EFFECT OF CYCLIC NUCLEOTIDES ON MITOTIC ACTIVITY OF CELLS OF THE DENTAL ANLAGEN AND ALVEOLAR BONE IN TISSUE CULTURE

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An important role in cell regulation, proliferation, and differentiation is nowadays ascribed to cyclic nucleotides [4, 8]. Cyclic nucleotides regulate fundamental biochemical processes which constitute the essence of reparative regeneration of connective tissue, including bone [1, 5, 6, 11, 13]. The writers have shown that proliferation and differentiation of cells and tissues are maintained in tissue cultures of parodontal tissues in the same way as takes place in the living organism [2, 3, 10].

The aim of the present investigation was to study the effect of cAMP, dibutyryl-cGMP (DB-cGMP), and dibutyryl-cAMP (DB-cAMP) on mitotic activity (MA) and differentiation on parodontal cells and tissues.

EXPERIMENTAL METHOD

Three series of investigations of explants of dental anlagen and alveolar bone of mouse embryos aged 15 days were undertaken: series I) control, series II) study of the effect of cyclic nucleotides on dental anlagen, series III) the study of their effect on alveolar bone.

In the control, 45 cultures of dental anlagen and alveolar bond (2 ×2 mm), and in the experimental series 30 cultures, from mouse embryos aged 15 days were studied. The THWP millipore filters with pore diameter 0.45 μ, thickness 25±5 μ, cut into squares with an area of 0.5 cm^2, were used. The squares of the filter were placed on a metal grid in a Petri dish with medium, and pieces of alveolar bone or pieces of dental anlagen were placed on them. The composition of the medium was: 85% of medium 199, 15% of bovine serum, 200 μg/ml of kanamycin, and 200 U/ml of polymyxin M sulfate. In series II and III the medium also contained DB-cAMP, cAMP, or DB-cGMP in concentrations of 10^{-6} and 10^{-8} M. A mixture of 50% O_2 and 50% N_2 was blown briefly through all the cultures and they were incubated at 37°C. The medium was changed every 24-48 h; its pH was 7.6. The duration of culture was 12 days. Explants were fixed by Bouin's method and embedded in paraffin wax; serial sections were cut to a thickness of 8-10 μ and stained with hematoxylin and eosin by Mowry's method. Mitotic coefficients (MC) were expressed in promille. Quantitative analysis of mitoses was carried out on the basis of counting the enameloblasts, cells of the dental papilla, and osteogenic cells of the dental anlage and alveolar bone every day throughout the period of culture. The results were subjected to statistical analysis by computer.

EXPERIMENTAL RESULTS

MC in cells of the dental papilla after addition of DB-cGMP and DB-cAMP in concentrations of 10^{-6} and 10^{-8} M did not differ significantly from the control level.

During the first four days a rapid rise of MA was observed, to reach a maximum on the 5th day. In the control MC = 28.956±0.899 ($x \pm t_{0.01} m$), whereas after addition of DB-cGMP in a concentration of 10^{-6} M, MC = 30.333±1.452, and in a concentration of 10^{-8} M MC = 28.333±1.45 ($P < 0.02$). The differences were not statistically significant (with 10^{-8} M DB-cGMP $t = 0.743 < t_{0.01} = 2.63$ and with 10^{-6} M DB-cGMP $t = 1.642 < t_{0.01} = 2.65$; with 10^{-8} M DB-cAMP $t = 2.089 < t_{0.01} = 2.65$, and with 10^{-6} M DB-cAMP $t = 0.508 < t_{0.01} = 2.565$). Later the value of MA fell.

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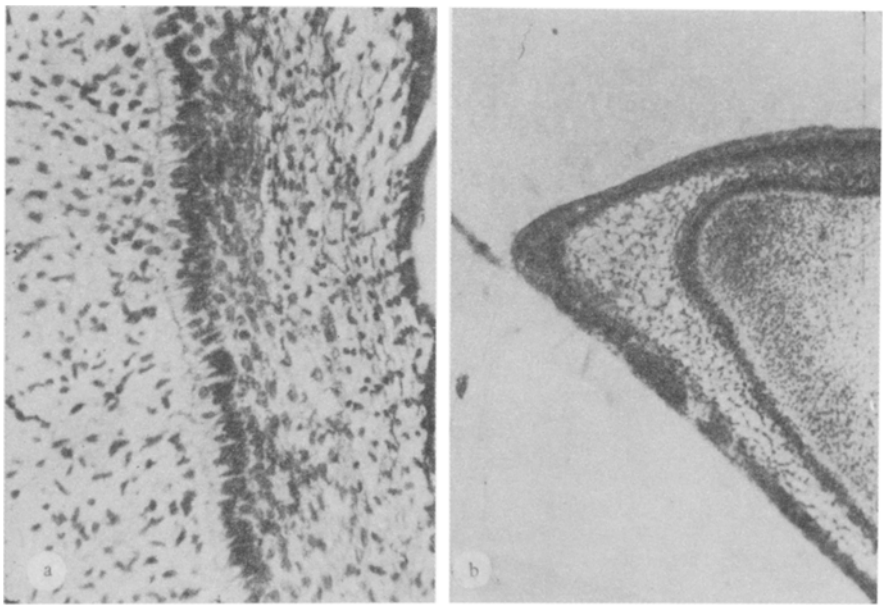

Fig. 1. Dental anlage of mouse embryo in tissue culture. a) Control culture after 4 days. Mitoses can be seen in levels of enameloblasts and dental papilla. Stained with hexatoxylin and eosin, 200 ×; b) dental anlage after 3 days in tissue culture on millipore filter under the influence of cGMP in a concentration of 10^{-6} M in medium; dentine formation has begun. Stained with hematoxylin and eosin, 100 ×.

Addition of cAMP to the tissue culture medium in concentrations of 10^{-6} and 10^{-8} M inhibited MA compared with the control: differences were significant (on the 5th day, with 10^{-8} M cAMP $t = 9.624 > t_{0.01} = 2.65$, with 10^{-6} M cAMP $t = 5.421 > t_{0.01} = 2.65$).

In the layer of enameloblasts MA reached a maximum on the 3rd day, MC = 34.133±1.229 ($P < 0.02$). Under the influence of DB-cGMP and DB-cAMP, MC during the first three days was significantly below the control level. For example, on the 3rd day, with DB-cGMP in a concentration of 10^{-6} M $t = 14.302 > t_{0.01} = 2.65$, and with DB-cAMP in a concentration of 10^{-6} M $t = 15.044 > t_{0.01} = 2.65$. Under the influence of cAMP, MA in the enameloblasts was shown to decrease and the differences compared with the control were significant.

The largest number of mitoses in the osteogenic cells of the alveolar bond in tissue culture was found on the 2nd day, namely 7.433±0.446 ($P < 0.02$). The number of mitoses then fell sharply. Differences between MC after the 3rd day in the control and experiment were significant (on the 4th day, with 10^{-6} M DB-cGMP $t = 13.915 > t_{0.01} = 2.664$, with 10^{-6} M cAMP $t = 8.040 > t_{0.01} = 2.664$).

The results show that the character of the effect of cyclic nucleotides on MA differs for different cells. Osteogenic cells are of mesenchymal origin and their mitotic index was 5-7%; enameloblasts were of epithelial origin and their mitotic index is 30-64% [12].

The character of differentiation of the bone and dental tissues in tissue culture was found to depend to a definite degree on cyclic nucleotides. Further development of the dental anlagen, which were distinctly formed and had a well marked structure, was observed. Mitoses were found in the layers of enameloblasts and odontoblasts (Fig. 1). Histochemical investigations showed that after the addition of DB-cGMP or DB-cAMP to the tissue culture medium, neutral mucopolysaccharides (MPC) were present on the 3rd day, compared with the 4th day in the control. Dentine production was observed on the 5th day, and enamel formation on the 7th day, i.e., two days earlier than in the control. The study showed that the stimulating action of DB-cGMP and DB-cAMP on dentine and enamel production is more effective than the action of cAMP. The stimulating effect of DB-cGMP and dB-cAMP on mineralization of the organic matrix is evidently due to the more rapid maturation of the cells.

In most cases cAMP exerts an inhibiting effect on actively proliferating cells, which was observed by us as regards the cells of the dental papilla and enameloblasts. A statistically significant difference in the effects of the dibutyryl derivatives of cAMP and cGMP evidently results from their ability to pass through the membrane, or a lowering of their affinity to receptors of cyclic nucleotides.

The addition of cyclic nucleotides to the tissue culture medium of alveolar bone usually suppressed bone resorption. In some areas the formation of osteoid tissue was already observed within 6 days as compared with the control, where osteogenesis was observed on the 10th day (Fig. 2). Figure 2 shows chondrocytes – cells with a highly irregular outer contour. Osteoblasts have a distinctly separated basophilic cytoplasm. The nucleus was usually off-center. Osteocytes are among intercellular bone substance. On the 7th day after the

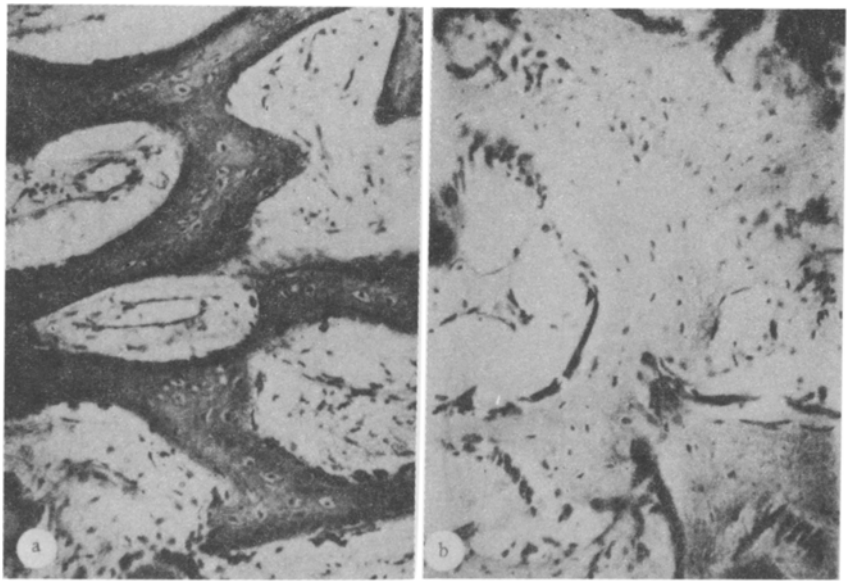

Fig. 2. Alveolar bone of mouse embryo in tissue culture: a) control culture af after 10 days; b) culture after 6 days of exposure to DB-cAMP in a concentration of 10^{-6} M in medium. Hematoxylin and eosin, 400 ×.

action of cyclic nucleotides the proliferation of osteoplastic elements and the formation of branching cell stands were observed. The periphery of the alveolar bone culture showed a finely looped network of newly formed lattice structure. Inside the lattice were found fibroblast-like osteogenic cells. Further intensification of the processes of osteogenesis was observed to the 12th day. An uneven distribution of MPS was found. The amount of neutral MPS in the mesenchymal cells was negligible, while in the preosteoblastic cells it was significant. The neutral MPS in the cytoplasm of preosteoblasts were shown in the form of fine multiple clumps. The content of neutral MPS was increased with the use of cyclic nucleotides as compared to the control.

All the cyclic nucleotides used increased mitotic activity of the osteogenic cells, inhibited resorption of alveolar bone, and stimulated the formation of new bone tissue; cAMP in a concentration of 10^{-6} M and DB-cGMP in concentrations of 10^{-6} and 10^{-8} M were more effective.

As regards osteogenic cells, the activating action of both cAMP and cGMP on their metabolic activity was observed. Such a picture is evidently characteristic of cell populations with a low proliferative pool. For example, the stimulating action of both cAMP and cGMP has been demonstrated on bone marrow stem cells [7, 9].

Stimulation of cell proliferation is the basis for the use of these cyclic nucleotides to stimulate osteogenesis.

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