EXPERIMENTAL BIOLOGY

Inhibition of Contractile Activity of Cultured Rat Cardiomyocytes in Response to Reduced Oxygenation

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Hypoxia completely abolishes the spontaneous contractile activity of cardiomyocytes. Once the original oxygenation is restored, the cells regain their functional activity, which exceeds the control level (particularly after a 2-h hypoxia). It can be assumed that the rapid restoration of cardiomyocyte functional activity is due to the preservation of a sufficiently high oxygen content and to the compensatory activation of glycolysis.

Key Words: *monolayer cardiomyocyte culture; hypobaric hypoxia; contractile activity; morphofunctional state of mitochondria*

In the whole organism, hypoxic hypoxia reflexively induces a complex of compensatory reactions associated with alterations in respiratory, cardiovascular, and neuroendocrine functions [2,7]. However, oxygen deficiency may have a direct effect on individual cells. For example, it has been found that insufficient oxygenation leads to the suppression of the contractile activity of smooth muscle cells and reduces their response to vasomotor stimuli [1]. Here we examined the effect of lowered oxygenation of the growth medium on the contractile activity of cultured cardiomyocytes (CMC) and on the morphology and function of their mitochondria.

MATERIALS AND METHODS

Primary 4-7-day monolayer cultures of rat CMC were used. The CMC suspension was obtained by trypsinization of minced hearts (ventricles) of 2-5-day-old rat pups. The cells were grown under stationary conditions at 37°C on glass slides in hermetically sealed penicillin flasks filled with medium 199 supplemented with 10% fetal calf serum.

Oxygenation was lowered by placing the flasks (either open or closed with cotton plugs) in a pressure chamber at a pressure of 400 mm Hg, which corresponds to an "altitude" of 5000 m above sea level. The pO_2 was 84 mm Hg, i.e., 2-fold lower than in normal air [6].

The cell cultures were held under conditions of hypoxia for 0.5, 2, and 4 h. They were studied and fixed immediately after the procedure.

The contractility of CMC (phase contrast microscopy) and the morphology and functional activity of mitochondria (fluorescence microscopy) after staining with the cationic stain Rhodamine 123 before placement in the pressure chamber were studied in living cells. Slides with cell cultures were incubated for 10 min at 37°C in medium containing 10 μg/ml Rhodamine 123, washed with Rhodamine-free medium, and returned to the original flasks.

The functional activity of mitochondria was assessed from the accumulation of Rhodamine 123. The intensity of fluorescence reflected the mem-

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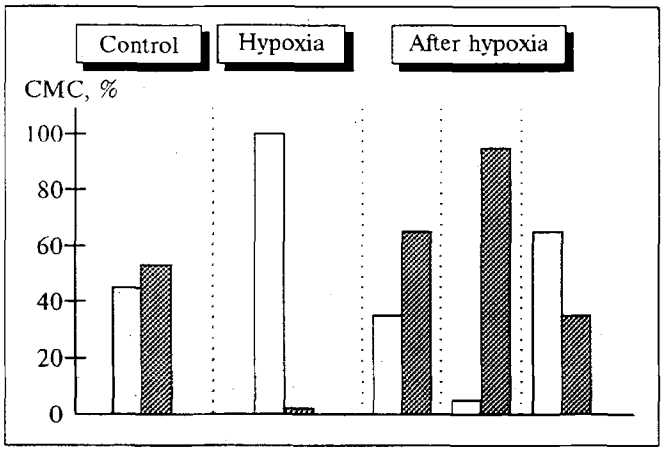

Fig. 1. Changes in the number of contracting (shaded bars) and noncontracting (white bars) cultured rat CMC in the norm and during and after hypoxia lasting 0.5 (*1*), 2 (*2*), and 4 (*3*) h.

brane potential of the mitochondria. i.e., their functional activity.

The ultrastructure of CMC was studied by transmission electron microscopy in a JEM-100B electron microscope.

The cultures were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2-7.4), postfixed with 1% OsO_4 in phosphate buffer, and embedded in Epon by the standard method.

Sections were cut on an LKB microtome and contrasted with lead citrate after Reynolds.

RESULTS

A 4-7-day culture of CMC consists of separate clusters of cells contracting in unison.

Under normal culturing conditions, 50-60% of cells contracted. After 0.5, 2, and 4 h of hypoxia all CMC stopped contracting (Fig. 1). A drop in cardiac contractile activity and a reduced frequency and force of CMC contractions were demonstrated on rats exposed to long-term hypoxia, as well as on isolated hearts and in tissue cultures [4,8,11,12,14]. This response of CMC to hypoxia is due primarily to alterations in oxygen uptake, decreased activity of oxidative enzymes, and disturbances orders of electron transport in the respiratory chain [3,8,11,12]. A decrease in the ATP and creatine phosphate contents and a drop of CMC functional activity have been demonstrated by numerous researchers [3,8,11,12,14,15].

A H^+ accumulation and pH drop (metabolic acidosis) have also been observed in hypoxia [3,9]. This probably also reduced the contractile activity of CMC. Previously we showed that contracting and noncontracting CMC in a tissue culture differ in the intracellular pH [5]. Cultured CMC contract at a pH of 6.7-6.8; when the pH of the culturing medium is <6.0, CMC stop contracting.

Restored to normal conditions, CMC regained their contractile activity (Fig. 1). After a 30-min hypoxia, the contractile activity of CMC was restored almost immediately (within 2-3 min), and 70-80% of cells contracted. After a 2-h hypoxia, CMC contraction was restored in 5-10 min, and the frequency of contractions of all CMC was higher, but this reverted to the baseline level after 30-60 min. After a 4-h hypoxia, CMC regained their contractile activity more slowly, and the number of contracting CMC was lower than in the control (30-40%).

It should be noted that after 0.5 h and, particularly, 2 h of hypoxia the contractile activity of CMC was not only restored but considerably higher than the baseline level. Presumably, this is a manifestation of the general phenomenon of functional stimulation caused by hypoxia [7].

It can be assumed that CMC regain their contractile activity due to replenishment of the pool of high-energy compounds on account of the compensatory activation of glycolysis. An increase in the glycolysis rate has been demonstrated by numerous researchers after both short- and long-term hypoxia on the organism, the isolated heart, and in tissue culture [2,7,9].

The slower and only partial restoration of CMC contractions after a 4-h hypoxia is probably a function of the acidosis caused by lactate accumulation during the longer period of glycolysis activation [9]. It has been reported that CMC cannot regain their contractility after prolonged hypoxia [11].

Thus, a 2-fold decrease in the oxygen content of the culture medium resulted in a complete arrest of spontaneous CMC contractions; after oxygenation the contractile activity of CMC not only was restored but even exceeded the baseline level within the first hour.

Staining with Rhodamine 123 allowed us to visualize two types of mitochondria in CMC: small elongated ones localized at the cell periphery between myofibrils and intensively fluorescing clusters of small and large round mitochondria in the perinuclear zone.

Mitochondrial fluorescence was preserved during hypoxia, indicating that the organelles remain functional. Their ultrastructure remained unchanged.

In some CMC, small intensely fluorescing mitochondria were accumulated in the perinuclear zone during 4-h hypoxia. Clusters of small closely-spaced mitochondria were seen on micrographs. Presumably, this is a consequence of their partial

fragmentation, a phenomenon caused by many damaging factors [10,13].

It should be noted that we chose a moderate hypoxia: the oxygen content in the culture medium was lowered less than 2-fold (53% of the control). In experiments with a culture of pig embryo renal cells, where the oxygen content was lowered 3-fold (30-35% of the control), a decrease in the intensity of fluorescence of mitochondria and their condensation were observed only after 6 h of hypoxia.

It can be assumed that the weak response of mitochondria to oxygen deficiency and the rapid restoration of contractile activity of CMC are due to the short-term hypoxia and sufficiently high oxygen content.

It should be taken into account that alterations in the oxygenation of cultured CMC differ considerably from those *in vivo*. Under normal conditions, pO_2 in the myocardium is several tens and in the mitochondria several mm Hg. The baseline oxygenation level in a CMC culture is considerably higher than that in the working myocardium. Nevertheless, a 2-fold reduction of this comparatively high oxygenation had a pronounced effect on the functional activity of CMC.

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