CHANGES IN cAMP LEVEL AND cAMP-BINDING CAPACITY OF TISSUE DURING HEALING OF EXPERIMENTAL WOUNDS OF DIFFERENT TYPES

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The concentration of the intracellular mediator cAMP undergoes considerable changes not only under the influence of hormones, but also during inflammatory and repair processes [12, 16]. The opinion is firmly held that the cAMP concentration is lowered in skin diseases accompanied by disturbance of proliferation and differentiation in cells of the epidermis, such as in psoriasis and hyperkeratosis [9]. Meanwhile views on the level and role of the nucleotide in wound healing are contradictory. Nosova and coworkers demonstrated a definite relationship between the course of wound healing and the cAMP level [4, 5] and observed that its level was raised in wound tissues through activation of adenylate cyclase. Meanwhile an effect on repair was obtained by the use of exogenous cAMP [1]. The cAMP level in known to depend on the activity of two enzymes (adenylate cyclase and phosphodiesterase). However, the level of cAMP-binding proteins also have been shown to play an important role in the regulation of the nucleotide concentration in the tissues [8, 10]. The ratio between free and bound forms of the nucleotide may be disturbed in pathology, and may also modify the physiological response of the cells. The question of bound forms of cAMP during wound healing has not been discussed in the literature.

The aim of this investigation was to study the cAMP concentration and cAMP-binding capacity of tissue in the course of healing of aseptic and infected wounds.

EXPERIMENTAL METHODS

Two series of experiments were undertaken on 220 male Wistar rats weighing 200-210 g. Aseptic and infected open wounds with an area of 400 mm^2 served as the experimental model. The model of an aseptic wound was produced by the method described previously [3]. To obtain a model of an infected wound, the edges and floor of the wound were additionally traumatized by toothed forceps, and 0.5 ml of a suspension of a 24-h culture of a pathogenic staphylococcus was injected into the wound surface ($1.5 \cdot 10^9$ staphylococci in 1 ml of physiological saline). In the course of healing, on the 1st-10th days after the operation, the cAMP concentration and binding capacity of protein kinases were determined in the wound tissue (granulation tissue). The cAMP concentration was determined by radioimmunoassay [11], using standard kits of reagents from Czechoslovakia. The results were expressed in picomoles per gram wet weight of tissue. The cAMP-binding capacity of the tissue was measured by the method described previously [10] and expressed per milligram of protein. The cAMP level and cAMP-binding capacity, determined in local tissues 5 min after wounding, were conventionally taken as the control.

EXPERIMENTAL RESULTS

Data on the cAMP concentration and binding capacity of protein kinases in the wound bed of aseptic wounds are given in Fig. 1. The cAMP concentration in wound tissues (control) 5 min after wounding was 463.5 ± 2.46 pmoles/g tissue and the binding capacity of protein kinase was 3.5 ± 0.2 nmole/g. Both the cAMP levels and the cAMP-binding capacity fell during the 24 h after the operation. The decrease in binding capacity of the proteins was more marked than the fall in the cAMP concentration. On the 2nd day the cAMP concentration continued to fall steadily, and by the 5th day (when granulation tissue was studied) the lowest

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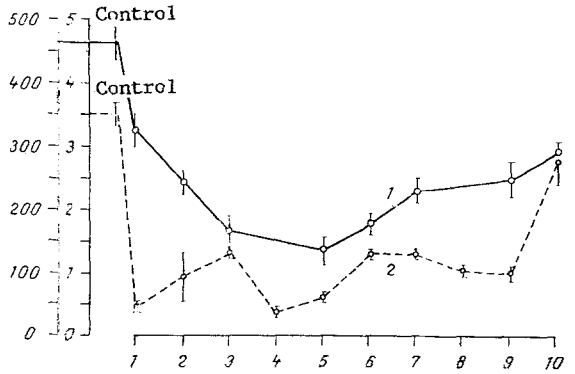

Fig. 1. Time course of cAMP concentration (1) and cAMP-binding capacity (2) of tissue during healing of aseptic wounds in rats. Abscissa) time after operation (in days); ordinate) on left – cAMP concentration (in pmoles/g tissue); on right – cAMP-binding capacity of tissue (in nmoles/mg).

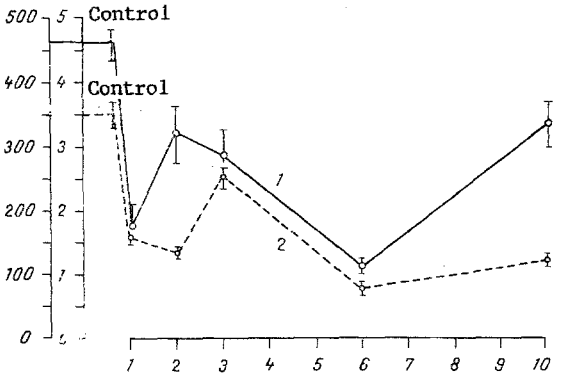

Fig. 2. Time course of cAMP concentration (1) and cAMP-binding capacity (2) of tissue during healing of infected wounds in rats. Legend as to Fig. 1.

level of the nucleotide was reached. By the 7th day of the experiment the cAMP concentration showed an increase, and on the 10th day it was significantly higher than on the 5th day ($p < 0.01$) but was not back to the control level. Until the 10th day a low binding capacity was determined with certain fluctuations from time to time, and not until the 10th day was this parameters back to the control value. Toward the end of the experiment, the ratio between free and bound forms of the nucleotide was thus changed.

A rather different picture was observed during healing of an infected wound (Fig. 2). After 24 h the cAMP level and binding capacity in tissues of the wound bed were significantly lower than in the control and than values recorded during healing of aseptic wounds. After the 2nd day the cAMP level rose, and on the 3rd day after the operation the concentration of the nucleotide in the granulation tissue reached a peak compared with that in the aseptic wounds. The binding capacity of the proteins, after falling on the 1st and 2nd days, also increased by this time synchronously with cAMP. On the 6th day the cAMP concentration again fell sharply, but by the 10th day it rose, and was significantly higher than on the 6th day ($p < 0.02$). The binding capacity at this time was significantly lower than in the control and in aseptic wounds.

The cAMP level determined in wound tissue consists of the sum of free and bound forms of the nucleotide; measurement of the binding capacity of protein kinases and other tissue proteins thus provides indirect information about the relative proportions of these forms, and enables the physiological effects of cyclic nucleotides to be evaluated from a different aspect. For instance, with a marked decrease in the cAMP-binding capacity in aseptic wounds

in the early inflammatory phase, a relatively high cAMP concentration can be deduced, and this is one of the factors reducing the intensity of inflammation [9], weakening the cellular reaction, reducing release of mediators of inflammation, and stabilizing the lysosomal membranes [2]. In this same period in the infected wound the relative level of the nucleotide was extremely low, causing weakening of the anti-inflammatory mechanisms of the wound tissue and leading to the development of suppurative inflammation.

In the period of completion of destruction and the beginning of granulation tissue formation (from the 3rd through the 5th days) the lowest cAMP levels and binding capacity were observed in the aseptic wound, evidence of a fall in the level of the nucleotide. The fall in the cAMP concentration in this series is evidently a positive background facilitating active cellular proliferation. The intracellular cAMP level in rapidly dividing cells is known to be maintained at low values [12, 16].

The results contradict those of investigations [4] which demonstrated an increase in the cAMP concentration during this period, possibly on account of differences in the experimental models of wounds.

The first peak of the total cAMP concentration in infected wounds was observed on the 3rd day after the operation, during a period of intensive suppurative inflammatory reaction. This increase in the nucleotide concentration may be due to activation of adenylate cyclase [6], and also due to cAMP release following destruction of macrophages and of the bacterial flora [15]. However, a parallel increase in the binding capacity of proteins, taking place under the influence of toxic products of the wound exudate [2, 8], led to the formation of bound nucleotide and ultimately to a fall in the concentration of free forms of cAMP. This causes intensification of suppurative inflammatory phenomena, such as phagocytosis, leukocytic infiltration, and histamine release, aimed at self-cleansing of the wound bed, although at the same time, it prolongs healing.

The binding capacity of the protein kinases, which we determined, does not directly reflect the phosphotransferase activity of the enzyme. However, the agreement between our own data on the increase in cAMP-binding capacity of proteins in the early period of the inflammatory reaction and data in the literature on increased protein kinase activity at this stage [6] confirms the existence of correlation between these parameters.

Disparity between the nucleotide level and binding capacity of the proteins was observed 10 days after the beginning of the experiment, when regenerative processes predominated over destructive. By this time the cAMP concentration rose in both aseptic and infected wounds, without any significant difference between these groups. However, the free nucleotide level is largely dependent on changes in binding capacity of protein kinases. In aseptic wounds active binding of cAMP takes place, as a result of which the relative concentration of the free form becomes low. Conditions of metabolism such as high binding capacity of protein kinases and other proteins, largely reflecting enzyme activity [10], in the presence of a low cAMP level, may potentiate collagen formation and proliferation of the epithelium [14]. According to data in the literature, the cAMP concentration correlates negatively with changes in the collagen concentration in the tissues during the period of their development [13], and administration of exogenous collagen leads to a fall in the cAMP level in wounds [7]. In the same period, the different ratio between the binding capacity of protein kinases and the nucleotide concentration in infected wounds is evidence of an increase in the free cAMP reserves, causing inhibition of proliferation and disturbing collagen formation. That is why in this period, during the treatment of infected wounds, it is evidently advantageous to lower the cAMP level, using imidazole preparations, for example, for these purposes. According to data in the literature, administration of imidazole in the phase of inflammation intensifies repair processes in a wound [7].

During healing of both aseptic and infected wounds, the fluctuations of the cAMP level observed thus depend largely on the ratio between concentrations of free and bound forms of the nucleotide. It is recommended that the role of the cyclic nucleotide in the pathogenesis of wounds be evaluated on the basis of assessment of the binding capacity of protein kinases. The character of the change in the cAMP level during healing of aseptic wounds differs from that in infected wounds, and this must be taken into account when corrective treatment is prescribed. The use of cAMP for the treatment of aseptic wounds can be regarded as not worthwhile, whereas in infected wounds, cAMP can be recommended for administration in the stage of the suppurative inflammatory reaction.

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ULTRASTRUCTURAL CHANGES IN NEOCORTICAL SYNAPSES DURING REHABILITATION OF MICE AFTER LONG-TERM PROTEIN-ENERGY DEFICIENCY

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Long-term protein-energy deficiency in the early postnatal period of development leads to significant changes in synaptic structure [7, 10, 13, 14]. A few investigations have shown that dietary rehabilitation as a rule does not lead to sufficiently full restoration of the morphological and functional characteristics of the synapses [8, 14]. In previous investigations the writers showed that a combination of dietary rehabilitation with the addition of carnitine to the diet sharply intensifies intracellular metabolism and thereby promotes repair of the ultrastructural changes arising in synapses after exposure of the organism to protein-energy deficiency in the early period of its postnatal development [8]. However, no quantitative electron-microscopic evaluation of changes in neocortical synapses during rehabilitation after protein-energy deficiency has hitherto been undertaken.

It was therefore decided to make a quantitative electron-microscopic study of the ability of dietary rehabilitation alone, and of dietary rehabilitation plus the addition of carnitine to the diet to restore the ultrastructure of synapses disturbed by exposure of mice to protein-energy deficiency.

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