Dysfunction of Ionic Channels in Cardiomyocyte Sarcolemma and Cardiac Arrhythmias

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The arrhythmic effects of Na^+ and Ca^{2+} intracellular imbalance were examined on rats with aconitine-induced cardiac arrhythmias. Under conditions of Na^+-dependent arrhythmogenesis, blockade of Ca^{2+}-channels with verapamil aggravated cardiac rhythm disturbances. Correction of ionic imbalance by intravenous injection of calcium preparations in aconitine-induced arrhythmia promoted recovery of stable sinus rhythm and decreased animal mortality. Intracellular imbalance of Na^+ and Ca^{2+} ions can underlie the arrhythmogenic effects of antiarrhythmic drugs.

Key Words: *cardiac arrhythmias; pathogenesis; ionic channels; arrhythmogenic effects of antiarrhythmic drugs*

Hyperpolarization of the sarcolemma caused by abnormal membrane repolarization and decreased resting potential is an important pathogenic mechanism of cardiac rhythm disturbances. In experiments, membrane hyperpolarization and cardiac arrhythmia (CA) are usually simulated by intravenous injection of KCl. Under conditions of hyperkalemia, outward potassium current during the final phase of hyperpolarization decreases due to increased concentration of K^+ in the intracellular fluid and the value of negative resting potential decreases. Normally resting potential is about -95 mV, but under conditions of hyperkalemia its value decreases with increasing of the extracellular potassium concentration. Hypopolarization of the membrane to -50 and -60 mV leads to activation of fast Na channels and spontaneous ectopic rhythm disturbances; at -30 and -35 mV these channels inactivate, but slow Ca channels open in sarcolemma, which leads to Ca-dependent CA caused by re-entry mechanism or trigger activity. Extreme hyperkalemia inactivates both Na and Ca channels and causes cardioplegia [6,7].

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During acute coronary syndromes in patients with coronary heart disease, membrane permeability for K^+ increases, and potassium ions leaks into the extracellular fluid from ischemized cardiomyocytes. Leakage of only 1% potassium from cells 2-fold elevates its extracellular level [5]. Local rise in potassium concentration can provoke various CA (depending on the degree of membrane hypopolarization).

There are no methods for evaluation of abnormal activation of ionic channels. Therefore, the antiarrhythmic drugs are routinely chosen by trial-and-error method, which frequently leads to block of ionic channels that are not involved in local arrhythmogenesis. Clinically, it manifested in antiarrhythmic drug inefficiency or even cause arrhythmogenic complications [2,3].

Our aim was to study the cardiac rhythm disturbances in rats under the effect of selective activation or blockade of Na and Ca channels.

MATERIALS AND METHODS

Experiments were carried out on random-bred male and female albino rats (n=50) weighing 160-200 g and randomized into five groups. Under nembutal

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narcosis (40 mg/kg intraperitoneally) the animals were fixed. ECG was recorded from the extremities in standard lead II; thereafter the rats of all groups were injected with 30 μg/kg aconitine via the femoral vein. The dose was chosen in such a way that the sustained extrasystolic bigeminy observed 2.5-4.0 min postinjection.

After recording of aconitine-induced extrasystole, group 1 rats (control) were intravenously injected with physiological saline of the same volume as the antiarrhythmic solution administered to rats of groups 2-5. ECG was recorded for 1 h or until fatal outcome. Group 2 rats were injected with intravenous bolus of verapamil (0.025%, 1 mg/kg) at the start of aconitine-induced CA. Group 3 rats received slow intravenous infusion of 0.25% ethacizin (2 mg/kg) after appearance of aconitine-induced CA. Group 4 rats were intravenously infused with 5% $CaCl_2$ (80-100 mg/kg) to the moment of heart rhythm normalization. Group 5 rats were preventively infused with 5% $CaCl_2$ (100 mg/kg), while aconitine (30 μg/kg) was administered 1 min after the start of calcium infusion.

RESULTS

All rats of control group 1 developed extrasystolic bigeminy. The degree of CA increased with the appearance of duplicates and triplets of polytopic extrasystoles, the episodes of paroxysmal monotopic tachycardia, torsades de pointes, ventricular fibrillation, which resulted the death of 9 rats (Table 1).

In all group 2 rats, CA was characterized by rapid and "aggressive" development: the extrasystolic bigeminy was replaced by polytopic grouped extrasystole, frequent episodes of torsades de pointes, ventricular flutter and fibrillation. Seven rats of this group died within 30 min postinjection.

In group 3 rats, the development of CA was less severe after blockade of Na channels by ethacizin, although 4 rats died of asystole.

In group 4 rats, extrasystolic bigeminy was replaced by regular sinus rhythm after injection of 5% $CaCl_2$ (0.1-0.2 ml). When extrasystole reappeared, similar injection favored stabilization of sinus rhythm. Only one rat died in this group because of pronounced sinus bradycardia probably caused by aconitine intoxication.

In group 5 rats, only one animal had extrasystolic CA, while other rats demonstrated moderate bradycardia and widening of *QRS* complex.

Our studies confirmed the fact that dysfunction of Na and Ca channels can initiate CA of various type. When fast Na channels are activated with aconitine, the intracellular concentration of Na^+

TABLE 1. Modulation of Ionic Channel Function in Aconitine Model of Cardiac Arrhythmia (AC, %; *n*=10)

Preparation, dose	VE	VPT	TdP	VF	AVB	VA	Survived
Aconitine, 30 μg/kg	10 (100±33)	6 (60±20)	7 (70±23)	8 (80±26)	6 (60±20)	9 (90±30)	1 (10±3)
Aconitine+verapamil, 1 mg/kg	10 (100±33)	7* (70±23)	9 (90±30)	8 (80±26)	7* (70±23)	10* (100±33)	0
Aconitine+ethacizin, 2 mg/kg	10 (100±33)	4 (40±13)	0	2 (20±6)	10 (100±33)	4** (40±13)	6** (60±20)
Aconitine+$CaCl_2$, 100 mg/kg	3 (30±10)	0	0	0	1** (10±3)	1** (10±3)	9** (90±30)
$CaCl_2$ (100 mg/kg) before aconitine	1** (10±3)	0	0	0	0	0	10** (100±33)

Note. VE, ventricular extrasystole; VPT, ventricular paroxysmal tachycardia; TdP, torsades de pointes; VF, ventricular fibrillation; AVB, atrio-ventricular block; VA, ventricular asystole. *$p<0.01$, **$p<0.05$ compared to the control.

ions elevates pronouncedly [4]. At this period, Na^+/K^+ pump works more intensively, and the exchange of Na^+ for Ca^{2+} proceeds at greater rate. Both processes evacuate the surplus of Na^+ ions from the cell. Probably, in our experiments the intracellular sodium concentration was far surpassed that calcium. Under these conditions, the block of Ca channels with verapamil not only decrease the entry of Ca^{2+} ions into the cells during the second phase of action potential, but it also impeded the donation of Ca^{2+} ions from sarcoplasmic reticulum. Thus, dysfunction of ionic channels aggravated the intracellular ionic imbalance, which resulted in the development of aconitine-induced CA and the death of animals. The pathogenic role of calcium deficit in cytosol was corroborated by the antiarrhythmic effect of intravenous $CaCl_2$ in group 4 rats and by its high efficiency in prevention of aconitine-induced CA in group 5 rats.

The ionic mechanisms of cardiac rhythm disturbances caused by dysfunction of ionic channels and intracellular imbalance of Na^+ and Ca^{2+} ions are little studied. Probably, the function of Ca-activated K channels is also distorted under these conditions, which promotes membrane depolarization.

This study confirms the possibility of arrhythmogenic side effects during drug-induced dysfunction of ionic channels. Our experimental paradigm can be used to assess the arrhythmogenic side effects of antiarrhythmic preparations.

Thus, the pathogenic relation between intracellular imbalance of Na^+ and Ca^{2+} ions and electrical instability of the myocardium is established. Under conditions of Na^+-dependent arrhythmogenesis, the block of slow Ca channels with verapamil decreases the intracellular concentration of Ca^{2+} ions, aggravates aconitine-induced CA, and increases mortality. Intravenous infusion of $CaCl_2$ during aconitine-induced CA demonstrates pronounced antiarrhythmic potency and prevents the fatal outcome.

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