EFFECT OF ADAPTATION TO STRESS AND TO PERIODIC HYPOXIA ON TRIGGERING ACTIVITY OF PAPILLARY MUSCLE CARDIOMYOCYTES OF THE RAT HEART

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Heterotopic triggering activity, activated by high-frequency stimulation, may play a role in the onset of arrhythmias associated with hypertrophy of the heart [9] and acute myocardial infarction in experimental animals [10]; it is the cause of the arrhythmias observed clinically in digitalis poisoning [8], idioventricular rhythm, and ventricular tachycardia associated with various heart diseases [8]. To understand the mechanism of trigger arrhythmias, it is essential that delayed post-depolarization and triggering activity which follows it be regularly reproduced by the action of factors increasing the Ca^{2+} concentration in the sarcoplasm, namely catecholamines [15], an excessive Ca^{2+} concentration in the perfusion fluid [9], and toxic doses of strophanthin [11]. Meanwhile these phenomena are abolished by ryanodine, an agent blocking Ca^{2+} release from the sarcoplasmic reticulum (SPR) [15]. This is in agreement with the view that disturbance of the function of SPR and of rhythmic release of Ca^{2+} from it is a key component of trigger arrhythmias [15]. It has recently been shown that adaptation to repeated short-term stress increases the ability of SPR of heart muscle to take up Ca^{2+}, and considerably increases its resistance to autolysis [13]. Adaptation to ueriodic hypoxia under pressure chamber conditions, as has recently been shown, also increases activity of the Ca^{2+}-pump of SPR without affecting resistance of elements of SPR to autolysis [1]. At the same time, adaptation to these two factors has a powerful antiarrhythmic action in acute ischemia and reperfusion [2, 5], in myocardial infarction [3, 12], and in postinfarct cardiosclerosis [4, 12]. The question arises whether ability of adaptation to suppress delayed postdepolarization and triggering activity plays a role in this antiarrhythmic effect.

The aim of this investigation was to assess the resistance of the papillary muscle of adapted animals to a factor inducing triggering activity, and then to compare the efficacy of the protection thus revealed during adaptation to stress and during adaptation to periodic hypoxia.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. Adaptation to stress was produced by fixing the animals in the supine position for 1 h on alternate days (eight immobilizations altogether). Adaptation to periodic hypoxia was carried out by daily ascents in a pressure chamber, in which the "altitude" was increased in stages up to 4000 m. A course of adaptation consisted of 40 sessions, each consisting of exposure to hypoxia for 5 h. Post-depolarization and triggering activity were induced by imposing a high frequency of stimulation on the papillary muscles, while the perfusion fluid contained isoproterenol. The experiments consisted of two stages: in stage I the effect of adaptation to hypoxia and stress on resistance of the papillary muscle cardiomyocytes to factors inducing delayed post-depolarization and triggering activity was studied; in stage II the effect of immobilization stress lasting 6 h on this resistance was investigated, and the protective effect of the two types of adaptation was assessed.

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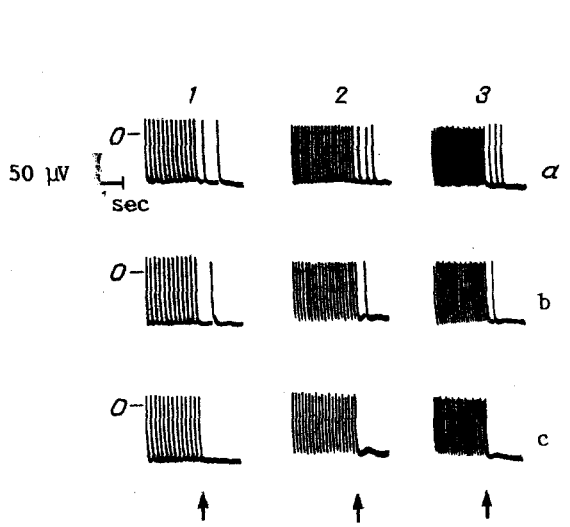

Fig. 1

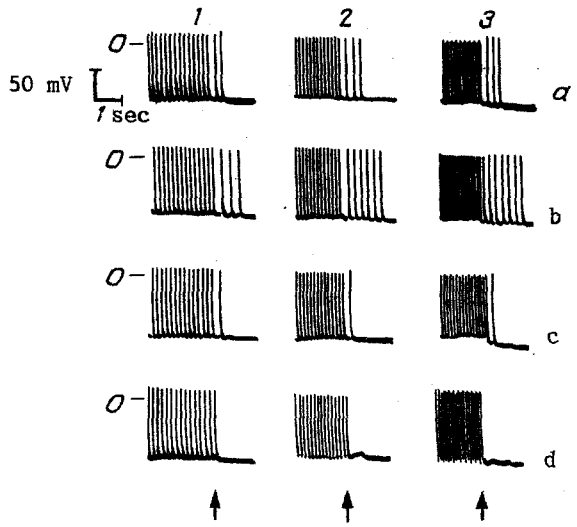

Fig. 2

Fig. 1. Action potentials of papillary muscle cardiomyocytes during increasing frequency of stimulation in testing burst of pulses, during administration of isoproterenol (10^{-8} M). a) Control, b) adaptation to hypoxia, c) adaptation to stress; arrow here and Fig. 2 indicates time of cessation of stimulation of preparation. Here and in Fig. 2: 1, 2, 3) frequency of pulses in testing burst 400 and 600 pulses/min respectively.

Fig. 2. Effect of stress and stress combined with adaptation to hypoxia and to stress on electrical activity of papillary muscle cardiomyocytes – action potentials during increasing frequency of stimulation in testing burst of pulses during administration of isoproterenol (10^{-8} M). a) Control, b) immobilization stress, c) immobilization stress combined with adaptation to hypoxia, d) immobilization stress combined with adaptation to stress.

The main experiment was as follows: animals were anesthetized with pentobarbital (50 mg/kg, intraperitoneally), after which the heart was quickly removed and the papillary muscles, not more than 0.6 mm in diameter, were isolated from the left ventricle. The isolated papillary muscles were perfused with standard Krebs–Henseleit solution at 35°C. saturated with a gas mixture consisting of 95% O_2 + 5% CO_2 (pH 7.3-7.4). The papillary muscle preparation was stretched to a length at which it developed maximal amplitude of contractions (the muscle worked under isotonic conditions). Before the beginning of the main experiment the preparations were stabilized for 60 min at a basic frequency of stimulation of 1 Hz. The preparations were stimulated through pointed silver electrodes, inserted directly into the preparation. Square pulses of current of twice the threshold strength and a duration of 1 msec were applied (SEN-3201 stimulator, from "Nihon Kohden," Japan). The program of stimulation included stimulation of the preparation by series of 30 pulses with an interval of 200, 150, and 100 msec between pulses. The interval between series was 1 min. The stimulation program was repeated 15 min after addition of isoproterenol ("Sigma") to the perfusion fluid in a concentration of 10^{-8} M. Electrical activity of the papillary muscle cardiomyocytes was recorded by a standard microelectrode technique, using "floating" microelectrodes and a set of equipment consisting of an MEZ-7101 microelectrode amplifier, RAT-1100 memory unit, VC-9 electronic oscilloscope, RELG-6201 recording camera, and RM-6000 polygraph ("Nihon Kohden," Japan). The results were subjected to statistical analysis by Fisher's exact method and by Student's test.

EXPERIMENTAL RESULTS

The results of recording action potentials in the three typical experiments illustrated in Fig. 1 show that triggering activity in the control animals began against a background of isoproterenol when the frequency imposed on the papillary muscles was 300 pulses/min, and at frequencies of 400 and 600 pulses/min the number of triggering impulses increased to

TABLE 1. Effect of Adaptation to Stress and to Periodic Hypoxia on Total Frequency of Cases of Delayed Post-Depolarization and Triggering Activity Arising in Papillary Muscle Cardiomyocytes after a Period of Testing Stimulation

Group of animals	Frequency of stimulation in testing burst of pulses, pulses/min		
	300	400	600
	number of preparations in which triggering activity appeared		
Control (n = 9)	5	8	9
Adaptation to hypoxia (n = 9)	3	3**	3**
Adaptation to stress (n = 8)	0*	2**	2***

Legend. *p < 0.02, **p < 0.01: statistically significant differences compared with control.

TABLE 2. Effect of Stress and Stress Preceded by Adaptation to Stress and to Periodic Hypoxia on Number of Automatic Excitatory Pulses Arising in Papillary Muscle Cardiomyocytes after Period of Testing Stimulation (M ± m)

Group of animals	Frequency of stimulation in testing burst of pulses		
	300	400	600
	number of pulses of excitation		
Control (n = 9)	2±0,2	3±0,3	3±0,5
Immobilization stress (n = 8)	3±0,4*	6±1,0*	7±1,4*
Immobilization stress preceded by adaptation to hypoxia (n = 8)	1±0,5**	1±0,5*,**	1±0,7*,**
Immobilization stress preceded by adaptation to stress (n = 8)	0	0	0

Legend. Statistically significant differences: *) compared with control, **) compared with values for immobilization stress.

three. Adaptation to hypoxia caused the number of triggering impulses at all frequencies to be not more than one, and adaptation to stress completely prevented the onset of triggering activity. Instead of triggering pulses at frequencies of 400 and 600 pulses/min only delayed post-depolarization was observed: this is usually a precursor of excitatory triggering pulses. The results of all three series of experiments are shown quantitatively in Table 1, which shows that in the control, with a frequency of stimulation of only 300 pulses/min, triggering activity occurred in more than half of the preparations, whereas at a frequency of 600 pulses/min it occurred in all cases. Triggering activity at frequencies of stimulation of 400 and 600 pulses/min occurred 2.5-3 times less frequently in papillary muscles taken from animals adapted to hypoxia than in the control. Adaptation to stress had an even stronger antitriggering effect: at all imposed frequencies triggering activity was absent, and the data in Table 1 show that only in two of eight cases was delayed post-depolarization observed in response to high frequencies of stimulation. In other words, the two different versions of adaptation were able to suppress triggering activity, but only in the case of adaptation to stress was this suppression total.

The results of recording action potentials, given in Fig. 2 and Table 2, reveal the effect of the immobilization stress endured by the animals and also of adaptation preceding stress on the number of triggering pulses arising in response to imposition of an increasing frequency of stimulation on the preparations. It will be clear from Fig. 2 that in typical experiments, imposition of an increasing frequency of stimulation from 300 to 600 pulses/min combined with administration of isoproterenol led to the appearance of two or three triggering pulses in the control preparations. In preparations taken from animals exposed to stress, resistance to induction of triggering activity was significantly lowered: as a result, in response to imposition of a frequency of 400-600 pulses/min the number of triggering pulses was more than twice as high as in the control. Adaptation to hypoxia, when preceding stress, not only completely abolished the potentiating effect of stress, but also reduced the number of triggering pulses by 2-3 times compared with the control. Adaptation to stress, just as in the experiments described above, completely abolished triggering activity according to both tests used, namely in

relation to both the frequency of onset and the number of triggering pulses arising in response to an equal frequency of stimulation. Adaptation to stress, moreover, undoubtedly gives a stronger antitriggering effect.

When the antitrigger effect of adaptation is assessed, two problems which require discussion are, first, its mechanism and, second, the reasons why the antitrigger effect of adaptation to stress was stronger than that of adaptation to hypoxia. For a provisional estimate of the mechanism of suppression of triggering activity, the data given above on the role of disturbance of the ability of SBR to take up and retain Ca^{2+} in the genesis of this phenomenon must first be taken into consideration. In this connection the fact established in our laboratory that adaptation to repeated stress [13], like adaptation to hypoxia [1], increases the rate of function of the Ca^{2+}pump of SBR, must be taken into account. This ability of the Ca^{2+}-pump of SBR must itself reduce the probability of appearance of triggering activity, which was in fact observed in the experiments described above with both types of adaptation. The more effective antitriggering action of adaptation to stress than adaptation to hypoxia, proved in the present experiments, is much more interesting. When this fact is explained it must be recalled that the general results of adaptation to repeated stress at the cell level is not limited to activation of individual cationic pumps or enzyme systems, but it leads to the formation of a phenomenon of adaptive stabilization of structures (PASS) [6, 14] which, besides stabilization of the cell nuclei [6] and mitochondria [6, 14], is manifested by an increase in resistance of elements of SPR of the heart muscle to autolysis [13]. In full agreement with this, leaking of Ca^{2+} into the incubation medium of preparations from the hearts of adapted animals, preserved for 4 days, was significantly depressed compared with the control [13]. It is this kind of stabilization of the SPR membrane with activation of its Ca^{2+}-pump that, in our view, constitutes the most likely mechanism of total suppression of triggering activity under the influence of adaptation to repeated stress – the main fact discovered in this investigation.

Exact proof was thus obtained of the powerful antiarrhythmic and, in particular, the antitriggering effect of adaptation to environmental factors such as stress and hypoxia.

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