EFFECT OF GLYCINE ON REPAIR PROCESSES IN TISSUES OF EXPERIMENTAL WOUNDS

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The ability of glycine, when injected repeatedly, to stimulate protein synthesis in intact and burned animals [4] and the use of glycine for parenteral feeding as a constituent of various mixtures of amino acids to promote wound healing [5] make the study of the possible effect of glycine on repair processes in wound tissues an important task. Since an excess of glycine gives rise to toxic effects *in vivo* [1], in the present investigation the effect of glycine in near-physiological concentrations on healing and on certain metabolic processes in wound tissues of experimental animals was studied. The area of the wound, the content of DNA, RNA, and collagen, the intensity of collagen synthesis, the concentration of cyclic nucleotides, and the blood levels of insulin and cortisol – important regulators of tissue metabolism – were determined. A parallel morphological analysis of the wound tissues was undertaken.

EXPERIMENTAL METHOD

Experiments were carried out on 150 male albino rats weighing 150-170 g. Full-thickness skin wounds measuring 2.5 X 2.0 cm (500 mm^2) were inflicted in the dorsal region. Animals of the two experimental groups were given glycine by intraperitoneal injection immediately after the operation and on the next 2 days; the first group recieved 0.5 mg and the second group recieved 2 mg in 0.5 ml of 0.14 M NaCl. The control animals (operation only) received 0.5 ml of the solvent. On the 7th day after the operation, at a time of intensive proliferative changes in the animals, the scab was removed and the area of the wound measured. The concentration of collagen and the rate of its biosynthesis were determined [7] in the wound tissues (skin and granulation); 2 h before the investigation the animals were given an intraperitoneal injection of $^{14}C_1$-glycine (specific radioactivity 47 mCi/mmoles) in a dose of 0.6 mCi/g body weight. The DNA and RNA content was determined in the granulations [3]. Cyclic nucleotides (cyclic AMP and GMP) were determined in the muscles and granulations on the 1st and 7th days after the operation by a radioimmunologic method using kits from the Radiochemical Centre, Amersham (England). The blood plasma insulin and cortisol levels in the intact and experimental animals were determined 15-60 min after injection of glycine in a dose of 0.5 or 2.0 mg per rat. The concentration of these hormones was determined by test kits from Hoechst and the Radiochemical Centre. Morphological analysis was carried out on the 7th day after the operation.

EXPERIMENTAL RESULTS

The area of the wound of the animals receiving glycine on the 7th day after its administration was significantly less than in the control animals: by 33% in group 1 ($P < 0.001$) and by 30% in group 2 ($P < 0.001$). Determination of nucleic acids showed that the DNA level in the granulations was 1.5 times higher ($P < 0.05$) in the animals of group 1 and 1.4 times higher ($P < 0.05$) in the animals of group 2 than in the controls. The RNA content in the granulations was unchanged by glycine. Investigation of collagen biosynthesis showed practically no difference in specific radioactivity in the granulations of the animals of group 1 on the 7th day compared with the control animals, whereas in the skin it had increased by 51% ($P < 0.05$). In the animals of group 2 no changes were found in the intensity of collagen synthesis in the skin, whereas in the granulations uptake of $^{14}C_1$-glycine into collagen was reduced by 26% ($P < 0.01$). The collagen content was changed only in the animals of group 2, in the skin it was increased by 20% ($P < 0.01$).

The study of the action of glycine on regulatory systems of the wound tissue cells showed that in the muscles of the animals of group 1 the cyclic AMP content was significantly increased (30%; $P < 0.05$) as early as after 1 day. The cyclic GMP concentration was unchanged. On the 7th day after the operation the cyclic AMP level was raised by 106% ($P < 0.05$) in the muscles and by 58.6% ($P < 0.05$) in the granulations compared with the corresponding values in the control animals (Fig. 1), whereas the cyclic GMP concentration in these tissues was virtually unchanged. The cyclic AMP/cyclic GMP ratio in the muscles and granulations of the experimental animals was thus increased by 1.6-2.0 times.

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TABLE 1. Effect of Glycine Administration to Experimental Animals on Changes in Wound Area, Nucleic Acid and Collagen Concentration in Granulations, and Rate of Collagen Biosynthesis in Skin and Granulations on 7th Day after Operation (M ± m)

Experimental conditions	Area of wound, mm^2	Nucleic acids, mg/g granulation tissue		Collagen			
				granulation tissue		skin	
		DNA	RNA	content, g/100 g tissue	rate of biosynthesis, cpm/mg protein	content, g/100 g tissue	rate of biosynthesis, cpm/mg protein
Control	450±52	1,98±0,43	5,83±1,28	11,32±0,88	913±18	42,63±3,97	218±9
Glycine injected intraperitoneally,							
0.5 mg, 3 times	303±46	3,05±0,23	5,52±0,72	9,41±0,14	939±17	48,51±4,32	330±4
P	<0,001	<0,05					<0,05
2.0 mg, 3 times	316±31	2,71±0,55	5,23±1,05	10,29±1,69	678±25	56,60±1,03	205±10
P	<0,001	<0,5			<0,05	<0,01	

TABLE 2. Insulin and Cortisol Concentrations (in μg/100 ml plasma) in Rats' Blood Plasma after Glycine Administration

Experimental conditions	Time of taking blood after injection of glycine, min	Intact rats			Fed on standard diet		
		fed on standard diet		starved for 24 h before beginning of experiment	fed on standard diet		starved for 24 h before beginning of experiment
		insulin	cortisol	insulin	insulin	cortisol	insulin
Control	—	20,8±2,2	13,1±0,3	34,7±3,4	7,0±2,9	22,3±1,5	19,3±3,0
Injection of glycine, mg							
0,5	15			36,8±3,0			
0,5	30	20,9±1,1	13,5±1,1	34,3±10,9	18,0±2,0	6,6±4,4	15,0±2,7
0,5	60	22,5±2,2	16,0±1,6		5,4±2,3	7,4±4,2	
2				45,2±7,6			

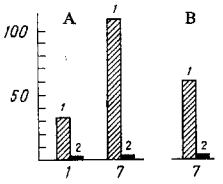

Fig. 1. Effect of glycine on cyclic nucleotide levels in wound tissues: in muscles (A) and granulations (B). Abscissa, time after operation (in days); ordinate, content of cyclic nucleotides (in % of control). 1) Cyclic AMP, 2) cyclic GMP.

Data on the cortisol and insulin levels in the rats' blood are given in Table 2. Clearly glycine did not affect their concentrations in the doses tested.

It can be concluded from the planimetric and biochemical data that glycine intensifies repair processes in the region of the wound. This was confirmed by morphological analysis of the granulation tissue. On the 7th day after the operation the manifestations of inflammation and edema were less marked in the experimental animals than in the control rats (no distinct inflammatory barrier was present). Fibroclasts were more numerous (by 20%, $P < 0.05$) in the granulation tissue than in the control, and mature cells predominated over juvenile and fibroblast-like cells. Peripheral epithelization of the wound also was more marked in the experimental animals.

Reduction in the area of the wound is a complex process which depends on several factors: growth of granulations, contraction of the surrounding skin, epithelization, and so on. Nowadays most authors [2] incline to the view that the

process of concentric indrawing of the wound edges is largely due to growth of granulation tissue, which actively contracts and draws in the wound edges. Under the experimental conditions used, glycine caused a significant increase in the DNA content in the granulations, regularly associated with an increase in the number of cells, including fibroblasts, their earlier maturation, and stimulation of peripheral epithelization. All these effects led to more rapid wound healing. This action of glycine is evidently based on its unique metabolic properties. Glycine is a precursor of many biologically active substances directly related to repair processes: purine bases, glutathione, α-ketoglutaric acid, and many others. Attention is also drawn to the increase in the cyclic AMP content discovered in the present investigation in granulation tissue in the early periods of wound healing and changes in the cyclic AMP/cyclic GMP ratio which, in turn, promote intensification of repair processes.

There is no doubt that administration of glycine in near-physiological doses triggers a system of cascade reactions the final result of which is to accelerate wound healing.

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PROLIFERATIVE ACTIVITY OF CELLS IN DYSHORMONAL FIBROADENOMATOSIS OF THE HUMAN BREAST

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In many cases the development of breast cancer is preceded by so-called dyshormonal dysplasia. These diseases can be regarded clinically and morphologically as precancerous, although not all forms of adenomatosis go on to cancer [2]. One condition that increases the risk of onset of malignant disease is the presence of considerable endocrine disturbances in women [7]. The main criterion for assessment of both dyshormonal dysplasias and malignant diseases is the degree and character of proliferation of the epithelium. Proliferative adenomatosis has been shown to become malignant more often than the nonproliferative form [5, 14]. Nevertheless, clarification of the precise character of proliferative activity of the epithelium from this point of view is beset by great difficulties. This explains the continued search for new methods making the differential diagnosis between dyshormonal fibroadenomatosis and malignant tumors of the human breast easier for both clinicians and pathologists [6].

The object of the present investigation was to study proliferative activity of the epithelium in dyshormonal fibroadenomatosis of the breast.

EXPERIMENTAL METHOD

The method of tissue culture of human breast affected by fibroadenomatosis in diffusion chambers implanted intraperitoneally in animals [3], and using morphological and autoradiographic methods of analysis of the growing cells [10] was used.

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