RHYTHM OF ORNITHINE DECARBOXYLASE ACTIVITY IN RAT PAROTID GLAND SLICES

U. N. Yarygin, N. V. Nechaeva,
V. I. Fateeva, E. D. Trushina,
and V. Ya. Brodskii

UDC 612.313.015.11:577.152.411

In rat parotid gland slices incubated in vitro ornithine decarboxylase activity and the rate of protein synthesis were determined. A circahoralian rhythm of enzyme activity preceding in-phase fluctuations in the range of protein synthesis was found. These results indicate a role of ornithine decarboxylase in maintenance of the circahoralian rhythm of the rate of protein synthesis in parotid gland cells.

KEY WORDS: salivary gland; biological rhythms; ornithine decarboxylase; protein synthesis.

Fluctuations in the protein content and uptake of amino acids into proteins, with a period ranging from tens of minutes to several hours, have been found in certain embryonic and differentiated tissues both in vivo and in vitro [2]. The mechanisms of formation and maintenance of these circahoralian biological rhythms are not clear. In this connection it is interesting to study changes with time in the activity of enzymes controlling protein metabolism. One such enzyme is ornithine decarboxylase, a key enzyme in the synthesis of spermidine and spermine, polyamines which stimulate anabolic processes in the cell [6-8].

The object of the present investigation was to study the temporal dynamics of ornithine decarboxylase activity in organ cultures of the rat parotid salivary gland, in which several circahoralian rhythms have previously been distinguished, including a circahoralian rhythm of the rate of protein synthesis [2, 5].

EXPERIMENTAL METHOD

Experiments were carried out on sections of the parotid gland of male Wistar rats weighing 120-150 g. The sections were incubated in Conway dishes on HUFS membrane filters in medium 199 with the addition of 20% bovine serum, 70 μg vitamin C, and 4 mg glucose per ml medium [3, 4]. Ornithine decarboxylase activity and the rate of protein synthesis were determined 12-14 h after explantation. Every 10 min, 15-20 fragments (2-3 mg tissue) were transferred into 10 volumes of cold Tris-HCl buffer, pH 7.4, containing 0.5 mM EDTA and 5 mM dithiothreitol, and homogenized. Samples were taken from the homogenate in order to determine the protein concentration, by Lowry's method. The rest of the homogenate was used for measurement of ornithine decarboxylase activity by the method of Russell and Snyder [13] with certain modifications. Into a test tube were introduced 200 μl homogenate, 200 μl buffer, 50 μl 5 mM solution of pyridoxal phosphate, and 0.20 μCi of 1-[^{14}C]-ornithine (Radiochemical Centre, Amersham, England; specific activity 60 μCi/mmole) in 10 μl distilled water. The plastic container with 0.2 ml Hyamine hydroxide (Koch-Light) was fixed to the wall of the tube. The tubes were tightly closed and incubated for 1.5 h at 37°C on a water bath. The reaction was stopped by the addition of 0.3 ml of 50% TCA, after which the tightly closed tubes were incubated for a further 1 h at 37°C so that all the $^{14}CO_2$ liberated could be absorbed by the Hyamine hydroxide. The container with the Hyamine was then removed from the tubes, placed in a scintillation flask, and its radioactivity determined on the SL-30 counter. The result was expressed in picomoles of product formed/mg protein · h. Preliminary experiments showed that the rate of formation of $^{14}CO_2$ is proportional to the quantity of homogenate over the range of reaction velocities from at least 0.5 to 30 pmoles $^{14}CO_2$/mg protein · h. The velocity of formation of the product measured under these conditions could therefore be taken to be proportional to the ornithine decarboxylase activity in the tissue and it could thus be used for quantitative assessment of that activity.

Laboratory of Cytology, Institute of Developmental Biology, Academy of Sciences of the USSR. Department of Biochemistry, Central Research Laboratory, Attached to the 4th Main Board, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 12, pp. 726-728, December, 1978. Original article submitted May 5, 1978.

0007-4888/78/8612-1657$07.50

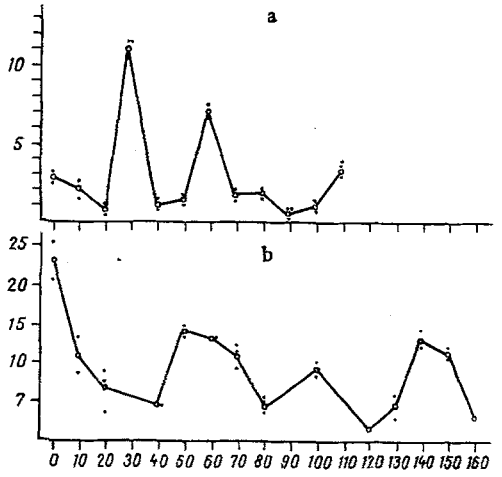

Fig. 1

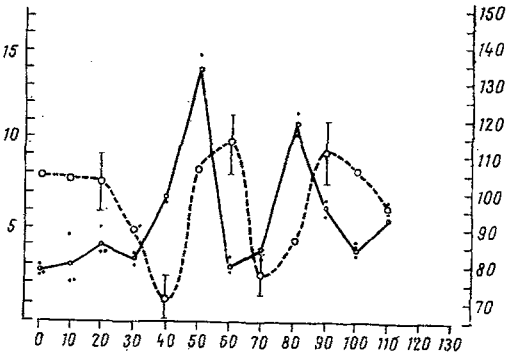

Fig. 2

Fig. 1. Examples of temporal dynamics of ornithine decarboxylase activity in rat parotid gland slices during organ culture. Each curve (a, b) is a result of measurement of parotid slices from one animal. Abscissa, time after beginning of sampling (in min); ordinate, ornithine decarboxylase activity (in pmoles $^{14}CO_2$/mg protein · h). At each time point values of 2 or 3 separate tests (filled circles) and their average (empty circles) are given.

Fig. 2. Dynamics of rate of protein synthesis (broken line) and ornithine decarboxylase activity (continuous line) in parotid gland slices during organ culture. Abscissa, time after beginning of sampling (in min); ordinate: on left – the same as in Fig. 1, on right – rate of protein synthesis (in % of mean level).

The velocity of protein synthesis was measured with other slices of the same gland. Radioactivity of proteins and of free [^{3}H]-lysine was determined after pulse labeling of the tissue. Slices of the gland (1.0-1.5 mg) were transferred every 10 min into 1 ml of medium containing 20 μCi [^{3}H]-lysine (Radiochemical Centre, Amersham, specific activity 20 Ci/mmole). After incubation for 10 min at 37°C the slices were frequently washed with cold medium containing an excess of unlabeled amino acid and treated with cold 5% perchloric acid. The radioactivity of the acid-soluble and protein fractions was determined. The combined radioactivity of the free [^{3}H]-lysine and the protein fraction characterized the permeability of the cells for the labeled precursor at each point in time. The velocity of protein synthesis was calculated as the ratio of incorporation into protein to the total incorporation [5].

EXPERIMENTAL RESULTS

Changes in ornithine decarboxylase activity during incubation of the parotid gland slices are illustrated in Fig. 1a, b. The results of these experiments indicate the existence of fluctuations of activity of this enzyme. The maximal activity was 5-10 times greater than the minimal activity. The period of the fluctuations was 30-40 min, which corresponds to the period of cyclic changes in the rate of protein synthesis discovered in the parotid gland previously [5].

Rhythms of ornithine decarboxylase activity and of the rate of protein synthesis were compared in the same experiment; to determine both parameters slices from the parotid gland of the same animal, incubated together, were used. The rhythm of enzyme activity was found to precede in phase the fluctuations in the rate of protein synthesis (Fig. 2). This difference in phase is evidence that stimulation of polyamine synthesis precedes the increase in protein production.

It can be concluded from these observations that ornithine decarboxylase plays a role in the maintenance of the circahoralian rhythm of protein synthesis in the cells of the parotid gland. Other evidence in support of a possible role of polyamines and of the enzymes controlling their synthesis in the regulation of circahoralian rhythms is given by the coincidence between the periods of fluctuations in the concentration of spermidine and spermine, on the one hand, and incorporation of amino acids into protein, on the other hand, observed in sea urchin blastomeres [9-12]. The short half-life of ornithine decarboxylase, namely about 10 min [1], is an indication of the regulatory function of this enzyme in the mechanisms of action of polyamines.

LITERATURE CITED

1. G. D. Berdysheva and K. G. Karpenchuk, Usp. Sovrem. Biol., 84, No. 1 (4), 81 (1977).
2. W. Ia. Brodsky (V. Ya. Brodskii), J. Theoret. Biol., 48, 167 (1967).
3. E. A. Luriya and I. E. P'yachenko, Byull. Éksp. Biol. Med., No. 6, 81 (1967).
4. N. V. Nechaeva, T. B. Aizenshtadt, and E. A. Luriya, Tsitologiya, No. 4, 465 (1970).
5. N. V. Nechaeva, "Biological rhythms in the secretory function of the salivary gland (quantitative cytochemical investigation)," Author's Abstract of Doctoral Dissertation, Moscow (1977).
6. E. Z. Tsvetnenko and V. S. Shugalei, Byull. Éksp. Biol. Med., No. 1, 28 (1978).
7. U. Bachrach, Function of Naturally Occurring Polyamines, New York (1973).
8. S. S. Cohen, Introduction to the Polyamines, Englewood Cliffs, New Jersey (1971).
9. S. Kusunoki and I. Yasumasu, Biochem. Biophys. Res. Commun., 68, 881 (1976).
10. Y. Mano, Develop. Biol., 22, 433 (1970).
11. Y. Mano, Arch. Biochem., 146, 237 (1971).
12. Y. Mano, Biosystems, 7, 51 (1975).
13. D. H. Russell and S. H. Snyder, Proc. Natl. Acad. Sci. USA, 60, 1420 (1968).