Simulation of Cardiac Hypopolarization Arrhythmias and Evaluation of Cardiomyocyte Membrane Hypopolarization in Experimental Animals

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A method for simulating cardiac hypopolarization arrhythmias was developed in order to study changes in cardiomyocyte membrane hypopolarization under the effects of antiarrhythmics and other drugs. The method is based on registration of K^+-induced arrhythmias after intravenous injection of a minimum arrhythmogenic dose of 1.5% KCl over 2 sec. Atrioventricular and intraventricular blockades without arrhythmias are recorded in first-degree membrane hypopolarization. The same changes and cardiac arrhythmias are characteristic of second-degree hypopolarization. Third degree is associated with transitory cardioplegia, fourth degree with heart arrest and animal death.

Key Words: *membrane hypopolarization; hyperkalemia; cardiac arrhythmias; arrhythmogenic effects of antiarrhythmic drugs*

Modern concepts of the pathogenesis of cardiac arrhythmias (CA) were appreciably extended due to the progress in experimental and clinical arrhythmology. It was found that many pathological processes led to the development of complex biomolecular changes in the myocardium significantly reducing K^+ concentration in the cytosol and increasing its extracellular level, which caused a decrease in the negative value of resting membrane potential (RMP), *i.e.* cardiomyocyte hypopolarization.

Registration of cardiomyocyte RMP in various areas of the heart in myocardial infarction showed that the negative RMP of involved myocytes was significantly lower than in similar areas of intact heart. Since the level of negative RMP depends on the number of K^+ ions released from cells during the final repolarization phase, we can speak about several factors participating in the mechanism of formation of RMP level: increase in extracellular K^+ concentration, decrease in intracellular K^+ concentration and in sarcolemma permeability for K^+ ions, and changed permeability of the plasma membranes for Na^+ ions [3,5].

We developed a method for evaluation of cardiomyocyte membrane hypopolarization in laboratory rats, which needs no opening of the chest, based on the use of arrhythmogenic effects of hypokalemia, and evaluated the effects of antiarrhythmic drugs on the degree of membrane hypopolarization and heart rhythm.

MATERIALS AND METHODS

The study was carried out on laboratory rats (n=35; 160-200 g) of both sexes. ECG in standard lead II from the limbs was recorded in animals fixed under Nembutal narcosis (35 mg/kg intraperitoneally). The animals were divided into 4 groups. Group 1 (control; n=5) comprised rats injected with saline (0.2 ml over 2 sec) into the femoral vein. ECG was recorded at the moment of solution injection and during subsequent 30 sec. Group 2 (n=10) animals were intravenously injected with 1.5% KCl solution

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in a previously determined minimum arrhythmogenic dose of 20 mg/kg over 2 sec (ECG was recorded incessantly until normalization of the sinus rhythm). The minimum arrhythmogenic dose of KCl was determined by gradual increase of the volume of 1.5% KCl solution, injected into the femoral vein over 2 sec. Hence, the dose after which the rhythm and conduction disorders persisted for 3-10 sec, was chosen empirically. The interval between repeated intravenous injections of KCl was at least 15 min.

Group 3 (n=10) rats were intravenously injected with 1% novocainamide solution, followed (after 5 min) by 1.5% KCl solution in doses of 20 mg/kg. ECG was recorded until normalization of the sinus rhythm. Group 4 rats (n=10) were infused with 0.5% amiodarone solution (Sanofi-Sinthelabo) in a dose of 10 mg/kg 5 min before KCl injection.

The data were statistically processed by the variation statistical methods using Student's coefficient.

RESULTS

The appearance of arrhythmogenic effects in hyperkalemia depends on the drug dose, its concentration in the solution, and rate of infusion. Injection of saline to group 1 animals in the same volume as in groups 2-4 and at the same rate did not change ECG. In group 2, ECG showed atrioventricular and intraventricular blocks and extrasystolic CA in all rats after injection of KCl (Table 1). The duration of rhythm disorders was no longer than 10 sec. It is obvious that hypopolarization of cardiomyocyte membranes played the main role in the pathogenetic mechanism of CA; short duration of this hypopolarization was due to the minimum arrhythmogenic dose of KCl.

In group 3, the course of K^+-induced CA was quite different: intraventricular and atrioventricular blocks were recorded 3-4 sec after injection of KCl, and cardioplegia developed, which lasted for 1-3 min. This was followed by rare idioventricular complexes. The rhythm increased over 2.0-2.5 min in 6 rats, and the idioventricular rhythm transformed into regular sinus bradycardia. In 3 rats the idioventricular rhythm progressively slowed down, respiration ceased, and the animals died. In group 4, the same ECG changes were observed. One rat died.

Four degrees of cardiomyocyte plasma membrane hypopolarization were distinguished in the analysis of ECG changes. The first degree included cases with deceleration of atrioventricular and intraventricular conduction without CA for 2 sec after intravenous injection of the minimum KCl dose. Second degree included the same changes with ectopic CA. Third degree was associated with short-term cardioplegia, concomitant with complete atrioventricular blocking with retained *P* waves without ventricular complexes. After reappearance of the *QRS* complexes, ectopic pacemaker activity appeared in some animals, which manifested by solitary or group extrasystoles on ECG. The duration of cardioplegia varied from 1 sec to 2-3 min in different rats. Fourth-degree hypopolarization was associated with heart arrest and animal death (Fig. 1).

Life-time closed-chest recording of hypopolarization CA opens new vistas for studies of the arrhythmogenic effects of antiarrhythmic drugs and cardiac glycosides and in studies of the pathogenesis of sudden death of coronary patients treated with Na^+ channel blockers.

One of the pathogenetic mechanisms of CA in coronary disease is elevated K^+ content in the extracellular space and its decreased cytosol concentration. Leakage of 1% K^+ ions from the cell 2-fold increases its content in the extracellular space [4]. This inhibits further release of K^+ from cells, some positively charged ions remain in the cytosol decreasing RMP by the end of the repolarization phase, which leads to a decrease in negative RMP value. In intact cardiomyocyte RMP varies from -90 to -95

TABLE 1. Effects of KCl, Novocainamide, and Amiodarone on K^+-Induced CA in Rats

Group	Atrioventricular blockade				Intraventricular blockade		Ventricular extrasystole		Cardioplegia		Survival	
	degree I-II		degree III									
	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
1	—	—	—	—	—	—	—	—	—	—	5	100
2	10	100	2	20±0	10	100	3	30±6	1	10±2	10	100
3	10	100	10**	100	10	100	7*	70±8	10**	100	7*	70±8
4	4**	40±7	7**	70±9	10	100	8*	80±9	8**	90±9	9	70±9

Note. *p<0.05, **p<0.01 *vs.* group 2.

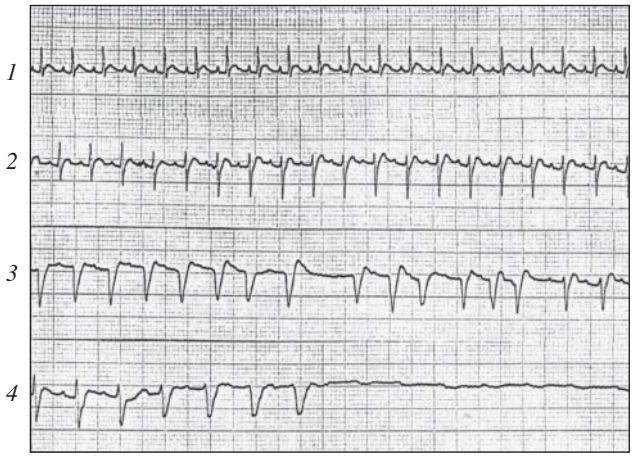

Fig. 1. Heart rhythm and conduction disorders in K^+-induced hypopolarization of the rat cardiomyocyte membranes. *1*) initial ECG in lead II from the limbs; *2*) first-degree hypopolarization of cell membranes: decreased wave amplitude, first-degree atrioventricular blockade, deep wave *S*, widened *QRS* complex as a result of disordered intraventricular conduction; *3*) second-degree hypopolarization: the same ECG changes, but more pronounced, and ventricular extrasystolic arrhythmia; *4*) third-degree membrane hypopolarization: complete atrioventricular blockade (cardioplegia).

mV, while its drop to -60 mV promotes activation of potential-dependent Na^+ channels and appearance of extra contractions of the heart. At lower negative values of resting potential (from -30 to -35 mV) Na^+ channels are inactivated, Na^+ current is replaced by slow Ca^{2+} current, promoting deceleration of conduction and formation of heart blocks and ectopic foci of arrhythmogenesis by the re-entry mechanism [3,4]. Further decrease in negative RMP values leads to inactivation of Na^+ and Ca^{2+} channels. Short-term cardioplegia develops and its duration depends on extracellular concentration of K^+. Dosed hyperkalemia is used for short-term heart arrest in clinical cardiosurgery. It is quite possible that cardiomyocyte hypopolarization in acute coronary syndromes is responsible for sudden death, particularly in patients with a history of myocardial infarction, treated with Na^+ channel blockers.

It seems that intravenous injection of novocainamide to animals decreases the concentration of Na^+ ions in the cytosol, impairs the function of Na^+, K^+-ATPase, and decreased the amount of K^+ entering the cell. Hence, the release of K^+ ions from the cell during the final repolarization phase (phase III) is insufficient, which causes a reduction of the negative RMP level. Depending on the membrane hypopolarization degree, pathological activation of ionic channels or cardioplegia develops and its duration depends on the degree of membrane hypopolarization [1,3]. Amiodarone blocking of K^+ channels also reduces the release of K^+ ions from the myocyte cytosol and prolongs phase III of action potential. It seems that amiodarone also leads to membrane hypopolarization, but the degree of hypopolarization, caused by this drug, was lesser than after novocainamide (Table 1). This is confirmed by shorter duration of cardioplegia and lesser number of animal deaths.

This method for evaluation of membrane hypopolarization, its degree, and registration of hypopolarization CA is very simple and can be easily used at experimental cardiology laboratories. However, accurate dosing of KCl solution and precise calculation of the rate of intravenous injection (using a chronometer) are essential for obtaining accurate reproducible results.

REFERENCES

1. D. K. Gatsby and L. E. Vith, *Cardiac Arrhythmias. Mechanisms, Diagnosis, Treatment* [in Russian], Ed. W. J. Mandel, Moscow (1996), Vol. 1, pp. 107-155.
2. A. I. Dyadyk, A. E. Bagrii, O. A. Prikolota, *et al.*, *Ukr. Medichn. Chasopis*, No. 3, 92-97 (2002).
3. H. S. Karagesian and W. J. Mandel, *Cardiac Arrhythmias. Mechanisms, Diagnosis, Treatment* [in Russian], Ed. W. J. Mandel, Moscow (1996), Vol. 1, pp. 263-303.
4. B. Suravits, *Cardiac Arrhythmias. Mechanisms, Diagnosis, Treatment* [in Russian], Ed. W. J. Mandel, Moscow (1996), Vol. 1, pp. 156-189.
5. J. Jalife, *Annu. Rev. Physiol.*, **62**, 25-52 (2000).
6. M. J. Janse, *ACC Curr. J. Rev.*, **8**, No. 2, 15-17 (1999).
7. J. A. Kastor, *Arrhythmias*, Philadelphia (2000).
8. Z. Qu, I. Kil, F. Xie, *et al.*, *Biophys. J.*, **78**, No. 6, 2761-2775 (2000).