Reduction of the Hypoxia-Induced Depression in the Intracellular Electrical Activity of the Ventricular Muscle Fibers of the Rabbit Fed on Food Containing Crataegutt®

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SUMMARY

The rabbits were divided at random into 2 groups: one was fed for 6 weeks on the rabbit food containing 5 mg Crataegutt® per 100 Gm food (CR group), and the other was fed for the same period on the commercial rabbit food and used as a control. Intracellular records were made from fibers in the ventricle of isolated rabbit heart. The inhibitory influences of hypoxia on the resting membrane potential and on the amplitude of action potential of the fiber of the CR group are significantly less than those of the control group. However, the inhibitory influence on the time courses of contraction of the papillary muscle was almost the same between these 2 groups.

Additional Indexing Words:

Hypoxia Intracellular potential of the ventricular muscle fiber Ventricular muscle fiber Rabbit heart Crataegutt® Contraction of the papillary muscle Sodium pump

It is well known that electrical activity of the cardiac muscle is closely related to cell metabolism. The shape of the action potential of cardiac muscle cells is markedly influenced by any factor reducing the utilization of metabolic energy (e.g., anoxia, fatigue, enzyme inhibitors). The most prominent change associated with the conditions reducing the utilization of metabolic energy is a progressive shortening of the action potential. Furthermore a reduction of amplitude, a rise in threshold, and a gradual depolarization may usually be observed. These responses of cardiac muscle cell are in marked contrast with those of nerve fibers: nerve fails to exhibit any prominent change in electrical activity associated with these conditions. If there was a drug that could conserve the cell metabolism of cardiac muscle cell against anoxia, the drug may perhaps delay the onset of these

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Received for publication November 7, 1975.
anoxia-induced changes of transmembrane potential. In the present study, experiments have been carried out on cardiac muscle cells of the isolated rabbit ventricles to distinguish the anoxia-induced responses between 2 groups of rabbits; one group was fed on rabbit food (pellets) containing Crataegutt® (a cardiac stimulant extracted from Crataegus oxyacantha and Crataegus monogyna: Willmar Schwabe, W Germany), and the other on commercial rabbit food. The experiments demonstrated that the onset of the anoxia-induced depression of transmembrane potentials was significantly reduced in the group fed on Crataegutt-containing food when compared with those of the control group.

**METHODS**

*Animals.* Experiments were performed on 2 groups of Japanese white rabbit *(Oryctolagus cuniculus)*, male or female, weighing from 2.2 to 2.8 Kg. Each animal was kept in an individual wire cage. The rabbits were divided at random into 2 groups of 10 each. One group was fed for 6 weeks on the rabbit food (pellets) containing 5 mg Crataegutt® per 100 Gm food. This group is called CR (Crataegutt) group hereinafter. The other group of animals was used as a control. They were fed for the same period on the commercial rabbit food. During the feeding period, food and water consumption, and body weight were measured in each of the animals. The time courses of changes in food consumption and body weight were compared between the control group and the CR group in Fig. 1. Analysis
Measurements of transmembrane potentials. Since blood coagulation in the small coronary vessels seem to produce a disturbance of excitability of the cardiac muscle, heparin (Novo, Denmark; 100 U/Kg body weight) was injected prior to the experiments into an ear vein to prevent blood coagulation. The rabbits were then killed by a blow on the head, and their hearts were excised quickly and dissected in cool modified Krebs-Henseleit solution. Isolated free walls of right ventricles were pinned to the wax-lined bottom of a 20 ml Lucite tissue chamber. The isolated ventricles were electrically stimulated by rectangular pulses from an electronic stimulator (Nihon Kohden, SEN-1101, Japan). Stimulating pulses were delivered to the preparations through a pair of chlorided silver electrodes inserted into the edge of the apical region. The pulses were 2 msec in duration, twice threshold, and about 1 Hz in frequency. The level of Krebs-Henseleit solution in the chamber was kept constant with a siphon. The modified Krebs-Henseleit solution had a following composition (mM): NaCl, 131; KCl, 5.6; CaCl₂, 2.5; MgCl₂ 1.0; NaHCO₃, 25; NaH₂PO₄, 1.0; glucose, 5.0. The solution in the reservoir bottle and in the tissue bath was equilibrated with 5% CO₂ in O₂ and had a pH of about 7.2. When the N₂-bubbled solution was used, it was equilibrated with 5% CO₂ in N₂. The solution continuously perfused the tissue bath at a constant flow rate (2 ml/min).

Intracellular recordings were obtained from the cardiac muscle cells on the surface of the ventricle by manually advancing micro-electrodes with a micromanipulator under visual control. Micro-electrodes were drawn with a commercial electrode puller (Narishige, Japan) from Pyrex cored tubing of about 1 mm o.d., and were filled directly with 3 M KCl. Cored tubings were made with an apparatus (Takahashi Shoten, C-555, Japan). The most satisfactory electrodes had resistances of about 20 MΩ. The microelectrode was connected by a chlorided silver wire to the input of a solid state preamplifier (WP Instruments, M 701, USA). A thick chlorided sliver wire served as the indifferent electrode. Potentials were observed on the screen of a cathode ray oscilloscope (Nihon Kohden, ATAC-250), and photographed with a Polaroid camera. To minimize vibration, the micro-manipulator carrying the micro-electrode and the tissue bath were placed on a table supported on a cushion of rubber balls.

Measurements of contractions. For recording contractions of the cardiac muscles, rabbit papillary muscles were excised from the left ventricles immediately after the excision of the free walls of the right ventricles. The basal part of the papillary muscle was pinned with a pair of stimulating electrodes to the wax-lined bottom of another Lucite tissue chamber. The tendon on tip of the papillary muscle was connected with a strain gauge transducer (Nihon Kohden, SB-1T) by a thread, and the mechanical activity was amplified and recorded with an ink-writing oscillograph (San-ei rectigraph, 8, Japan). The other experimental arrangements were similar to those in measurements of transmembrane potentials.
Results

Effects of hypoxia on resting and action potential of the control group. The control response shown in Fig. 2 (record A) is typical of those obtained from the ventricular muscle fibers studied in the present experiments. When perfusion of the tissue bath was switched from the standard oxygenated Krebs-Henseleit solution to the N₂-bubbled solution, the resting potential decreased and so also did the rate of rise of the action potential, its amplitude, and duration (Fig. 2, record B and C). The effects of hypoxia were noticed at about 15 min after perfusion with the N₂-bubbled solution, and were clearly distinguished at about 30 min after the perfusion. In some preparations, there was no or only local response at 30 min after the perfusion.

Similar experiments were carried out in the 10 different preparations of the ventricular muscles of the rabbits. Thus the means (±S.E.) of resting membrane potential, peak of action potential, and several points in the plateau and repolarizing phase were calculated from the potentials recorded at 15 min and 30 min after the perfusion with N₂-bubbled solution (Fig. 3).

Effects of hypoxia on resting and action potential of the CR group. The control response shown in Fig. 4 (record A) is typical of those obtained from the ventricular muscle fiber of the preparation obtained from the CR group. When

![Figure 2](image_url)

Fig. 2. An example of the hypoxia-induced depression in the transmembrane potential of ventricular muscle fiber of the heart of the control group. A: a recording of potential in the control Krebs-Henseleit solution. B: the potential recorded from the same fiber after 15 min of perfusion with the N₂-bubbled solution. C: the potential from the same fiber after 30 min of perfusion with the N₂-bubbled solution.
perfusion of the tissue bath was switched from the standard oxygenated Krebs-Henseleit solution to the \( \text{N}_2 \)-bubbled solution, the resting potential decreased and so also did the rate of rise of the action potential, its amplitude, and duration (Fig. 4, record B and C). The effects of hypoxia were also noticed at about 15 min after perfusion with \( \text{N}_2 \)-bubbled solution, and were clearly distinguished at about 30 min after the perfusion. There was no preparation that produced no or local response at 30 min after the perfusion.

Similar experiments were performed in the 10 different preparations of the ventricular muscles of the rabbits. Thus the means (±S.E.) were also calculated from the potentials recorded at 15 min and 30 min after the perfusion with \( \text{N}_2 \)-bubbled solution (Fig. 5).

The differences between the means of the CR group and the corresponding means of the control group were calculated using Student's \( t \) test.
resting potential of fibers of the CR group at 15 min after perfusion with N₂-bubbled solution was significantly higher than that of the control group (P<0.05). At 30 min after perfusion with N₂-bubbled solution the resting potential and the peak of action potential of fibers of the CR group were significantly higher than that of the control group (P<0.01 and P<0.05 respectively).

*Effect of hypoxia on the time course of contraction.* Contraction of the papillary muscle of the left ventricle of the rabbit was recorded before and after switching the perfusion of the tissue bath from the standard Krebs-Henseleit solution to the N₂-bubbled solution. When perfusion solution of the tissue bath was switched from the standard solution to the N₂-bubbled solution, the amplitude of contraction declined almost exponentially. The declining tendency in the amplitude of contraction was similar in the control and the CR group. Similar experiments were performed in the different preparations of the papillary muscles in the control and CR groups. The means (±S.E.) of per cent decreases in these 2 groups were calculated (Fig. 6). The differences between the means of the CR group and the corresponding values of the control group were calculated using Student’s t test. The differences were not significant in the whole period of the time courses.
DISCUSSION

The principal conclusion permitted by the present studies is that the inhibitory influences of hypoxia on the membrane potential and on the amplitude of action potential of ventricular muscle fiber of the CR group are significantly less than those of the control group. Harth\textsuperscript{10} reported that, in 3 healthy volunteers, inhalation of a gas mixture containing 8% \( \text{O}_2 \) in \( \text{N}_2 \) produced hypotension, tachycardia, and appearance of negative T in ECG, and that these symptoms were alleviated after intravenous injection of 2 ml of alcohol solution of CR. Frank and Heymanns\textsuperscript{12} also reported that, in 10 healthy volunteers, the solution of CR improved the anoxic symptoms produced by inhalation of a gas mixture containing 8% \( \text{O}_2 \) in \( \text{N}_2 \). Harth\textsuperscript{11} showed that a preceding administration of the CR solution prolonged the appearance of cyanosis due to hypoxia in the rat. Trunzler and Schuler\textsuperscript{13}
showed the results that the CR solution enhanced the recovery from anoxic depression of contraction force induced by perfusion with a solution bubbled with 10% O₂ in N₂ in the isolated and perfused heart of guinea pig. However, at least some part of these effects of the CR solution seems to be due to the effect of alcohol, the solvent of the CR solution, for alcohol is known to produce dilatation of the coronary vessels. The present experiments provided, for the first time, the evidence that administration per os of CR significantly diminished the anoxic influences on the electrical activity of the cardiac muscle fibers.

The mechanism of this action of CR remains to be fully defined, for as yet there is no generally acceptable concept of the mode of interaction between metabolism and electrical activity of the heart. Among the different influence of metabolic inhibition, an analysis of the loss of plateau of action potential, which is paralleled by a reduction in contractile strength, has been of primary interest. Haas, Kern, and Einwächter⁶) studied the influences of the metabolic inhibitor, 2, 4-dinitrophenol (DNP), on membrane currents in frog atria by means of the sucrose-gap technique and in tracer experiments. They proposed that under normal conditions an electrogenic potassium inward transport may be involved in the mechanism sustaining the plateau of the cardiac action potential, and that the metabolic inhibitor may suppress the
activity of the pump. Whether this hypothesis could be adequate to explain the loss of plateau in anoxia, or not, it may not be the explanation for the mechanism of the action of CR: the present study showed that the difference between the hypoxia-induced shortening of plateau in the CR group and that of the control group was not significant. Changes in resting potential during anoxia may possibly due to the result of an increased uptake of sodium and loss of potassium by the cardiac muscle cell. Kardesch, Hogancamp, and Bing\textsuperscript{14} have suggested that it is the active sodium pump mechanism that is injured by anoxia. If this is true, then the mechanism of action of CR may be in protecting the activity of the sodium pump against anoxia.

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