EFFECT OF CYCLIC GUANOSINE MONOPHOSPHATE ON SOME INDICES OF CARBOHYDRATE METABOLISM OF MUSCLE TISSUE DURING WOUND HEALING

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Since the discovery of cyclic guanosine monophosphate (GMP) by Price et al. [7, 9] many observations have been made to confirm the important role of this nucleotide in the regulation of many different processes in the cell and, in particular, in cell proliferation and division [6, 10, 11]. Meanwhile the effect of cyclic GMP on the course of energy-forming and oxidation–reduction processes [2, 4] has been very inadequately studied, especially in pathological states. There have been only isolated investigations of this problem, yet such studies during wound healing would be particularly important, for the state of energy formation largely determines the intensity of the course of repair processes during wound healing.

The object of this investigation was to study the effect of cyclic GMP on glycogen metabolism and also on the activity of oxidation–reduction enzymes lactate dehydrogenase (LDH) and malate dehydrogenase in the muscle tissue of a wound at different stages of healing.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-220 g. A linear skin wound was inflicted on the animals and a nichrome coil (0.4 × 3 cm) was implanted beneath the skin in the dorsal region to induce granulation tissue formation. Cyclic GMP was injected intraperitoneally into the rats in a dose of 0.5 mg per animal 30 min after the operation. The investigation was carried out on the 3rd, 5th, and 7th days after the operation.

The following indices were investigated in the muscle tissue of the wound: the glycogen concentration [8], gluconeogenesis, as reflected in the incorporation of ^{14}C-glycine into glycogen. For this purpose, 2 h before sacrifice, the animals were given an intraperitoneal injection of ^{14}C-glycine (specific activity 670 μCi/mg) in a dose of 0.6 μCi/g body weight. Activity of the following enzymes also was determined in the muscles of the wound: glycogen phosphorylase [3], expressed in micrograms phosphorus liberated per milligram protein per minute, and also LDH [12] and malate dehydrogenase [5].

EXPERIMENTAL RESULTS

Data characterizing glycogen metabolism in the muscle tissue in the region of the wound are given in Table 1. During the course of wound healing on the 5th day after the operation considerable activation of glycogen metabolism was observed: Gluconeogenesis was increased sharply (threefold), activity of glycogen phosphorylase was increased, and consequently the glycogen concentration fell, evidence of predominance of breakdown of glycogen over its synthesis. In the experimental animals treated with cyclic GMP after the operation this intensification of glycogen metabolism was observed sooner after the operation — on the 3rd day.

Investigation of activity of LDH, which catalyzes the final stage of glycolysis (Fig. 1), showed that in wounded animals not treated with cyclic GMP the activity of this enzyme was

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TABLE 1. Effect of Cyclic GMP on Glycogen Metabolism in Muscle Tissue of Wound (M ± m)

Experimental conditions	Day after operation	Glycogen concentration, mg%	Incorporation of ^{14}C, cpm/10 mg	Glycogen phosphorylase activity, μg phosphorus/mg protein/min
Intact rats	—	326,5±24,09	1553±397,76	4,24±0,57
Wounded rats				
without cyclic GMP	3rd	226,7±35,89	1279±274,27	4,50±0,35
with cyclic GMP		97,8±30,17*	4502±820,09*	5,73±0,38*
without cyclic GMP	5rd	143,5±6,10*	4918±836,01*	6,66±0,44*
with cyclic GMP		407,6±41,10	1472±494,25	5,20±0,16
without cyclic GMP	7th	313,3±36,48	1398±518,81	4,88±0,67
with cyclic GMP		347,8±14,12	1582±681,15	4,93±0,24

*Differences statistically significant compared with corresponding values in intact animals.

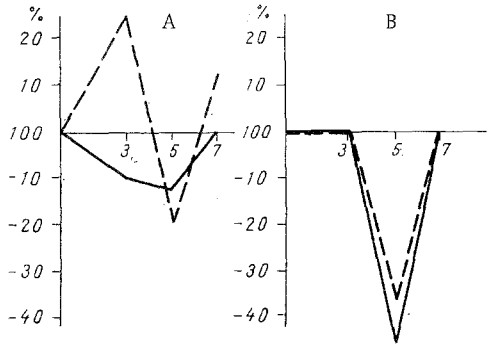

Fig. 1. Effect of exogenous cyclic GMP on LDH (A) and malate dehydrogenase (B) activity. Abscissa, days after operation; ordinate, LDH and malate dehydrogenase activity (in %). Continuous line — control (wounded) animals; broken line — wounded animals treated with cyclic GMP. Corresponding values for intact animals taken as 100%.

slightly reduced on the 3rd day after the operation (by 10%; $P < 0.05$), on the 5th day it still remained depressed (by 12.6%; $P < 0.05$), and it was restored to normal on the 7th day after the operation.

In the wounded animals treated with cyclic GMP, a marked increase in LDH activity (by 25%; $P < 0.05$ compared with the corresponding values in intact animals) was observed on the 3rd day after the operation, and by the 5th day after the operation, just as was the case in animals not treated with cyclic GMP, this increase gave way to a decrease. On the 7th day after the operation LDH activity was a little higher than in the controls.

The dynamics of malate dehydrogenase activity at different times after the operation remained practically the same in wounded animals receiving or not receiving cyclic GMP.

The results are evidence that the effect of cyclic GMP is to cause earlier activation of metabolism of glycogen, the main energy reserve of muscle tissue, in the muscles of the wound. Correlation was found between changes in activity of glycogen phosphorylase, the enzyme of glycogenolysis, and activity of LDH, the enzyme of the final stage of glycolysis, under the influence of cyclic GMP: Both were increased on the 3rd day after the operation, indicating considerable intensification of glycolytic processes in muscle tissue under the influence of this nucleotide.

This early mobilization of the energy resources of muscle tissue during wound healing is important, for muscles are one of the sources of formation of granulation tissue during wound healing.

The results of this investigation are in harmony with earlier observations showing the more rapid rate of wound healing under the influence of cyclic GMP [1], and they demonstrate the activating effect of cyclic GMP on energy formation in the muscle of a wound, an effect which evidently contributes to the intensification of repair processes.

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EFFECT OF THE PERIOD OF PREGNANCY ON GROWTH-REGULATING PROPERTIES OF THE MATERNAL BLOOD SERUM *in vitro*

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The use of blood sera from various animals and man as additional components in media for organ and tissue culture *in vitro* is widely familar [6, 7]. More recently many investigations have been published in which homologous sera were used for this purpose: rat [5, 8-10], rabbit [3], etc. By means of allogenic rat serum, New [8] cultured whole embryos and thereby proved that the sex of the animal from which the serum was obtained is of no importance.

In investigations to study the nature and mechanisms of action of growth-regulating factors in embryogenesis of the lung, conducted by the method of primary monolayer culture of embryonic rat lung [2], we have used different homologous blood sera from females and, in particular, blood serum from pregnant animals. With the onset of pregnancy, various specific biological substances, whose composition and concentration vary depending on the stage of pregnancy, enter the blood stream and may perhaps be reflected in the character of growth of the monolayer. The investigation described below was devoted to the study of this problem.

EXPERIMENTAL METHOD

A cell suspension for primary monolayer culture was obtained from the lungs of 19-day Wistar rat fetuses. The technique of preparation of the material and of culture was described previously [2]. In the present investigation 10% homologous serum from rats at the stages of 9, 9.5, 10, 12, and 18 days of pregnancy was added to the medium No. 199. According to the classification suggested by Professor A. P. Dyban's laboratory [1], this corresponded to stages 10, 11, 12, 14-15, and 20-21 of embryonic development of rats. At each time five series of experiments were performed. The numerical data were analyzed on the Nairi-K computer. The significance of differences was evaluated by Student's t-test.

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