LITERATURE CITED

1. S. M. Bychkov and M. M. Zakharova, Vopr. Med. Khim., No. 3, 227 (1979).
2. S. M. Bychkov and M. D. Kolesnikova, Biokhimiya, No. 1, 204 (1969).
3. S. M. Bychkov and S. A. Kuz'mina, Byull. Éksp. Biol. Med., No. 3, 284 (1977).
4. S. M. Bychkov and S. A. Kuz'mina, Byull. Éksp. Biol. Med., No. 11, 562 (1977).
5. S. M. Bychkov and V. N. Kharlamova, Biokhimiya, 33, 840 (1968).
6. J. S. Brimacombe and J. M. Webber, Mucopolysaccharides, Amsterdam (1964), p. 48.
7. T. E. Hardingham, in: The Methodology of Connective Tissue Research, Oxford (1976), p. 153.

EFFECT OF IMIDAZOLE ON SOME METABOLIC PROCESSES IN WOUND TISSUES IN RATS

M. A. Zaidenberg, S. A. Pisarzhevskii, G. N. Dudnikova, and A. A. Karelin

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KEY WORDS: wounds; imidazole; collagen; DNA; RNA.

The participation of cyclic nucleotides in cell proliferation and differentiation makes the study of their effect on repair processes and, in particular, during wound healing, particularly urgent. It was shown previously that administration of exogenous cAMP [2] and cGMP [3, 4] to animals after operations gives rise to a broad spectrum of changes in wound tissue metabolism, and thus evidently gives rise to an increase in the collagen content of the granulations. It has also been shown that a single injection of cGMP immediately after an operation in a near-physiological dose causes early activation of collagen biosynthesis in the granulations, skin, and muscles of wounds, activation of biosynthesis of various muscle proteins, and early mobilization of energy resources – glycogen metabolism and glycolysis [5]. The changes in metabolism thus observed contribute to the intensification of repair processes, as has been confirmed by morphological study and measurement of the area of wounds in control and experimental animals in the course of healing.

The facts so far obtained suggest that substances modulating the activity of the guanylate cyclase system may have a significant influence on metabolism and the regenerative capacity of injured tissues. One such substance is imidazole, an inactivator of 3',5'-AMP phosphodiesterase and an inhibitor of enzymic hydrolysis of 3',5'-GMP. Imidazole thereby alters the relative levels of cAMP and cGMP in the direction of an increase in concentration of the latter [7]. Imidazole also is interesting because it is a component of carnosine, which is known for its physiological activity [6] and its wound-healing action [9], and also of levamisole, which stimulates certain immune processes. The object of this investigation was to study the effect of imidazole administration on metabolic processes characterizing regeneration in wound tissues.

EXPERIMENTAL METHOD

Experiments were carried out on 70 male albino rats weighing 150-170 g with experimental wounds: a linear skin incision with a nichrome coil, inducing granulation tissue formation [4], implanted subcutaneously. The animals were divided into three groups with 10-12 rats in each group: 1) control animals (undergoing the operation), and 2 and 3) experimental animals. Imidazole was injected intraperitoneally into the experimental rats in 0.5 ml of 0.14 M NaCl solution 30 min after the operation in a dose of 5 mg (group 2) or 10 mg (group 3). The animals were investigated on the 5th day after the operation. The collagen content was measured [8] in the wound tissues (skin, muscles, and granulations). In addition, the content

A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow.
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TABLE 1. Effect of Imidazole on DNA, RNA, and Collagen Content in Wound Tissues in Rats

Experimental conditions	Granulation tissue			Skin	Muscles
	DNA, μg/mg	RNA, μg/mg	collagen, g/100 g	collagen, g/100 g	
Control	2,57±0,40	3,70±0,06	2,41±0,34	6,63±0,57	1,54±0,16
Injection of imidazole					
5 mg	3,42±0,24	5,41±0,67	3,57±0,37	8,40±0,69	1,50±0,21
P	<0,02 (+33%)	<0,02 (+46%)	<0,02 (+48%)	<0,05 (27%)	
10 mg	4,45±0,56	7,44±0,46	4,25±0,30	9,40±0,70	1,46±0,17
P	<0,001 (+73%)	<0,001 (+101%)	<0,001 (+76%)	<0,02 (+40%)	

of DNA and RNA [1] was determined in the granulation tissue, and in the animals of group 2 collagen biosynthesis was investigated *in vitro* in the system in [11], modified by the writers. As labeled precursor D,L-proline-3,4,5-$^3H_3$ (specific activity 3.5 Ci/mmole) was used. The specific radioactivity of hydroxyproline was determined by the method in [10]. A morphological analysis of the granulation tissue by autoradiography also was undertaken on group 2. To obtain autoradiographs pieces of granulations weighing 50 mg were incubated for 8 h at 37°C [11] in the presence of 24 μCi of D,L-proline-3,4,5-$^3H_3$. The autoradiographs were prepared with M photographic emulsion on sections 1-2 μ thick. Exposure was 7 days at 4°C. Grains of reduced silver were counted above 100 fibroblasts and in the intercellular space in the immediate vicinity of these cells. The numerical results were subjected to statistical analysis by Wilcoxon's test.

EXPERIMENTAL RESULTS

Injection of imidazole into the animals undergoing operation (Table 1) increased the DNA and RNA concentrations in the animals of group 2 compared with the control by 33% ($P < 0.02$) and 46% ($P < 0.02$) respectively, and in the animals of group 3 by 73% ($P < 0.001$) and 101% ($P < 0.001$) respectively. Meanwhile the collagen concentration in the granulations was increased by 48% ($P < 0.02$) and 76% ($P < 0.001$) in the animals of groups 2 and 3 respectively. Changes in the intensity of anabolic processes in the granulations were confirmed by the results of a study of biosynthesis: in the experimental animals specific radioactivity, determined as incorporation of labeled hydroxyproline, was increased after injection of imidazole by 60% compared with the control. Determination of collagen in the skin also showed an increase in its concentration in the animals of group 2 by 27% ($P < 0.05$) and in the animals of group 3 by 40% ($P < 0.02$). Collagen biosynthesis was activated in these experiments (+20%). No changes were found in the collagen content in muscles adjacent to the coil.

The results of the morphological study showed that on the 5th day after the operation signs of inflammation were rather less well marked in the wounds of the experimental animals than in the control rats. Granulation and epithelization of the wound edges proceeded more actively. Foci of granulations had a marked tendency to merge and consisted of large, juicy fibroblasts with oval or round nuclei, containing two or three eccentrically arranged nucleoli. The basophilic cytoplasm of these cells had outgrowths of different lengths. The fibroblasts themselves were arranged in parallel rows close to the newly formed capillaries. Quantitative analysis of the autoradiographs showed that the label in the wounds of the control rats was located mainly above cells, and only a very small fraction of it was above the intercellular space. In the rats receiving imidazole the amount of label above and close to the fibroblast was 2.5 times greater than in the control animals. Most label was projected above the intercellular space under these circumstances, and together with the overall larger number of grains of silver in the experimental animals, this is evidence of an increased rate of protein synthesis and a more rapid outflow of proteins into the extracellular space.

It can be concluded from these data that injection of imidazole into animals undergoing operations intensifies biosynthesis in the wound tissues. It can also be postulated that the wound-healing action of carnosine is due to its structural component, namely the imidazole ring, which activates the guanylate cyclase system.

LITERATURE CITED

1. E. M. Zhantieva and N. V. Bogoyavlenskaya, Vopr. Med. Khim., No. 5, 545 (1972).

2. M. A. Zaidenberg, R. N. Korotkina, and A. A. Karelin, in: Cyclic Nucleotides [in Russian], Kiev (1980), p. 43
3. M. A. Zaidenberg, R. N. Korotkina, I. M. Nosova, et al., in: The Cyclase System and its Role in the Regulation of Cell Metabolism [in Russian], Tashkent (1978), p. 114.
4. M. A. Zaidenberg, R. N. Korotkina, I. M. Nosova, et al., Vopr. Med. Khim., No. 6, 770 (1980).
5. R. N. Korotkina, I. M. Nosova, M. A. Zaidenberg, et al., Byull. Éksp. Biol. Med., No. 9, 361 (1980).
6. S. E. Severin, Vest. Akad. Med. Nauk SSSR, No. 9, 31 (1976).
7. N. A. Fedorov, Usp. Sovrem. Biol., 82, No. 1 (4), 34 (1976).
8. J. Bergman and R. Loxley, Anal. Chem., 35, 1961 (1963).
9. K. Nagai and T. Jamane, Heterocycles, 10, 277 (1978).
10. M. Rojkind and E. Gonzalez, Anal. Biochem., 57, 1 (1974).
11. J. Uitto, Biochem. Biophys. Acta, 201, 438 (1970).

EFFECT OF CALMODULIN AND FATTY ACIDS ON Ca-DEPENDENT ADENOSINE TRIPHOSPHATASE IN THE MUCOSA OF THE SMALL INTESTINE

V. A. Yurkiv, S. E. Severin, and V. I. Shvets

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Cells of the mucosa differ from cells of many other tissues in that the area of their cytoplasmic membrane is several times greater than the area of the intracellular membranes. This feature of the morphology of the plasma membrane in the mucosa is connected with its high transport activity. A decisive role in many secretory processes (transport of amino acids and sugars, diffusion of water, active and passive transport of Na^+, K^+, HCO_3^-, Cl^-) through the membrane is played by Ca^{++} ions.

Just as in other tissues, the Ca^{++} concentration in the cytoplasm of mucosal cells is several orders of magnitude lower than in the extracellular medium. There is a continuous passive inflow of Ca^{++} inside the cells, which is considerably increased by the action of various stimulators of secretory processes: hormones and neuromediators, and also many biologically active substances which enter the intestine together with the food [1, 2]. To maintain the Ca^{++} concentration gradient in the plasma membranes of the mucosa, systems of Ca^{++} transport against their concentration gradient must operate. In some tissues transport of this kind is effected by Na–Ca exchange [1]. Active transport of Ca^{++} on account of functioning of a special Ca-ATPase in the plasma membrane also is known. This mechanism of Ca^{++} release from the cell has been demonstrated most convincingly for erythrocytes [10]. The Ca-ATPase activity of erythrocytes can be regulated by calmodulin [5, 6]. This substance is found in many tissues, including the mucosa of the small intestine [3].

Calmodulin evidently activates the Ca-ATPase of the sarcolemma of the heart by increasing the rate of Ca-dependent phosphorylation of membrane proteins [11]. Activation of Ca-ATPase in erythrocytes is achieved by direct interaction between the enzyme and calmodulin [8]. Taking this into account, a one-stage method of isolating homogenous Ca-ATPase from erythrocytes based on affinity chromatography of solubilized membranes on calmodulin-sepharose has been developed.

The mechanism of active transport of Ca^{++} from cells of the mucosa into the intercellular space has not been established. The existence of Ca-ATPase in the cytoplasmic membranes

Laboratory of Molecular Bases of Pathogenesis of Infectious Diseases, Central Research Institute of Epidemiology, Ministry of Health of the USSR, Moscow. Department of Chemistry and Technology of Fine Organic Compounds, M. V. Lomonosov Moscow Institute of Fine Chemical Technology. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 6, pp. 62-65, June 1983. Original article submitted July 7, 1982.

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