CIRCAHORALIAN RHYTHM OF CYCLIC AMP CONCENTRATION IN RAT PAROTID GLAND SLICES

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UDC 576.34:591.431.6

The cyclic AMP concentration was measured by a radioimmunoassay method in slices of rat parotid gland after incubation for 12-14 h. In each concrete experiment only one gland was used. Pieces for measurement of cyclic AMP concentration were taken at 10-min intervals for 2 h. Rhythmic changes in the cyclic AMP concentration with a period of 20-50 min were found. The period of the cyclic AMP rhythm is similar to the period of fluctuations in other parameters discovered previously on the same object: the dry weight of the cells, rate of protein synthesis, and ornithine decarboxylase activity.

KEY WORDS: parotid salivary gland; cell rhythms; cyclic AMP.

In slices of rat parotid gland cultured in vitro cyclic changes are observed in several cytological and biochemical parameters with a period of about 1 h [1, 5, 6]. The mechanism of these circahoralian rhythms and their significance in cell physiology are still largely unexplained. Accordingly it is interesting to look for circahoralian fluctuations in the concentration of biologically active substances that play the role of metabolic regulators. The present investigation was devoted to the study of changes in the cyclic AMP concentration during incubation of rat parotid gland slices.

EXPERIMENTAL METHOD

Experiments were carried out on parotid gland slices from male Wistar rats weighing 120-150 g. Slices from the gland of one rat were used in each experiment. Pieces taken from one gland were minced and placed on HUFS membrane filters in medium No. 199, with the addition of 20% bovine serum, 70 mg vitamin C, and 4 mg glucose (per ml of medium). The slices were incubated in Conway dishes [3, 4]. The cyclic AMP concentration in the parotid gland slices was determined 12-14 h after explantation. For this purpose, slices from two dishes (about 4-5 mg tissue) were collected every 10 min on a scalpel cooled in liquid nitrogen and transferred to a glass homogenizer, containing 600 μl of ethanol cooled to $-10°C$. The tissue was carefully homogenized and the homogenate transferred to a test tube, which was placed for 2 min in a boiling water bath. The samples were then centrifuged at 3000g for 15 min, the supernatant was dried in vacuo, and the cyclic AMP concentration in it determined by radioimmunoassay [7].* ^{3}H-Cyclic AMP with specific radioactivity of 20-30 Ci-mmole ("Radiochemical Centre," Amersham, England) was used. The residue was redissolved in water and the protein concentration measured by Lowry's method. The cyclic AMP concentration was expressed in picomoles/mg tissue protein.

EXPERIMENTAL RESULTS

Data on changes in the cyclic AMP concentration in the parotid gland slices during incubation are shown in Fig. 1. The concentration of the nucleotide was measured every 10 min for a period of 2 h. The cyclic AMP concentration in the parotid gland slices fluctuated with a period of 20-50 min. Minimal concentrations were between 33 and 50% of maximal. The mean concentration of cyclic AMP and the amplitude and period of

*Antiserum against cyclic AMP was generously provided by G. Ya. Bakalkin and E. N. Komissarova.

Laboratory of Cytology, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 12, pp. 711-712, December, 1979. Original article submitted February 14, 1979.

0007-4888/79/8812-1467$07.50

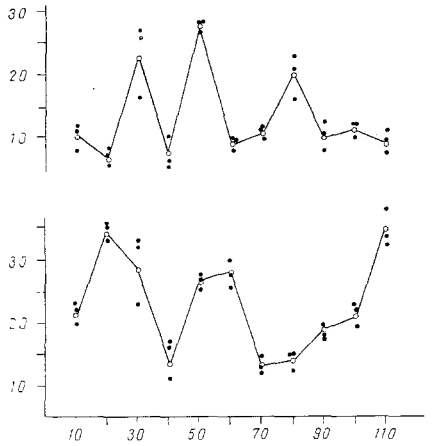

Fig. 1. Dynamics of cyclic AMP concentration in rat parotid gland slices during organ culture. Abscissa, time (in min) from beginning of sampling; ordinate, cyclic AMP concentration (in pmoles/mg protein). Values of individual samples (filled circles) and their mean value (empty circles) are shown at each time point. Each curve represents result of measurement on slices of gland from one animal.

the fluctuations differed a little in different experiments, probably on account of individual differences in metabolism of the rat from which the gland was obtained in order to prepare the slices.

So far as the writers are aware, circahoralian fluctuations in cyclic AMP concentration are here described in nondividing cells for the first time. A circahoralian rhythm of concentration of cyclic nucleotides, the period of which coincided with the period of the mitotic cycle, was discovered during a study of the early stages of cleavage of sea urchin eggs [8].

The cyclic AMP concentration is an important parameter of a universal cellular system of regulation, including membrane receptors of hormones and other biologically active substances; adenylate cyclases catalyzing cyclic AMP synthesis, coupled with these receptors; protein kinases responsible for phosphorylation of proteins, activated by these cyclic nucleotides, and so converting various enzymes from the inactive into the active form; and phosphodiesterases breaking down cyclic AMP [2]. In the present experiments the parotid gland cells were not exposed to any hormonal or other influences from outside the tissues. The results therefore indicate, in the writers' view, the existence of an endogenous (relative to the tissue of the gland or to the individual cell) circahoralian rhythm of changes in the parameters of the cellular regulatory system, incorporating cyclic AMP.

The period of the cyclic AMP rhythm in parotid gland slices is similar to periods of fluctuations in other parameters discovered previously on the same object: the dry weight of the cells, the velocity of protein synthesis, and ornithine decarboxylase activity [1, 5, 6].

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REACTION OF LYMPHOID TISSUE DURING COMPENSATORY REGENERATION OF THE SALIVARY GLANDS IN MICE

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UDC 612.6.03:612.313-08:612.42

After unilateral removal or burns of a single submaxillary salivary gland and also after amputation of the lower incisors in CBA mice the ability of the spleen cells to form antibodies and to take part in the graft versus host reaction was studied and the number of hematopoietic stem cells was counted by the splenic colonies method. Each of the experimental procedures on the salivary glands was shown to be accompanied by increased migration of stem cells, and the ability of the lymphocytes to induce a graft versus host reaction was increased. The ability of splenic lymphocytes to react to an additional antigenic stimulus is enhanced by amputation of the lower incisors, which was followed by enlargement of the salivary glands, and is sharply reduced by removal or burns of the submaxillary salivary gland, neither of which induces hypertrophy of the salivary glands.

KEY WORDS: immune reactions; hypertrophy of salivary glands; amputation of lower incisors.

Regeneration of the liver and compensatory hypertrophy of the kidney are accompanied by a combination of changes in the functional properties of the lymphoid organs [1-4]. To decide to what extent these changes are characteristic of regenerative processes and how they correlate with the regenerative power of the organs, the reactive properties of splenic lymphocytes were studied during various forms of regenerative growth of the salivary glands in mice. This organ was chosen as the test object because of wide variation in its ability to undergo hypertrophy, ranging from absence after unilateral sialadenectomy to intensive hypertrophy of the glands following repeated amputation of the lower incisors.

EXPERIMENTAL METHOD

Experiments were carried out on 300 CBA mice. The antibody-forming capacity of spleen cells to sheep's red blood cells (SRBC) was studied by Jerne's method [5] 4, 17, and 48 h after unilateral sialadenectomy, burns of one submaxillary salivary gland, and amputation of the lower incisors. At the specified time after the operations the mice were killed, a suspension of their spleen cells was prepared, and was injected in a dose of 1×10^7 cells into lethally irradiated syngeneic recipients together with 2×10^8 SRBC. Control recipients received the same number of SRBC and spleen cells from intact mice. The number of antibody-forming cells (AFC) was determined on the 8th day after transfer of the lymphocytes and immunization. Changes in the ability of the lymphocytes to give a graft versus host reaction (GVHR) were determined at the same times after the operation by Möller's method [6]. The number of hematopoietic stem cells (CFU) in the spleen of the experimental animals also was determined by counting macroscopic colonies 8 days after transplantation of splenocytes of irradiated recipients in the usual way [7].

EXPERIMENTAL RESULTS

The experiments showed that each of the experimental procedures on the salivary glands (extirpation, unilateral burns, or repeated amputation of the lower incisors) was accompanied by an increase in the migrating capacity of the stem cells, as reflected in an increase in the number of exogenous colonies in the spleen of recipients of splenocytes of the experimental mice. This was clearly revealed 17 h after the beginning of

Laboratory of Growth and Development, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 12, pp. 712-714, December, 1979. Original article submitted April 4, 1979.

0007-4888/79/8812-1469$07.50