MECHANISM OF ACTION OF COLLAGEN ON REGENERATION

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Collagen preparations have recently found wide application as stimulators of healing of wounds, burns, and trophic ulcers [4]. The mechanism of the wound-healing effect of collagen has been discussed in the literature. In particular, it has been postulated [8] that the mechanism of action of collagen is closely bound with its resorption in the wound by macrophages. Under these circumstances macrophages secrete a factor which is evidently responsible for stimulating repair processes and which acts on fibroblasts. At the same time, the role of source of supply of amino acids, whose action on the regulatory systems of the cell as a whole is the subject of an extensive literature [1, 11], to the wound tissues, has been ascribed to the macrophages. However, the mechanism of action of collagen on regeneration and wound healing processes has not yet been explained.

It was accordingly decided to undertake the following tasks: 1) to study the effect of collagen in a state of resorption on the system of universal regulators of intracellular metabolism, responsible for neurohumoral influences on the cell, namely the wound tissue cyclic nucleotide system; 2) to compare the action of collagen on the cyclic nucleotide system of wound tissues together with parenteral action of a complete set of amino acids characteristic of collagen (collagen hydrolysate); 3) to determine whether the action of "collagen" amino acids on the cyclic nucleotide system is due to the action of glycine, the relative percentage of which in collagen is greater than that of any other amino acid.

EXPERIMENTAL METHOD

An excised wound in the spinal region, into which a Fluoroplast ring was sutured [3], was used as the experimental model. Collagen was obtained from bovine dermis by alkaline-salt treatment and by drying on a KRS-spray dryer [4]. The collagen hydrolysate was obtained by acid hydrolysis of collagen with 6N HCl at 136°C for 6 h. The acid was removed after hydrolysis by evaporation and by washing twice or three times with distilled water and evaporation, the dry hydrolysate was dissolved in physiological saline, purified with activated charcoal, and the pH adjusted to 7.0. The amino acids were used in near-physiological doses, and the dose of glycine used corresponded to the amount of it contained in the quantities of hydrolysate used. Collagen was applied to the wound locally in a dose of 133.3 mg/kg body weight [4]. The hydrolysate was injected in a dose of 11.1 mg/kg immediately after the operation and on the following day 2 h before sacrifice. Glycine was injected intraperitoneally by a similar scheme in a dose of 3.3 mg/kg.

The material was collected on the first day after trauma, i.e., at times when active resorption of the injected collagen was taking place but had not yet ended. The muscle in the floor of the wound was investigated. The content of cyclic nucleotides was determined by a radioimmunologic method. Cyclic AMP phosphodiesterase activity was determined by Hunt and Kemp's method in the modification of Tkachuk et al. [5]. The basal phosphodiesterase activity and also activity of the enzyme against the background of the stimulating effect of imidazole were investigated. The final concentration of imidazole was 20 mM. The protein content was measured by Lowry's method [9]. Altogether 54 male rats weighing 150 g were used.

EXPERIMENTAL RESULTS

Administration of collagen led to a decrease of 48.2% in the cyclic AMP concentration in the muscle in the floor of the wound ($P < 0.05$). The cyclic GMP level was unchanged. The use of hydrolysate led to a similar response of the cyclic nucleotides, although by a rather lesser degree. For instance, the cyclic AMP concentration was reduced by 12.5% although the cyclic GMP concentration was unchanged. Glycine gave the opposite effect. The cyclic AMP concentration was increased by 77.4% ($P < 0.05$), the cyclic GMP level was reduced by 17.3%.

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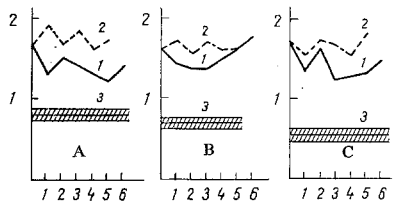

Fig. 1. Effect of collagen (A), collagen hydrolysate (B), and glycine (C) on cyclic nucleotide concentrations in wound muscle.

TABLE 1. Basal and Imidazole-Stimulated Activity of Cyclic AMP Phosphodiesterase (in pmoles cyclic AMP/min/mg protein) in Wound Muscles (M ± m)

Experimental conditions	Phosphodiesterase activity		Activation, %
	basal	imidazole-stimulated	
Control	217±19,5	476±39,8	+119,3
Collagen	186±17,3	310±30,1	+66,6
Hydrolysate	195±20,2	308±32,7	+57,9

Legend. Imidazole was added to incubation medium up to a final concentration of 20 mM.

The basal phosphodiesterase activity was virtually unchanged by collagen and its hydrolysate (Table 1). *In vitro,* imidazole caused activation of phosphodiesterase in all series of the experiment, but the degree of activation differed: Whereas in animals undergoing the operation imidazole increased phosphodiesterase activity by 119% ($P < 0.05$), in animals receiving collagen and its hydrolysate the increase in phosphodiesterase activity was 66.6 and 57.9%, respectively.

The investigations thus showed a decrease in the cyclic AMP concentration in the wound tissues under the influence of collagen, probably due to inhibition of adenylate cyclase. A similar action of collagen has been observed, incidentally, in relation to platelet adenylate cyclase [6]. Meanwhile, although collagen did not change the basal phosphodiesterase activity, it reduced the imidazole-stimulated phosphodiesterase activity, evidence of a disturbance of the functional properties of the enzyme and of the possibility of a potential inhibitory effect of collagen on phosphodiesterase.

Previous investigations [2] showed that in the early stages of healing the cyclic nucleotide concentration rises in wound tissues, as a result of intensified liberation of catecholamines, and also possibly in connection with increased production of mediators of inflammation: histamine, serotonin, and prostaglandins [7, 10]. Very probably the effect of collagen observed in the present experiments on the cyclic AMP concentration results in modification of the phenomena of inflammation and lies at the basis of the subsequent stimulating action of collagen on regeneration processes.

When the effect of collagen on the cyclic nucleotide system of wound tissues is compared with the parental action of the complete set of amino acids characteristic of collagen in collagen hydrolysate, a noteworthy feature is that the response of the cyclic nucleotides to collagen and to its hydrolysate is in the same direction. Changes in the cyclase system induced by collagen preparations may be due, it can be tentatively suggested, to amino acids liberated during intracellular hydrolysis; the specificity of action of collagen, moreover, is determined by the complete set of amino acids characteristic of collagen, and not by any single amino acid, glycine in particular.

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