The concentration of conjugated dienes (CD), often used as an indirect marker for the production of free oxygen radicals, increased by 141% (from 91 to 218 nmol) after calculation per gram of tissue (Table 1) However, evaluation of CD concentration directly in phospholipids revealed no change, suggesting that phospholipids in cardiac tissue after L-NAME treatment were not damaged additionally, by increased level of free oxygen radi cals The increase of the CD concentration in the cardiac tissue is therefore a consequence of the elevated phospholipid concentration

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Hypokalemia-Induced Ultrastructural, Histochemical and Connexin-43 Alterations Resulting in Atrial and Ventricular Fibrillations

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Abstract. Perfusion of the isolated guinea pig heart with hypokalemic solution provide simple model for examination of the molecular mechanisms involved in the incidence

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of atrial and/or ventricular fibrillations The results point out that dispersion of the metabolic and subcellular alterations and heterogenously impaired intercellular coupling might account for electrical disturbances and desynchronization of the myocardium thus facilitate occurrence of fibrillation

Key words: Hypokalemia — Histochemistry — Ultrastructure — Connexin 43 — Atrial and ventricular fibrillations

Hypokalemia is a common medical condition occurring during the treatment of the hypertension and is known to have serious consequences resulting in electro-mechanical disturbances of the heart (Steiness and Olesen 1976) Taken into consideration that dietary sodium intake is very high and that the use of thiazide diuretics when dietary sodium is not restricted commonly produces potassium losses (Anderson *et al* 1991), the occurrence of hypokalemia is potentially enormous Moreover, hypokalemia is frequently associated with cardiovascular, gastrointestinal, urogenital diseases (Janko *et al* 1992) and transient hypokalemia can be induced by increase of catecholamines (Seck *et al* 1996) as well as by exercise

The understanding how hypokalemia affects cardiac metabolism, structure and function is still incomplete, although these conditions are associated with a high risk for arrhythmias, such atrial and ventricular fibrillations (Friedensohn *et al* 1991, Miletich *et al* 1997) Reduction in serum K^+ influences myocardial excitability by increasing of the membrane potential, diastolic depolarization, duration of action potential and refractory period and by decreasing of conduction velocity (Akita *et al* 1998) The arrhythmogenic potential of hypokalemia is thought to result from electrical inhomogeneity, alterations in conduction, changes in automaticity and disturbances in Na pump kinetics (Wong *et al* 1993)

To elucidate more the low kalium related alterations of the myocardium leading to arrhythmias, the aim of this study was to examine the vulnerability of the isolated guinea pig heart to hypokalemia-induced atrial and/or ventricular fibrillations and to analyze the involvement of the ultrastructure, histochemistry and connexin-43 protein in the initiation of these arrhythmias

The experiments were performed on the hearts of young ($n = 6$), adult ($n = 8$) and old ($n = 6$) guinea pigs of both sexes Animals were sacrificed by stunning followed by carotid exsanguination and the aorta of excised heart was immediately cannulated for perfusion with crystalloid 37 °C Tyrode solution oxygenated by 95% O_2 & 5% CO_2 at the pressure of 70 mm Hg After 15 min stabilization with standard solution (2 8 mmol/l K^+) the heart was perfused with K^+ deficient (1 4 mmol/l) ones Bipolar epicardial electrocardiograms from the left atria and ventricle were continuously monitored The atrial and/or ventricular tissue for electronmicroscopic, histochemical and connexin-43 (gap junction channel protein involved in electrical coupling) examination were taken during hypokalemic perfusion, at the onset of fibrillation as well as 15 min after incidence of fibrillation Subcellular alterations were evaluated in randomly chosen transmural myocardial samples The histochemical enzyme activities of α-glucan phosphorylase, succinic dehydrogenase, β-hydroxybutyrate dehydrogenase (involved in energetic metabolism) and 5-nucleotidase (involved in cell membrane function) as well as immunoreaction of connexin-43 (using monoclonal mouse anticonnexin-43 Ab and FITC goat antimouse IgG, Zymed lab Inc) were performed on the cryostat sections

Results and Discussion

The findings revealed that in our model sustained atrial and ventricular fibrillations appeared in each heart during 15–30 min of hypokalemic perfusion, independent on the age and sex These fibrillations were irreversible and sustained after reintroduction of standard solution In other animal models and in the human, hypokalemia is associated particularly with polymorphic tachycardia, Torsade de Pointes (Lazzara *et al* 1993), which often degenerate into fibrillation (Chew and Ong 1993)

Hypokalemia induced inhomogeneity of the myocardial changes They were characterized by disseminated areas of decreased enzyme activities, by heterogenously diminished or absent immunopositivity of connexin-43 and by heterogenously distributed ischemia-like subcellular alterations of the cardiomyocytes in the atrial or ventricular tissue The alterations consisted of mitochondrial injury of various degree, electronlucent nuclei, moderate cytoplasmic edema and nonuniform pattern of sarcomers with occurrence of hypercontraction of myofibers and sporadically even contraction bands This feature of the injury was observed in our previous studies dealing with either ischemia-related arrhythmias (Tribulova *et al* 1993, Ravingerova *et al* 1995) or electrically-induced atrial or ventricular fibrillations (Manoach *et al* 1987, Tribulova *et al* 1999) It points out a close relationship between heterogenously injured, but still viable cardiomyocytes and occurrence of fibrillations Hypokalemia, similar like in previous models, caused focal ultrastructural impairment of intercellular connections that coincided with diminished immunostaining of gap junction protein connexin-43 This strongly indicates disturbances in intercellular communication and in electrical, metabolic and mechanical couplings as well as spatial dispersion of alterations throughout myocardium These alterations resulted in desynchronization of contraction-relaxation process which was manifested by variations in the pattern of myofibres

The hypothesis and pilot studies related to structure-function relationship and occurrence of fibrillations were performed by Manoach *et al* (1987) Today it is more and more accepted that disturbances in intercellular couplings are highly arrythmogenic and gap junctions might be fundamental structural substrate involved in incidence of malignant arrhythmias (Severs *et al* 1994, Peters *et al* 1997) Subcellular and connexin-43 alterations correlate with histochemical findings of heterogenously decreased activities of the enzymes involved in myocardial energy production This is in accordance with marked decline of ATP observed by others (Peterson *et al* 1995) Reversibly and irreversibly altered cardiomvocytes clearly indicate disturbances in electromechanical coupling and synchronisation of the myocardium These changes should precede apparent ion disbalance resulting in electrophysiological instability of the heart Available data suggest that hypokalemia decrease Na,K-ATPase activity and potassium efflux (Wong *et al* 1993) which in turn increases cytoplasmic free calcium via Na/Ca exchanger (Shapiro *et al* 1998) Excess of Ca^{2+} inhibits intercellular coupling at the gap junctions as well as provokes spontaneous Ca^{2+} release from the sarcoplasmic reticulum Both conditions favor precipitation of arrhythmias and fibrillation Furthermore, tachyarrhythmias and fibrillation themselves aggravate already existing ion, metabolic and structural alterations and cause persistence of arrhythmias

Summarizing these data we suggest that dispersion of myocardial alterations, particularly at the intercellular junctions might account for disturbances in electrical coupling and synchronization and consequently facilitate fibrillation

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