural substrates (Spach and Heidlage 1995, Tribulová et al. 1999). More attention to the latter is paid in this review.

As for VF, consistent with theoretical and experimental findings elucidating this arrhythmia, proposed mechanisms focused on "rotors" (Winfree 1974) as transient, unstable object and VF was explained in terms how mother rotors break up into daughter rotors to form turbulent state seen in epicardial maps. Recent work suggests (Chen et al., 2000) that emphasis on the breakup of rotors as the driving force for VF is misplaced. It is proposed (Zaitsev et al., 2000) that the high frequency source is stable rotor, thus reviving the importance of the rotor as the organizing center for VF, with fibrillatory conduction away from the rotor playing a secondary role in the arrhythmia. Mechanisms implicated in initiation and/or maintaining VF are still controversial. Fundamental knowledge about cellular basis of this arrhythmia is essential for designing novel and more effective approaches to prevention and/or treatment, including ICD patients. In this respect cell-to-cell communication via gap junction connexin channels can play a key role as suggest numerous studies included in this article.

The general classification of cardiac arrhythmias assumed that all disturbances of rhythm result from one of two primary abnormalities in electrical activity. The first is an abnormality in impulse initiation and the second, an abnormality in impulse propagation, whereby both may co-exist (Hoffman and Rosen 1981). The former is associated particularly with triggered activity and/or abnormal automaticity, whereas the latter with block of conduction and re-entry. It was hypothesized that intercellular electrical coupling and communication mediated by gap junctions may determine conduction velocity and that alterations in gap junction distribution and/or defective cell-to-cell coupling contribute to abnormal conduction facilitating occurrence of re-entrant arrhythmias, such as AF or VF (Spach et al., 1982, Spach and Starmer 1995, Manoach and Watanabe 1995, Tribulová et al. 2001, Saffitz 1999, Axelsen et al. 2007, Fialová et al. 2007a).

Taking into account that functional cell-to-cell coupling and communication via gap junction connexin channels is crucial for rapid electrical signal propagation and cardiac muscle synchronization, in turn, disorders in cell-to-cell coupling and communication may be a key factor in arrhythmogenesis. In this article we focus, therefore, on gap junction and connexin channels alterations that may underlie proarrhythmia substrates implicated in the development of atrial or ventricular fibrillation in various experimental conditions as well as clinical settings.

Cardiac gap junctions: structure, composition and distribution.

The subcellular structures responsible for myocardial electrical current flow propagation from one cardiac cell to another are specialized connections termed gap junctions (nexuses). There are two types, i.e. end-to-end and side-to-side junctions (Fig. 1A and Fig 1B). End-to-end type is a part of intercalated disc and it predominates in the cardiac muscle. Lateral gap junctions are much less abundant and occur more often in atrial than ventricular tissues. Conventional patterns of gap junction distribution that can be revealed by immuno-labeling (Fig. 1C) underlie uniform anisotropic impulse propagation throughout myocardium (Joyner 1982, Spach and Heidlage 1995).

Gap junctions that link adjoining cardiomyocytes ensure cell-to-cell electrical coupling and direct communication via clusters of intercellular channels (connexons) composed of proteins, termed connexins (Fig. 2 A, B). The heart expresses several connexin isoforms that differ in conductance, whereby connexin-43 (Cx43) predominates in ventricular as well as atrial tissues (Dhein 1998). Besides, Cx40 is abundant in the latter. The half-life of connexins in the adult rat heart is surprisingly short, only about 1.3 hours (Goodenough et al. 1996). The cardiac connexins are phosphoproteins and changes in phosphorylation have been implicated in the regulation of connexin turnover kinetics (Laird 1996). Phosphorylation also appears to play a key role in channel gating that determines channel conductance (Lampe and Guarneri

1993). Expression and/or gating of connexin channels have been shown modulated by various endogenous and exogenous compounds, e.g. hormones, growth factors, eicosanoids, narcotics, protein kinases as well as by abnormal elevation of intracellular ions, such as Na^+, Ca^{2+} and H^+ (see for details ref. Salameh and Dhein 2005).

An effective pump function of the heart requires electrical activation of the myocardium in a specific temporal and spatial pattern and this process is depending in large part on the distribution and function of gap junctions. Accordingly, gap junction connexin channels are crucial in direct intercellular communication and myocardial synchronization. Thus, it is very likely that reduced number and/or disturbed myocardial distribution of gap junctions as well as impairment and/or dysfunction of connexin channels can promote cardiac arrhythmias, including AV and VF. Indeed, numerous studies support this assumption.

Cardiac gap junction and Cx43 alterations implicated in occurrence of AF.

In humans incidence of AF is known to increase in aged or cardiomyopatic heart. These conditions are characterized by myocardial structural remodeling since the heart changes its structure and function in response to aging, disease or injury. Thus, it alters the structure of the cardiomyocytes and extracellular matrix by activating intracellular signaling cascades. Consequently it results in myocardial hypertrophy and/or fibrosis (Spach and Heidlage 1995, Tribulová et al. 1999, Janse and DeBakker 2001, Mukherjee et al. 2006). Importantly, myocardial structural remodeling is accompanied with gap junction remodeling (Saffitz et al., 1999, Tribulová et al. 1999, van der Velden et al. 2000, Kostin et al. 2001, Severs 2001, Tribulová et al. 2002, 2002a) that may be triggered by changes in signaling molecules in response to overall remodeling characteristics (Teunissen et al. 2004). In general, remodeling of gap junctions, i.e. changes in topology of connexin channels, is linked with electrical remodeling contributing to conduction alterations (Papageorgiou et al. 1996, Spach et al. 1982, Wijffels et al. 1995), hence to be considered as an arrhythmogenic substrate.

In agreement with it, it has been shown that burst pacing of guinea pig atria in isolated heart preparation resulted in prolonged AF or fibrillo-flutter in old guinea pigs (Fig. 3) exhibiting pronounced changes in extracellular matrix composition and less gap junction profiles (Fig. 4B), while not in young characterized by normal ultrastructure and numerous gap junctions (Fig. 4A). In parallel, Cx43 expression was significantly decreased in the atria of old comparing to young guinea pigs (Tribulová et al. 1999, Tribulová et al 2002a). Likewise Cx40-deficient mice exhibited high atrial vulnerability to fast pacing-induced tachyarrhythmias (Hagendorff et al. 1999). Reduction in Cx40 was detected in human with chronic AF (Polontchouk et al. 2001) as well as in chronic model of pacing-induced sustained AF in goats (van der Velden et al. 2000). It indicates that down-regulation of connexins may also contribute to persistence of AF.

It should be stressed that aging and cardiovascular diseases are, in addition to structural and gap junction remodeling, characterized by abnormal Ca^{2+} handling (Dhalla 1988, Jiang et al., 1993, Lakatta and Guarneri 1993). Accordingly, it was hypothesized that aged or diseased heart due to altered Ca^{2+} handling is less able to maintain Ca^{2+} homeostasis, while prone to develop Ca^{2+}-overload. The latter leads to subcellular injury and particularly to impairment of cell-to-cell coupling (or even uncoupling) at the gap junction channels (deMello 1986, Salameh and Dhein 2005). Consequently it can facilitate occurrence of malignant reentry arrhythmias (Merrilat et al. 1990, Kihara and Morgan 1991, Tribulová et al. 2002, Tribulová et al. 2003) and prevent their termination and sinus rhythm restoration (Manoach et al. 2007). Fast pacing-induced elevation of intracellular Na^{+} (Simor et al. 1997) and consequent activation of Na/Ca exchanger can result in disturbances of Ca^{2+} homeostasis. Indeed, Ca^{2+} overload has been indicated by presence of hypercontracted sarcomeres and dehiscence of fascia adherens junctions upon intermittent burst pacing (Fig. 4C). Notably, these subcellular changes occurred early (after 5-10 minutes of burst pacing) and in majority of cardiomyocytes

of old guinea pig atria. Unlike to old, young guinea pigs developed high Ca^{2+} -induced injury in minor population of cardiomyocytes and upon prolonged (>1 hour) fast pacing. In addition, immunolabeling revealed that diminished expression and inhomogeneous myocardial distribution of Cx43 in old guinea pig myocardium aggravated due to burst pacing prior occurrence of AF (Tribulová et al. 1999, Tribulová et al. 2002a). These findings suggest acute impairment of cell-to-cell coupling due to, at least in part, pacing-induced Ca^{2+} elevation. Interestingly, pretreatment of perfused heart with heptanol, a compound that deteriorates electrical coupling and induces cell-to-cell uncoupling, promoted inducible AF in aged rat model (Hayachi et al. 1998).

Cardiac gap junction and Cx43 alterations implicated in occurrence of VF.

Experimental and clinical studies showed that diseased or failing heart is prone to develop VF, which is main cause of sudden cardiac death particularly during heart attack. Slow conduction and fractionated electrograms were recorded in the infarcted human heart (De Bakker et al. 1993, Gardner et al. 1985). Myocardial tissue analysis revealed pathological substrates, including a decrease and miss-localization of gap junctions, which correlated with location of reentrant circuits in the border zone of infarction (Peters et al. 1997). Interestingly, Cx43-deficient mice exhibited markedly slowing of myocardial conduction that facilitates re-entrant arrhythmias and sudden arrhythmic death (Lerner et al. 2000, Gutstein et al. 2001). Down-regulation and/or abnormal distribution of myocardial Cx43-positive gap junctions linked with increased susceptibility to hypokalemia-induced ventricular fibrillation (Fig. 5) have been reported in rats suffering from diabetes mellitus (Okruhlicová et al. 2002) spontaneous or L-NAME-induced hypertension (Tribulová et al. 2002, Tribulová et al. 2003, Fialová et al. 2007a). While diabetes was associated with myocardial fibrosis, young spontaneously hypertensive rats were characterized by left ventricular hypertrophy and L-NAME-induced hypertension was accompanied by both hypertrophy and fibrosis of the heart

(Tribulová et al. 2002a). Reduced gap junction coupling in areas of fibrosis could disrupt wave-front propagation and hence interfere with uniform and synchronized cardiomyocyte function. Likewise, abnormal distribution and lateralization of gap junctions in hypertrophied myocardium can affect uniform anisotropic conduction (Spach et al. 2000). There are changes in electrical properties in the early stage of left ventricular hypertrophy before arrhythmia occurrence (Bacharová 2007). It would be interesting to know alterations in Cx43 expression in early juvenile period of SHR, which is critical for hypertension (Zicha et al. 2006). It appears that structural and gap junction remodeling associated with electrophysiological remodeling (Severs 2001, Teunisset et a. 2004) can create arrhythmogenic substrate for triggering and sustaining of cardiac arrhythmias. Myocardial gap junction remodeling linked with increased vulnerability to VF was detected also in hypertriglyceridemic rats (Tribulová et al. 2007), an experimental model of metabolic syndrome (Zicha et al. 2006a).

Perfusion of the heart with K^+ deficient solution leads to an increase in intracellular Ca^{2+} concentration (Fig. 6A). Diabetic or hypertensive rat hearts with abnormal Ca^{2+} handling (Dhalla et al. 1988, Balke and Shorofsky 1998) were much prone to develop high Ca^{2+}-induced ventricular premature beats, compared to controls (Tribulová et al. 2003). Furthermore, acute Ca^{2+} overload may contribute to disturbances in coordinated contraction likely due to cell-to-cell uncoupling (de Groot and Coronel 2004). The latter was indicated according non-uniform sarcomere patterns of adjacent cardiomyocytes connected by gap junctions (Fig. 7), i.e., relaxed sarcomeres in one versus contracted in neighboring (Fig. 7B). Total cell-to-cell uncoupling was indicated when two neighboring cardiomyocytes differ in structural appearance, i.e. one with negligible while another with irreversible injury (Fig. 7C). In such case, the uncoupling prevents propagation of “injury current” (de Mello 1989). Myocardial inhomogeneities in Cx43 distribution including patchy areas with abolished immunostaining (Fig. 8) as well as suppression of Cx43 phosphorylation (Tribulová et al.

2003) strongly suggested cell-to-cell coupling disorders. Similarly, electrical uncoupling due to acute ischemia-induced dephosphorylation of Cx43 (de Groot and Coronel 2004) and its intracellular ventricular redistribution (Beardslee et al. 2000) were observed. Suppression of phosphorylated isoforms of Cx43 in spontaneously hypertensive rat hearts (Fig. 9A) (Tribulová et al. 2003) and young (Fig.9C) (Lin et al. to be published) unlike to old thyroid hormone-treated rats (Fig. 9B) (Tribulová et al. 2005) was associated with higher vulnerability of the former to hypokalemia-induced VF (Tribulová et al. 2004). Thus, defects in Cx43 expression, phosphorylation and channel function, particularly when heterogeneously distributed throughout myocardium renders the heart prone to VF.

How to prevent AF and VF by targeting cardiac gap junctions?

Potential mechanisms controlling the level of intercellular communication in the heart include regulation of connexin turnover (synthesis and degradation), cellular distribution and phosphorylation. There are numerous data showing that some compounds can up-regulate Cx-43 via modulation either synthesis or degradation and enhance gap junctional communication (see review Salameh and Dhein 2005). In contrast, much less information is about causal relationship between the Cx-43 up-regulation and decreased susceptibility to re-entry arrhythmias as well as whether attenuation or regression of gap junctions remodeling will abolish arrhythmogenic substrate resulting in lower arrhythmia incidence. Nevertheless, very recent studies showed (Haugan et al. 2005, Axelsen et al. 2007) that an increase of gap junctional conductance by specific peptide, rotigaptide, can prevent atrial conduction slowing or re-entrant ventricular tachycardia in ischemic heart. Suppression of ischemia-induced dephosphorylation of connexin seems to be one of the mechanisms involved. This mechanism was thought to be involved in the antiarrhythmic effects of ischemic preconditioning (Schulz et al. 2007)**.** It seems that modulation Cx43 channels function via phospohorylation, which is often linked with intracellular ATP levels (Turner et al 2004) may be powerful tool to prevent

cell-to-cell uncoupling and to enhance gap junctional communication. Similarly, in isolated heart model the abolishment of Ca^{2+} overload, which was facilitated by stobadine, resulted in termination of VF and sinus rhythm restoration (Fig. 6B), most likely due to restoration of intercellular coupling (Tribulová et al. 2001). This assumption is supported by the findings of others (Dekker et al. 1999, Merrilat et al. 1990). Furthermore clinical studies indicate that compounds or interventions preventing or attenuating disease-related structural remodeling protect against atrial or ventricular fibrillation (Hanna et al. 2006, Wachtell et al. 2006). While experimental studies showed significant antifibrillating effects of lipid lowering compounds (atorvastatin) and omega-3 fatty acids in hypertriglyceridemic and aged male and female SHR rats despite structural and gap junction remodeling was not eliminated (Fialová et al. 2007, Tribulová et al. 2007). However, expression and phosphorylation of Cx43 was increased. It is important to note that the increased intrinsic level of myocardial Cx43 expression in either healthy or hypertensive female than to male rat hearts (Tribulová et al. 2005, Fialová et al. 2007) is very likely implicated in their decreased susceptibility to VF compared to male (Mitošíková et al to be published).

Conclusions and perspectives

It is very interesting that despite of various etiologies (aging, diabetes, hypertension, hyperthyroidism) and triggering factors (fast burst pacing, ischemia or hypokalemia) a similar features of myocardial subcellular and Cx43 changes have been detected prior occurrence of either atrial or ventricular fibrillation in experimental conditions. The findings suggest that 1/ the structural substrates for re-entry mechanism underlying cardiac fibrillations appear to be remodeling of both myocardial architecture (hypertrophy, fibrosis) and gap junctions (abnormal distribution, decreased number); 2/ occurrence of atrial or ventricular fibrillation is triggered by acute events, which induce sudden disturbances in electrolyte homeostasis, Ca^{2+} overload and Cx43 dephosphorylation deteriorating (or even block) of cell-to-cell cell

coupling and communication; 3/ heterogeneous or focal myocardial distribution of both pre-existing gap junction abnormalities due to age or disease and sudden connexin channels dysfunction due to acute pathophysiological conditions can significantly contribute to myocardial electrical instability rendering the heart prone to malignant arrhythmias.

Taking into account all above mentioned factors and a key role of the gap junction connexin channels in the cardiac arrhythmogenesis, it appears that aimed modulation of intercellular communication to prevent spatial electrical heterogeneities in viable myocardium, is promising way to fight life-threatening arrhythmias and sudden death in human. Nevertheless, more detailed analysis and further studies are needed to address this issue looking for innovative therapeutic approaches.

Conflict of Interest

There is no conflict of interest.

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References

AXELSEN AN, HAUGAN K, STAHLHUT M, KJØLBYE, HENNAN JK, HOLSTEIN-RATHLOU NH, PETERSEN JS, NIELSEN MS: Increasing gap junctional coupling: A tool for dissecting the role of gap. *J Membrane Biol* **216:** 23-35, 2007.

ALLESIE MA, LAMMERS WJEP, BONKE IM, HOLLEN J: Intra-atrial reentry as a mechanism for atrial flutter induced by acetylcholine and rapid pacing in dog. *Circulation* **70**: 123-135, 1984.

BACHAROVÁ L: Electrical and structural remodeling in left ventricular hypertrophy- a substrate for a decrease in QRS voltage? *A.N.E.* **12**: 260-273, 2007.

BALKE CW, SHOROFSKY SR: Alterations in calcium handling in cardiac hypertrophy and heart failure. *Cardiovasc Res* **37**, 290-299, 1998.

BEARDSLEE MA, LERNER DL, TADROS PN, LAING JG, BEYER EC, YAMADA KA, KLÉBER AG, SCHUESSLER RB, SAFFITZ JE: Dephosphorylation and intracellular redistribution of ventricular connexin43 during electrical uncoupling induced by ischemia. *Circ Res* **87**: 656-662, 2000.

CHEN J, MANDAPATI R, BERENFELD O, SKANES AC, JALIFE J: High frequency periodic sources underlie ventricular fibrillation in the isolated rabbit heart. *Circ Res* **86**: 86-93, 2000.

DE BAKER MJT, CAPELLE JL, JANSE MJ, TASSERON S, VERMEULEN JE, DE JONGE N, LAHPOR JR: Slow conduction in the infarcted human heart. "Zigzag" course of activation. *Circulation* **88:** 915-926, 1993.

DE GROOT JR, CORONEL R: Acute ischemia-induced gap junctional uncoupling and arrhythmogenesis. *Cardiovasc Res* **62**: 323-334, 2004.

DEKKER LRC, CORONEL R, VAN BAVEL E, SPAAN AE, OPTHOF T: Intracellular Ca^{2+} and delay of ischemia-induced electrical uncoupling in preconditioned rabbit ventricular myocardium. *Cardiovasc Res* **44:** 101-102, 1999.

DE MELLO WC: Interaction of cyclic AMP and Ca^{2+} in the control of electrical coupling in the heart fibers. *Bioch Biophys Acta* **888**: 91-99, 1986.

De MELLO WC: Cell coupling and healing-over in cardiac muscle. In: *Physiology and pathophysiology of the heart*. SPERELAKIS N (ed). Kluwer Academic Publisher 541-549, 1989,

DHALLA NS, LIU X, PANAGIA V, TAKEDA N: Subcellular remodeling and heart dysfunction in chronic diabetes. *Cardiovasc Res* **40**: 239-247, 1988.

DHEIN S: Gap junction channels in the cardiovascular system: pharmacological and physiological modulation. *Trends Pharm Sci* **19**: 229-241, 1998.

FIALOVÁ M, DLUGOSOVÁ K, KNEZL V, OKRUHLICOVÁ L, DŘÍMAL J, TRIBULOVÁ N. Omega-3 fatty acids protect male and female aged hypertensive rat heart againts ventricular fibrillation. *Acta Physiologica* **19**, supp.658: 88, 2007.

FIALOVÁ M, DLUGOŠOVÁ, OKRUHLICOVÁ L, KRISTEK F, MANOACH M, TRIBULOVÁ N. Adaptation of the heart to hypertension is associated with maladaptive gap junction connexin-43 remodelling. *Physiol Res* in press (2007a).

GARDNER PI, URSEL PC, FENOGLIO JJ, WIT AL: Electrophysiologic and anatomic basis for fractionated electrograms recorded from healed myocardial infarcts. *Circulation* **72**: 596-611, 1985.

GASPO R, BOSH RF, TALAJIC M, NATTEL S: Functional mechanisms underlying tachycardia-induced sustained atrial fibrillation in a chronic dog model. *Circulation* **96**: 4027-4035, 1997.

GOODENOUGH DA, GOLIGER JA, PAUL DL: Connexin, connexons and intercellular communication. *Annu Rev Biochem* **65:** 475-502, 1996.

GRAY RA, PERTSOV AM, JALIFE J: Spatial and temporal organization during cardiac fibrillation. *Nature* **392**: 75-78, 1999.

GUTSTEIN DE, MORLEY GE, TAMADONN H, VAIDYA D, SCHNEIDER MD, CHE J: Conduction slowing and sudden arrhythmic death in mice with cardiac-restricted inactivation of connexin43. *Circ Res* **88:**

HAGENDORFF A, SCHUMACHER B, KIRCHHOFF S, LUDERITZ B, WILLECKE K: Conduction disturbances and increased atrial vulnerability in Connexin40- deficient mice analyzed by transesophageal stimulation. *Circulation* **99**: 1508-1515, 1999.

HANNA IR, HEEKE B, BUSH H, BROSIUS L, KING-HAGMAN D, DUDLEY SC Jr, BESHAI JF, LANGBERG JJ: Lipid-lowering drug use is associated with reduced prevalence of atrial fibrillation in patients with left ventricular systolic dysfunction. *Heart Rhythm* **3**: 881-6, 2006.

HAUGAN K, OLSEN KB, HARTVIG L, PETERSEN JS, HOLSTEIN_RATHLOU NH, HENNAN JK, NIELSEN MS: The antiarrhythmic peptide analog ZP123 prevents atrial conduction slowing during metabolic stress. *J Cardiovsc Electrophysiol* **16:** 537-545, 2005.

HAYACHI H, WANG Ch, MYIAUCHI Y, OMICHI Ch, PAK HN, ZHOU S, OHARA T, MANDEL WJ, LIN SF, FISHBEIN MC, CHEN PS, KARAGUEUZIAN HS: Aging-related increase to inducible atrial fibrillation in the rat model. *J Cardiovasc Electrophysiol* **13**: 801-808, 2002.

HOFFMAN BF, ROSEN MR: Cellular mechanisms for cardiac arrhythmias. *Circ Res* **49**: 1-15, 1981.

JANSE MJ, DE BAKKER JMT: Arrhythmia substrate and management in hypertrophic cardiomyopathy: from molecules to implantable cardioverter-defibrillators. *Europ Heart J* **3**: 15-20, 2001.

JIANG MT, MOFFAT MP, NARAYAN N: Age-related alterations in the phosphorylation of sarcoplasmic reticulum and myofibrillar proteins and diminished contractile response to isoproterenol in intact rat ventricle. *Circ Res* **72**: 102-111, 1993.

JOYNER RW: Effect of the discrete pattern of electrical coupling on propagation through an electrical syncytium. *Circ Res* **50**: 192-200, 1982.

KIHARA Y, MORGAN JP: Intracellular calcium and ventricular fibrillation. *Circ Res* **68**: 1378-1389, 1991.

KOSTIN S, KLEIN G, SZALAY Z, HEIN S, BAUER EP, SCHAPER J: Structural correlate of atrial fibrillation in human patients. *Cardiovasc Res* **54**: 361-379, 2002.

LAIRD DW: The life cycle of a connexin: gap junction formation, removal and degradation. *J Bioenerg Biomembr* **28**: 311-317, 1996.

LAKATTA EG, GUARNIERI T: Spontaneous myocardial calcium oscillations: are they linked to ventricular fibrillation? *J Cardiovasc Electrophysiol* **l 4**: 473-489, 1993.

LAMPE PD, LAU AF: Regulation of gap junctions by phosphorylation of connexins. *Arch Biochem Biophys* **384**: 205-215, 2000.

LERNER DL, YAMADA KA, SCHUESSLER RB, SAFFITZ JF: Accelerated onset and increased incidence of ventricular arrhythmias induced by ischemia in Cx43-deficient mice. *Circulation* **101**: 547-552, 2000.

MANOACH M, WATANABE Y: How can we facilitate spontaneous termination of ventricular fibrillation and prevent sudden cardiac death? *J Cardiovasc Electrophysiol* **6**: 584-590, 1995.

MANOACH M, TRIBULOVÁ N, VOGELEZANG D, THOMAS S, PODZUWEIT T: Transient ventricular fibrillation and myosin heavy chain isoform profile. *J Cell Mol Med* **11:** 171-174, 2007.

MERRILAT JC, LAKATTA EG, HANO O, GUARNIERI T: Role of calcium and the calcium channel in the initiation and maintenance of ventricular fibrillation. *Circ Res* **67**: 115-1123, 1990.

MUKHERJEER, HERRON AR, LOWRY AS, STROUD RE, STROUD MR, WHARTON JM, IKONOMIDIS JS, CRUMBLEY AJ, SPINALE FG, GOLD MR: Selective induction of matrix metalloproteinases and tissue inhibitor of metalloproteinases in atrial and ventricular myocardium in patients with atrial fibrillation. *Am J Cardiol* **97**: 532-537, 2006.

OKRUHLICOVÁ Ľ, TRIBULOVÁ N, MIŠEJKOVÁ M, KUKUČKA M, ŠTETKA R, SLEZÁK J, MANOACH M: Gap junction remodelling is involved in the susceptibility of

diabetic rats to hypokalemia-induced ventricular fibrillation. *Acta Histochemica* **104**: 387-391, 2002.

OLSSON SB: Atrial fibrillation – where we stand today? *J Int Med* **250**: 19-28, 2001.

PAPAGEORGIOU P, MONAHAN K, BOYLE NG, SEIFERT MJ, BESWICK P, ZEBEDE J, EPSTEIN LM, JOSEPHSON ME: Site-dependent intra-atrial conduction delay. *Circulation* **94**: 384-389, 1996.

PETERS NS, COROMILAS J, SEVERS NJ, WIT AL: Disturbed connexin 43 gap junction distribution correlates with location of reentrant circuits in the epicardial border zone of healing canine infarcts that cause ventricular tachycardia. *Circulation* **95**: 988-996, 1997.

POLONTCHOUK L, HAEFLIGER JA, EBELT B, SCHAEFER T, STUHLMANN D, MEHLHORN U, KUHN-REGNIER F, DE VIVIE ER, DHEIN S: Effects of chronic atrial fibrillation on gap junction distribution in human and rat atria. *J Am Coll Cardiol* **38**: 883-891, 2001.

SAFFITZ JE, SCHUESSLER RB, YAMADA KA: Mechanisms of remodelling of gap junction distribution and the development of anatomic substrates of arrhythmias. *Cardiovas Res* **42:** 309-317, 1999.

SALAMEH A, DHEIN S: Pharmacology of gap junctions. New pharmacological targets for treatment of arrhythmia, seizure and cancer? *Biochim Biophys Acta* **1719**: 36-58, 2005.

SEVERS NJ: Gap junction remodelling and cardiac arrhythmogenesis: cause or coincidence? J Cell Mol Med **5:** 355-366, 2001.

SCHULZ R, BOENGLER K, TOTZECK A, LUO Y, GARCIA-DORADO D, HEUSCH G: Connexin 43 in ischemic pre- and postconditioning. *Heart Fail Rev***12:**261-266, 2007.

SIMOR T, LORAND T, GASZNER B, ELGAVISH GA: The modulation of pacing-induced changes in intracellular sodium levels by extracellular Ca in isolated perfused rat hearts. *J Mol Cell Cardiol* **29**: 1225-1235, 1997.

SPACH MS, HEIDLAGE JF: The stochastic nature of cardiac propagation at a microscopic level. An electrical description of myocardial architecture and its application to conduction. *Circ Res* **76**: 366-380, 1995.

SPACH MS, HEIDLAGE JF, DOLBER PC, BARR RC: Electrophysiological effects of remodeling cardiac gap junctions and cell size. *Circ Res* **86**: 302-311, 2000.

SPACH MS, KOOTSEY JM, SLOAN JD: Active modulation of electrical coupling between cardiac cells of the dog. A mechanism for transient and steady variations in conduction velocity. *Circ Res* **51**: 347-362, 1982.

SPACH MS, STARMER CF: Altering the topology of gap junctions a major therapeutic target for atrial fibrillation. *Cardiovasc Res* **30**: 336-344, 1995.

TEUNISSEN BE, JONGSMA HJ, BIERHUIZEN MF: Regulation of myocardial connexins during hypertrophic remodeling. *Eur Heart J* **25:** 1979-89, 2004.

TRIBULOVÁ N, DUPONT E, SOUKUP T, OKRUHLICOVÁ L, SEVERS NJ: Sex differences in connexin-43 expression in left ventricles of aging rats. *Physiol Res* **54**: 705-708, 2005.

TRIBULOVÁ N, FIALOVÁ M, DLUGOŠOVÁ K, KNEZL V, OKRUHLICOVÁ L, KRISTEK F, ZICHA J, KUNEŠ J. Mzocardial gap junction remodelling in hypetriglyceridemic rat heart is associated with increased vulnerability to ventricular fibrillation. *Cardiol* **15** (Suppl.1): 32-33S, 2006.

TRIBULOVÁ N, KNEZL V, OKRUHLICOVÁ Ľ, DŘÍMAL J, LAMOŠOVÁ D, SLEZÁK J, STYK J: L-thyroxine increases susceptibility of young rats to low K^+ -induced venticular fibrillation while facilitates sinus rhythm restoration in old one. *Exper Physiol* **89**: 629-636, 2004.

TRIBULOVÁ N, MANOACH M, VARON D, OKRUHLICOVÁ Ľ, ZINMAN T, SHAINBERG A: Dispersion of cell-to-cell uncoupling precedes of low K^+-induced ventricular fibrillation. *Physiol Res* **50**: 247-259, 2001.

TRIBULOVÁ N, OKRUHLICOVÁ Ľ, IMANAGA I, HIROSAVA N, OGAWA K, WEISMANN P: Factors involved in the susceptibility of spontaneously hypertensive rats to low K^+- induced arrhythmias. *Gen Physiol Biophys* **22**: 369-382, 2003.

TRIBULOVÁ N, OKRUHLICOVÁ Ľ, NOVÁKOVÁ S, PANCZA D, BERNÁTOVÁ I, PECHANOVÁ O, WEISMANN P, MANOACH M, SEKI S, MOCHIZUKI M: Hypertension-related intermyocyte junction remodeling is associated with higher incidence of low K^+- induced lethal arrhythmias in isolated rat heart. *Exp Physiol* **87**: 195-205, 2002.

TRIBULOVÁ N**,** OKRUHLICOVÁ Ľ, VARON D, MANOACH M, PECHANOVÁ O, BERNÁTOVÁ I, WEISMANN P, BARANČÍK M, STYK J, SLEZÁK J: Structural substrates involved in the development of severe arrhythmias in hypertensive rat and aged guinea pig hearts. In: *Cardiac Remodeling and Failure,* SINGAL P, DIXON I, KIRSCHENBAUM L, DHALLA NS (Eds): Kluwer Academic Publishers, Boston, USA, pp. 377-398, 2002a.

TRIBULOVÁ N, SEKI S, MANOACH M, TAKEDA H, OKRUHLICOVÁ Ľ, MOCHIZUKI S: Restoration of basal cytoplasmic Ca^{2+} and recovery of intermyocyte coupling precede stobadine-induced ventricular defibrillation in whole heart preparation. *Europ Heart J* **22**: A547, 2001a.

TRIBULOVÁ N, VARON D, POLACK-CHARCON S, BUSCEMI P, SLEZÁK J, MANOACH M: Aged heart as a model for prolonged atrial fibrilo-flutter. *Exp Clin Cardiol* **4**: 64-72, 1999.

TURNER MS, HAYWOOD GA, ANDREKA P, YOU L, MARTIN PE, EVANS H, WEBSTER KA, BISHOPRIC NH: Reversible connexin 43 dephospohorylation during hypoxia and reoxygenation is linked to cellular ATP levels. *Circ Res* **95:** 726-733, 2004.

VAN DER VELDEN HMW, AUSMA J, ROOK MB, HELLEMONS AJCGM, VAN VEEN TAAB, ALLESSIE MA, JONGSMA HJ: Gap junctional remodeling in relation to stabilization of atrial fibrillation in the goat. *Cardiovasc Res* **46**: 476-486, 2000.

WACHTELL K, DEVEREUX RB, LYLE PA: Atrial fibrillation and renin-angiotensin system. *Nippon Rinsho* **65**: 569-74, 2007.

WANG YG, HUSER J, BLATTER LA, LIPSIUS S: Withdrawal of acetylcholine elicits Ca-induced delayed afterdepolarizations in cat atrial myocytes. *Circulation* **96**: 1275-1281, 1997.

WIJFFELS MCEF, KIRCHHOFF CHJHJ, DORLAND R, ALLESIE A: "AF begets AF" a study in awaked chronically instrumented goats. *Circulation* **92**: 1954-1968, 1995.

WINFREE AT: Rotating solutions to reaction diffusion equations in simply connected media. *SIAM-AMS Proc* **8**: 13-31, 1974.

WITKOWSKI FX, LEON LJ, PENKOSKE PA, GILES WR, SPANO ML, DITTO WL, WINTRE AT: Spatiotemporal evolution of ventricular fibrillation. *Nature* **392**: 78-82, 1998.

ZAITSEV AV, BERENFELD O, MIRONOV SF, JALIFE P, PERTSOV AM: Distribution of excitation frequences on the epicardial and endocardial surfaces of fibrillating ventricular wall of the ship heart. *Circ Res* **86**: 408-417, 2000.

ZICHA L, DOBEŠOVÁ Z, KUNEŠ J: Late effects of early intervention: transient captopril treatment of SHR in juvenile critical period for hypertension. *Phys Res* **55**: 5P, 2006.

ZICHA L, PECHÁŇOVÁ O, ČAČÁNYIOVÁ S, CEBOVÁ M, KRISTEK F, TŐRŐK J, IMKO F, DOBEŠOVÁ Z, KUNEŠ J: Hereditary hypertriglyceridemic rat: A suitable model of cardiovascular disease and metabolic syndrome? *Phys Res* **55**: 49-63, 2006.

ZIPES DP, Wellens HJJ: Sudden cardiac death. *Circulation* **98:** 2334-2351, 1998.

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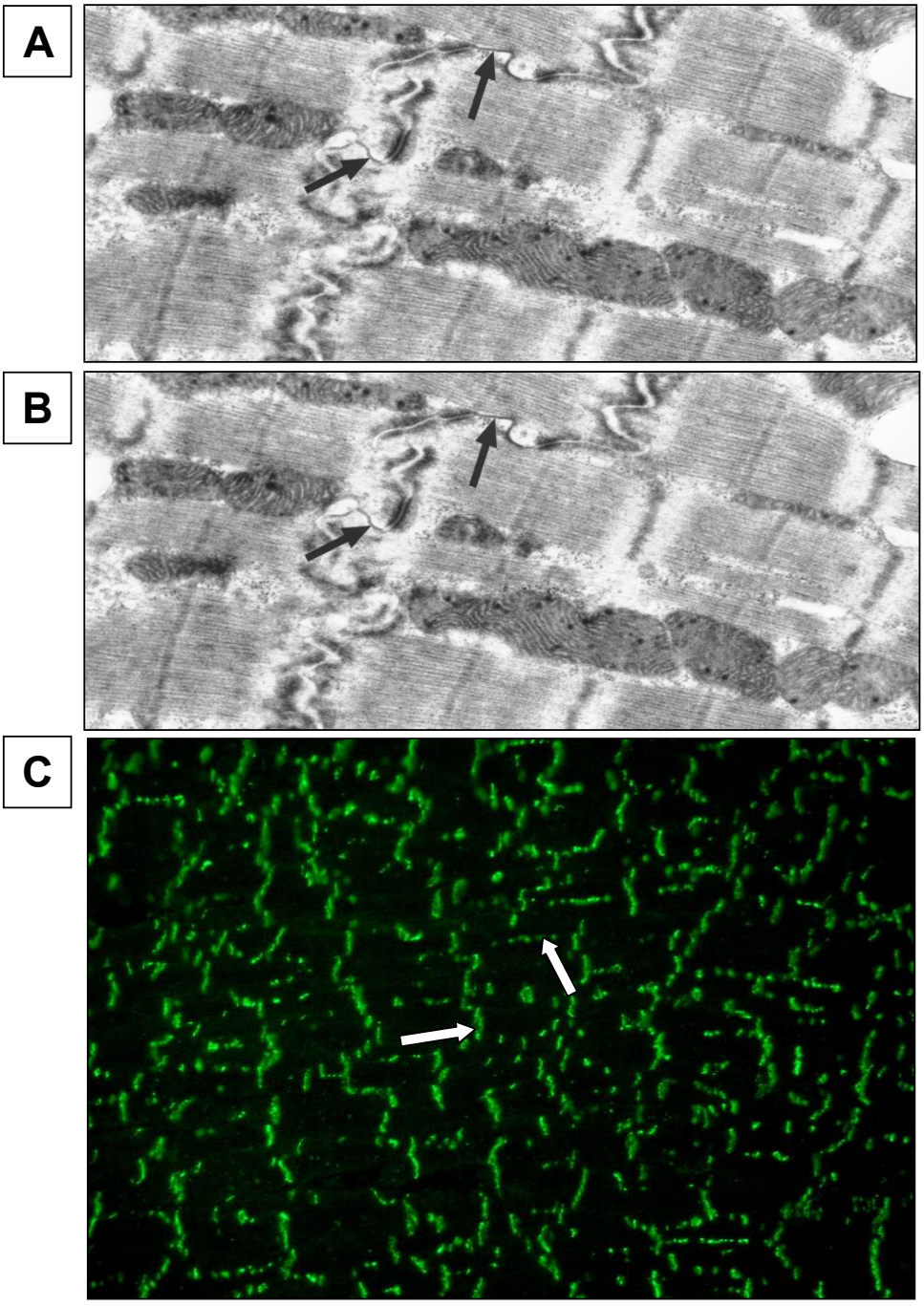

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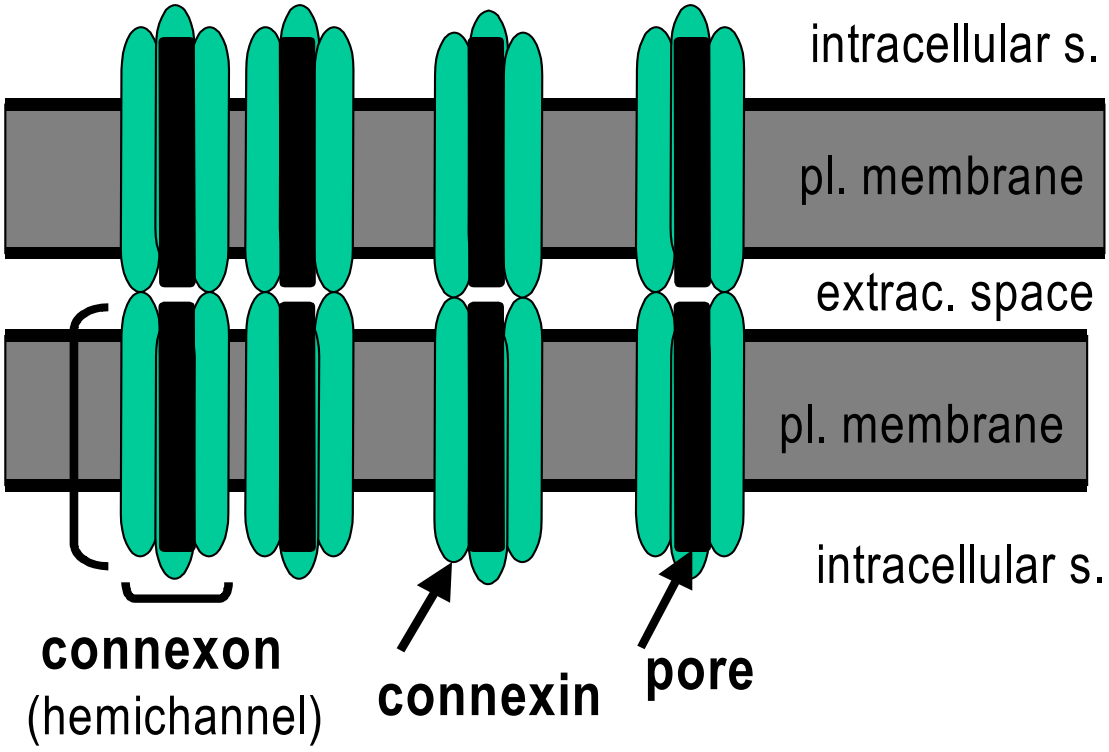
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connexon
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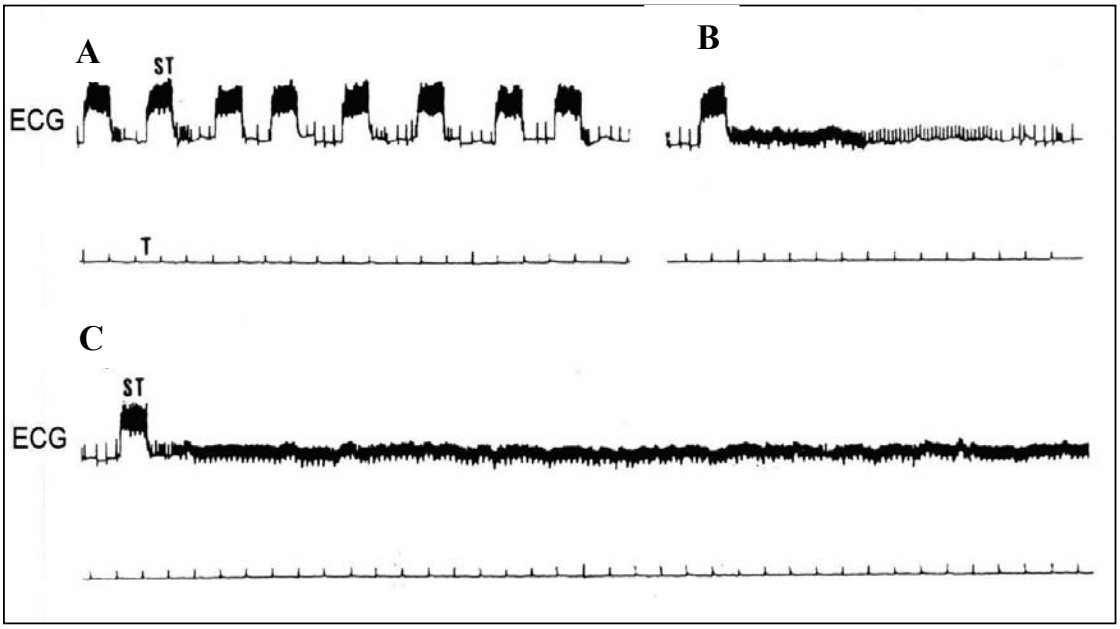
Fig. 3.



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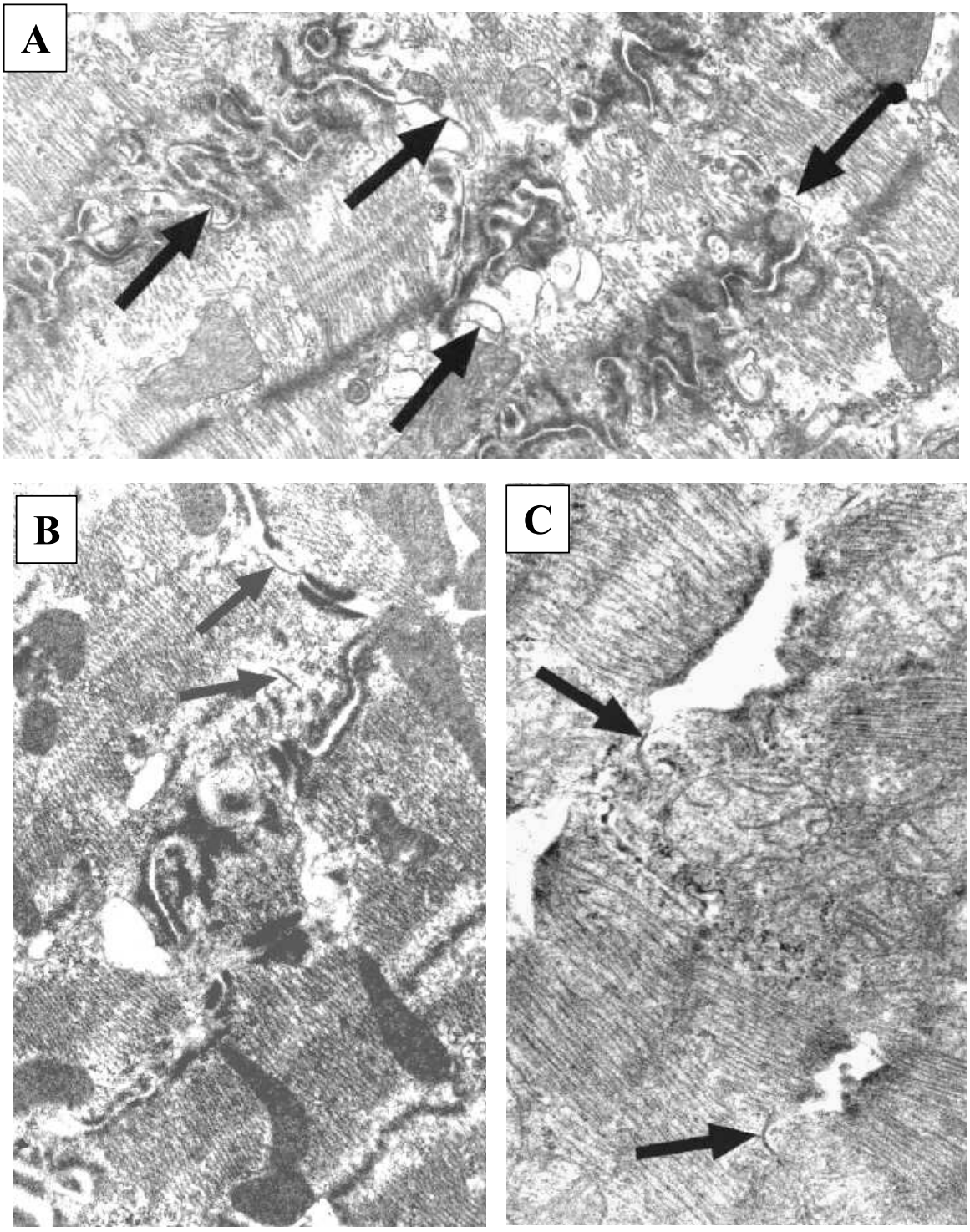

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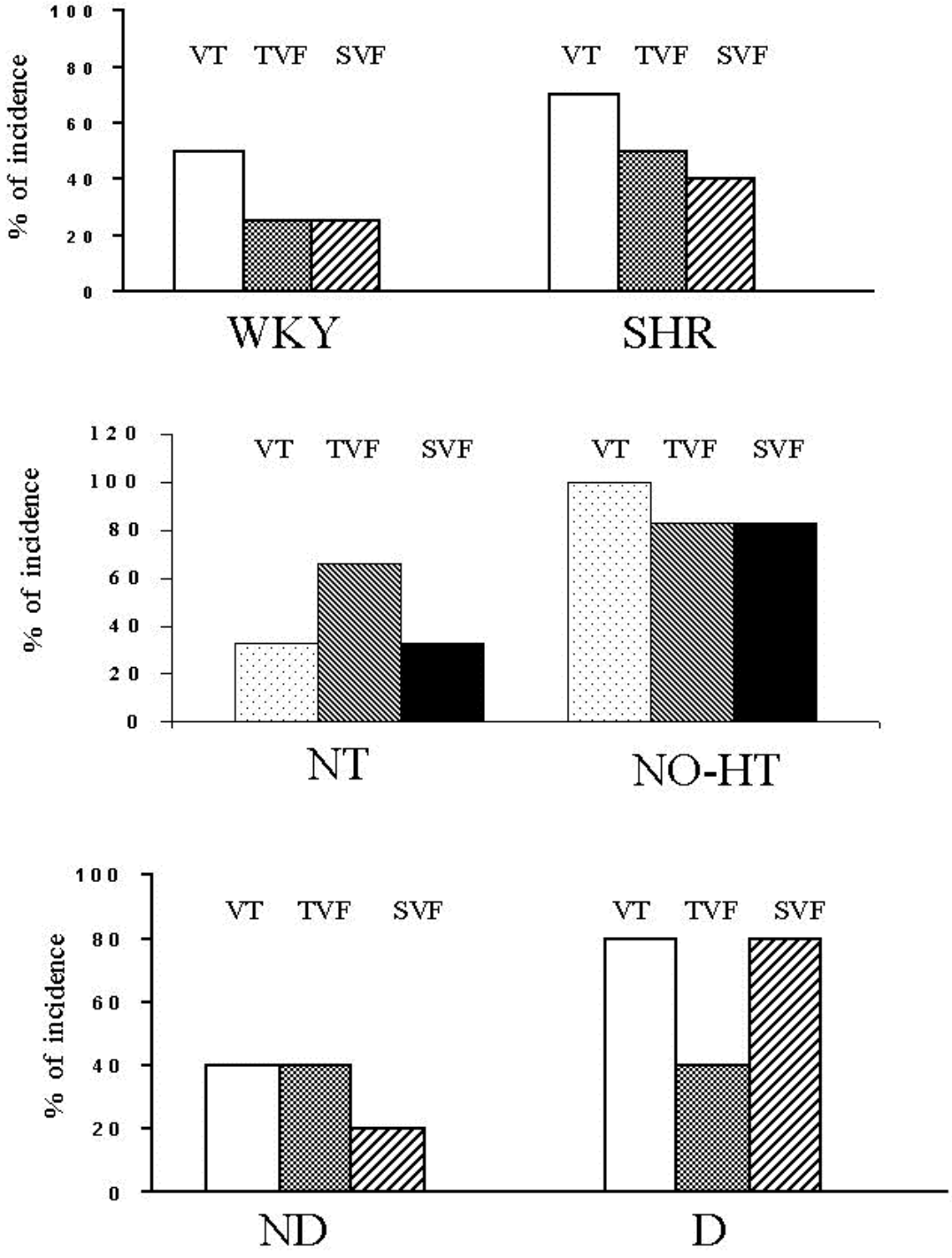
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Fig. 6.

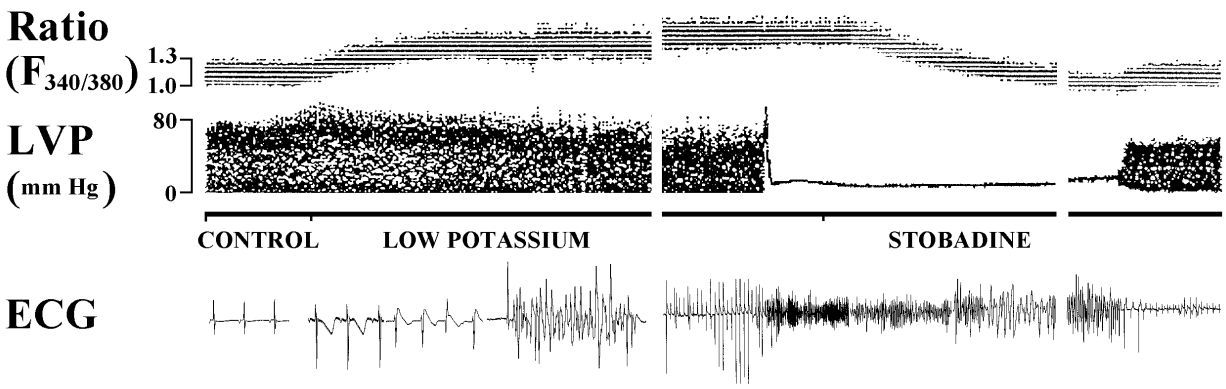
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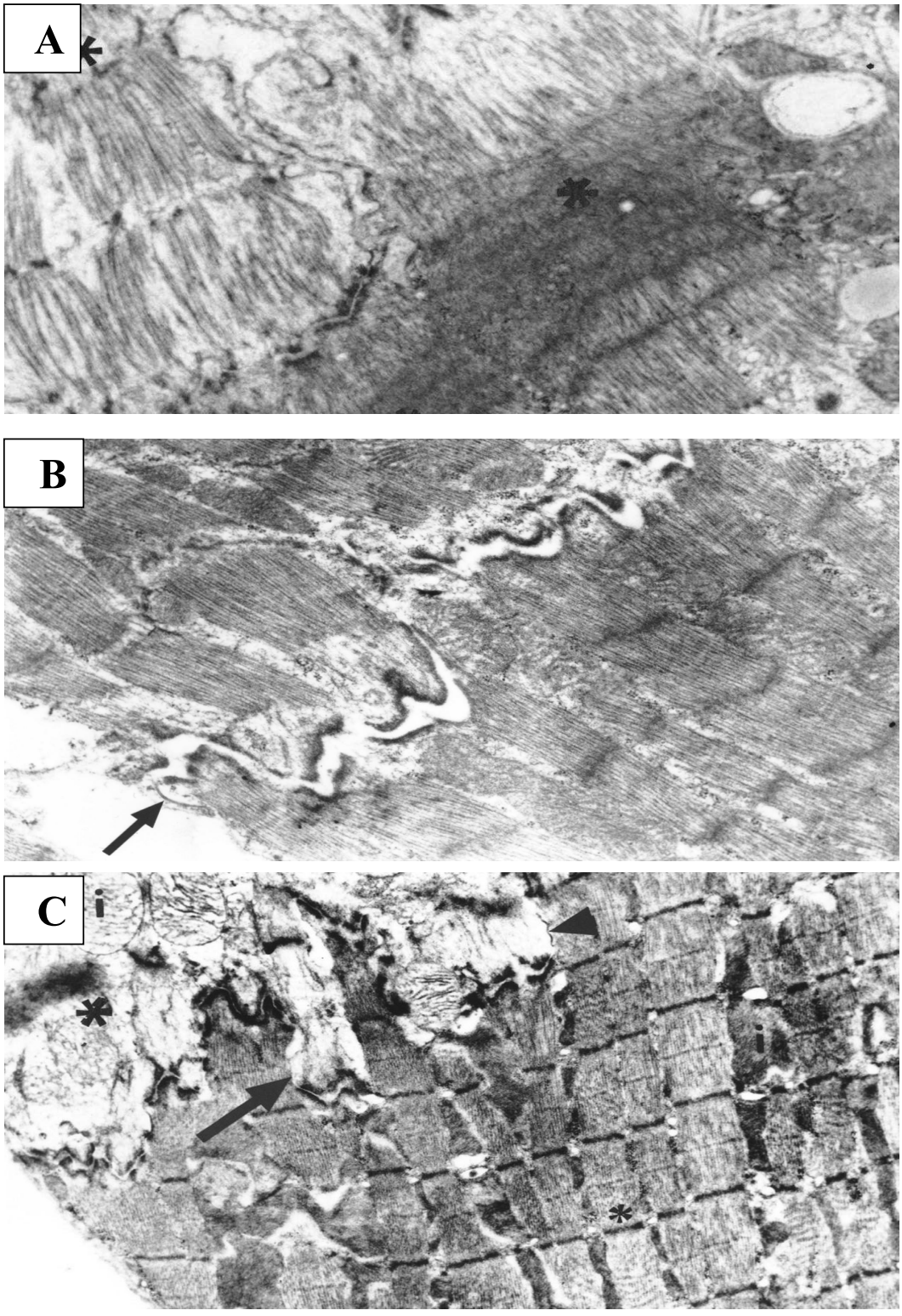
Fig. 7.



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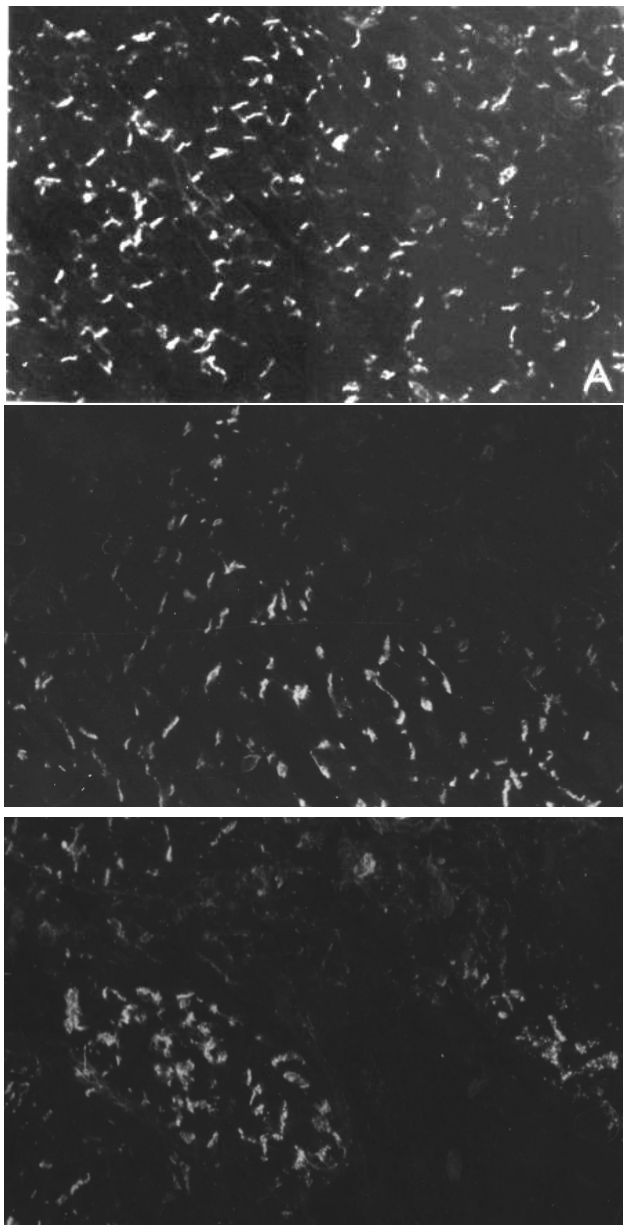

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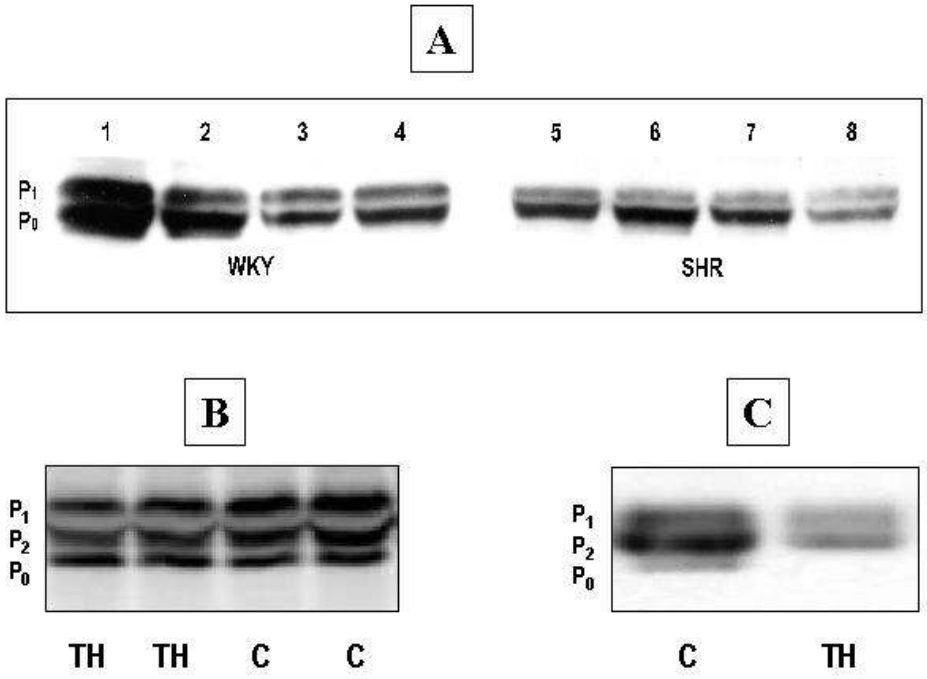

Legends to figures

Fig.1. Electronogram of rat ventricular cardiomyocytes coupled by intercalated disc-related end-to-end type (A, arrows) and lateral side-to-side type (B, arrow) of gap junctions. Myocardial distribution of both end-to-end (thick arrows) and side-to-side (thin arrows) of Cx43-positive gap junctions is revealed by immunolabeling using specific anti-Cx43 antibodies (C). Magnification 14 000 x (A,B), 80 x (C).

Fig.2. Details of gap junction structure (arrows), as seen in electron microscope by higher magnification - 30 000 x. There are two closely apposed unit membranes (black lines arrows) separated by "gap" with transverse densities (middle line). Diagram of gap junction structure shows gap junction channels composed of proteins termed connexins. Each intercellular channel comprises an abutting pair of connexons (hemichannels).

Fig.3. Electrocardiograms of the atria of guinea pig hearts subjected to electrical burst stimulation to induce atrial fibrillation. Note that intermittent pacing (A) did induce only short-lasting arrhythmias in young (B), while prolonged (15 min) atrial fibrillo-flutter in old guinea pig heart (C). T – time in seconds, (adapted from Tribulova *et al*. 1999).

Fig. 4. Subcellular features of cell-to-cell junctions of young (A) and old (B, C) guinea pigs atria. Note numerous gap junctions (arrows) and undulated fascia adherens junctions with narrow intercellular space in young (A), while reduced number of gap junctions (arrows) and flattened fascia adherens junctions with widened extracellular space in old guinea pig myocardium (B). Burst pacing of old guinea pig atria, lasting for 5-15 minutes, induced subcellular alterations indicating cytosolic Ca^{2+} disturbances and dehiscence of fascia

adherence junctions in the vicinity of (functionally very likely altered) gap junctions (arrows, C). Magnification 18 000 x.

Fig. 5. Incidence of hypokalemia-induced malignant arrhythmias (VT – ventricular tachycardia, TVF – transient ventricular fibrillation, SVF – sustained ventricular fibrillation) in the hearts of rats suffering from spontaneous (SHR) or L-NAME-induced hypertension (NO-HT), or diabetes (D). Note much higher incidence of SVF in disease-affected hearts compared to age-matched healthy controls (adapted from Tribulová *et al.* 2002a).

Fig. 6. Original recording of cytosolic $[Ca^{2+}]i$ and left ventricular pressure (LVP) alterations as well as ECG changes occurred prior hypokalemia-induced VF, during VF and before sinus rhythm restoration facilitated by stobadine (10^{-6} m/l) in isolated heart preparation. Note a pronounced increase of $[Ca^{2+}]i$ due to perfusion of the heart with K^+-deficient solution that was accompanied by transient arrhythmias, which degenerated upon 15-20 min to VF, while baseline $[Ca^{2+}]i$ restoration preceded sinus rhythm restoration.

Fig. 7. Subcellular alterations and impairment of cell-to-cell junctions due to hypokalemia-induced Ca^{2+} overload. Note in A - contraction bands (asterisk) in the vicinity intercellular junctions; in B - relaxed versus contracted cardiomyocytes strongly indicating impaired coupling at the gap junctions (arrow) in the vicinity of dissociated fascia adherence junctions; in C – subcellular features indicating cell-to-cell uncoupling when almost normal cardiomyocyte (right) is connected with irreversibly-injured one (left). Such patterns of deteriorated coupling heterogeneously distributed throughout myocardium preceded occurrence of ventricular fibrillation. Magnification 18 000 x (A, B), 15 000 x (C).

Fig. 8. Immunolabelling of Cx-43 in the ventricles of isolated rat hearts perfused with standard (A) and low K^+ solution (B, C). Note uniform distribution of Cx43-positive gap junctions in (A), while heterogeneously abolished Cx43 staining during 15 min of low-K^+ perfusion prior occurrence of sustained VF (B, C). Magnification 80 x.

Fig. 9. Representative immunoblots of rat hearts Cx-43 showing its expression and phosphorylated isoforms (P1, P2). Note pronounced reduction of myocardial Cx-43 phosphorylation in spontenously hypertensive rats (A- SHR, lines 5,6 *vs* WKY, lines 1,2) and T_4-treated young (C) while not in old rat hearts (B) compared to untreated age-matched controls. TH- T_4-treated rats, C- control rats.