SELECTIVE PACEMAKER BLOCKING AND ATRIAL AUTOMATICITY DISTURBANCE DURING INDUCTION OF LIPID PEROXIDATION

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Induction of lipid peroxidation (LPO) by addition of H_2O_2 to the perfusion fluid of the isolated working heart not only induces LPO in the myocardium [5], but initially also exerts a positive ionotropic effect similar to the action of catecholamines, which is followed by bradyarrhythmia and arrest of the spontaneously contracting atrium; these disturbances of function, moreover, can be prevented by a water-soluble antioxidant of the hydroxypyridine class [3]. In this last investigation, the positive ionotropic effect of H_2O_2, namely an increase in the force of contractions, was maintained also during the bradyarrhythmia which preceded cardiac arrest.

It was accordingly postulated that under certain conditions induction of LPO does not necessarily disturb the function of the contractile myocardium, but may selectively damage the conducting system, and in particular, the pacemaker, and this in turn may cause disturbances of automaticity followed by arrest of the heart.

To test this hypothesis the mechanical activity of the isolated atrium and action potential (AP) of a cardiomyocyte were recorded simultaneously during spontaneous activity of the isolated atrium, acted upon by an LPO inducer, and later, after atrial arrest, the same parameters were recorded during direct electrical stimulation of the contractile myocardium.

EXPERIMENTAL METHOD

Thin strips of right atrial myocardium of Wistar rats were used as the test object. Myocardium was excised from the atrium so that the sinus node remained intact and the strips preserved their spontaneous contractile activity. The strips of myocardium were fixed in a 5-ml perfusion chamber, where they were surrounded by Krebs' solution, aerated with carbogen (pH 7.3-7.4, 30°C). One end of the strip was attached to the wall of the chamber immovably, the other end to the lever of a 6MKh1S mechanotron to record mechanical activity. The signal from the mechanotron was led through an amplifier to one input of an S1-18 oscilloscope. The signal from an intracellular microelectrode, introduced into an atrial cardiomyocyte, was led through a cathode follower and UPT-2 amplifier to the other input of the oscilloscope.

The standard microelectrode technique was used. The microelectrodes were made from Pyrex glass on an ME-4 semiautomatic machine. The length of the microelectrode neck was 25-30 mm and its resistance 10-30 MΩ. For work with floating microelectrodes the microelectrode tip was cut off at the level of the neck with a diamond needle file. The microelectrode was suspended on platinum wire 50 μ in diameter, bent to form two turns of a coil. The electrode was introduced into the beating preparation with the aid of a micromanipulators.

H_2O_2 was added to the incubation medium in a concentration of 5 mg/liter. To study the effect of antioxidant OP-6 it was added to the perfusion solution in a concentration of 150 mg/liter, and 10-15 min later H_2O_2 was added in a concentration of 5 mg/ml.

AP and spontaneous mechanical activity were recorded by means of the FOR-2 photo-optical recorder, and traces were obtained of at least 10 contractions and AP, with tape winding speed of 25 mm/sec. A few contractions and AP also were recorded at a speed of 50 mm/sec to obtain a more accurate estimate of the parameters of AP.

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The force of contractions was recorded on the preparation with an initial length L = 0.3-0.5 L_{max}, where L_{max} is the length of the preparation at which the isometric force developed by it is maximal. The initial length of the preparation was chosen to be small for convenience of introduction of the microelectrode into a weakly beating preparation.

After cessation of spontaneous activity under the influence of H_2O_2 the muscle was stimulated electrically through massive platinum electrodes, arranged parallel to the preparation (field stimulation), and contractions and AP were again recorded.

The effects of H_2O_2 were studied on 10 preparations and the antioxidant effect of OP-6 on eight preparations.

EXPERIMENTAL RESULTS

Spontaneous electrical and mechanical activity disappeared simultaneously under the influence of H_2O_2 on average after 18 ± 3 min: the minimal duration of blockade of spontaneous activity was 7 min and the maximal 30 min. The time course of the effect of H_2O_2 was quite characteristic: in most preparations the spontaneous contraction rate increased immediately after addition of H_2O_2, then fell gradually, and contractions ceased. The regularity of the contractions was always disturbed by H_2O_2: in different preparations and at different moments of time irregular intervals were observed between contractions (Fig. 1). However, in some preparations spontaneous activity was arrested suddenly after an increase in the frequency of contractions.

The strength of isometric contractions was significantly increased (by 3.7 times) by H_2O_2 and stabilized on average after 6 min.

The amplitude of AP was unchanged relative to the control by H_2O_2 but their duration at the 90% repolarization level rose steadily with time from 30 ± 2.2 to 54 ± 3.6 msec, i.e., by 1.8 times (Fig. 2), which probably depended on an increase in the calcium inflow into the cardiomyocyte. This hypothesis is supported by two facts. First, an increase in duration (Fig. 2a) took place mainly on account of the appearance and subsequent enlargement of the AP plateau, which is due to the slow Ca current. Second, an increase in the duration of AP was regularly accompanied by an increase in the force of contractions (Fig. 2b), which can most probably be explained by the positively ionotropic effect of excess Ca. The problem whether Ca inflow into cardiomyocytes is increased during activation of LPO in the plasma membrane because of more intensive functioning of slow Ca channels or whether this is due to the formation of lipid clusters, which has been demonstrated during LPO activation [1], requires further study. Antioxidant OP-6, in a concentration of 150 mg/liter, increased the time from the beginning of action of H_2O_2 to arrest of spontaneous contractions on average by almost 2.5 times, if it acted on the preparation before the peroxide. The mean time until arrest of spontaneous contractions under the influence of OP-6 was 42 ± 5 min; the shortest duration of blockade of spontaneous contractions was 31 min, the longest 120 min. The antioxidant prevented the effect of H_2O_2 on the duration of AP. For instance, at the 20th minute of the action of OP-6 the mean duration of AP was 32 ± 3 msec, falling to 28.5 ± 4 msec at the 40th minute. The increase in the force of contractions under the influence of H_2O_2 preceded by OP-6 was 130%, just over one-third of the increase in the force of contractions produced by H_2O_2 alone.

The LPO inducer thus profoundly disturbed automaticity of the atrium, where the cardiac pacemaker is located, and the antioxidant considerably limited this phenomenon. The main result, from the standpoint of the aim of this investigation, was obtained by electrical stimulation of the atria after their complete arrest. It was found that pacing of this kind in every case caused regular contractions, and their duration and magnitude were approximately the same as those recorded before arrest of spontaneous atrial electrical activity, i.e., at the height of action of the LPO inducer (Fig. 2c, d). This means that cessation of spontaneous atrial electrical activity (atrial arrest) took place before conditions when the contractile myocardium was able to respond to excitatory impulses reaching it with a sufficiently strong contraction.

This situation may perhaps be due to disturbance of the ability of the sinus node, when damaged by LPO induction, to generate excitatory impulses which usually maintain the rhythm of the spontaneously contracting atrium.

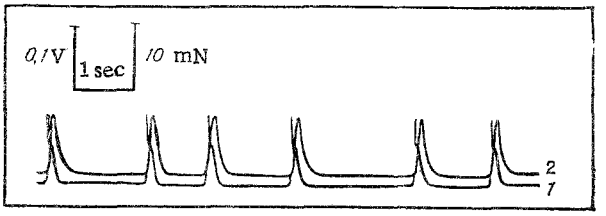

Fig. 1. Irregularity of spontaneous electrical (1) and mechanical (2) activity.

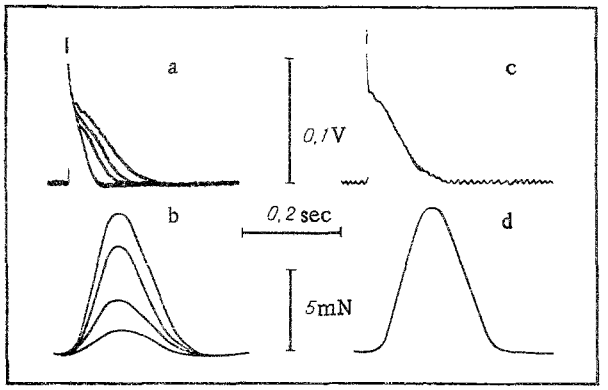

Fig. 2. Effect of H_2O_2 on AP of cardiomyocytes and force of contractions of isolated right atrium. a) Superposition of AP after addition of H_2O_2 (gradual increase in duration of AP under the influence of H_2O_2); b) increase in force of spontaneous contractions under the influence of H_2O_2 (the increase in contractions correlates closely with the increase in the duration of AP); c) AP arises during external stimulation after arrest of spontaneous contractions under the influence of H_2O_2; d) contraction corresponding to the given AP.

When the results are assessed it must be recalled that LPO activation is the key stage of stress-induced damage to the heart [2], which lowers the electrical threshold for fibrillation of the heart significantly and plays a role in sudden cardiac arrest [6, 7]. We also know that in persons frequently subjected to stress or other loading factors accompanied by LPO activation [4] a block of the right branch of the bundle of His and other parts of the conducting system is frequently observed. Accordingly the results suggest that during stress and in other situations activating LPO selective damage to the conducting system may be observed, and antioxidant protection of the heart deserves study as a factor in the prevention of cardiac arrhythmias, fibrillations, and arrest.

LITERATURE CITEE

1. V. E. Kagan, Yu. V. Arkhipenko, L. V. Belousova, and Yu. P. Kozlov, Biofizika, No. 4, 625 (1977).
2. F. Z. Meerson, V. E. Kagan, Yu. P. Kozlov, et al., Kardiologiya, No. 2, 81 (1982).
3. F. Z. Meerson, V. A. Saltykova, V. V. Didenko, et al., Kardiologiya, No. 5, 61 (1984).
4. L. L. Prilipko, O. M. Orlov, S. M. Ivanova, et al., Dokl. Akad. Nauk SSSR, 265, No. 4, 1010 (1983).
5. R. Barsacchi, P. Camici, U. Bottigli, et al., Biochim. Biophys. Acta, 762, 241 (1983).
6. B. Lown, R. L. Verrier, and R. Corbalan, Science, 132, 834 (1973).
7. B. Lown, R. A. Desilva, P. Reich, and B. I. Muranski, Am. J. Psychiat., 137, 1325 (1980).