POSSIBLE ROLE OF ORNITHINE DECARBOXYLASE IN CONTROL OF THE CIRCAHORALIAN RHYTHM OF PROTEIN SYNTHESIS

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UDC 612.398.015.36:"514"

KEY WORDS: biological rhythms; protein synthesis; ornithine decarboxylase; 1,3-diaminopropane; cadaverine.

A circahoralian rhythm of activity of ornithine decarboxylase (ODC), an enzyme which, in eukaryotes, limits the velocity of polyamine synthesis, was discovered by the writers in rat parotid gland slices. Fluctuations of ODC activity had a period equal to that of the circahoralian rhythm of protein synthesis (30-60 min) but preceded it in phase [5]. Addition of putrescine (1,4-diaminobutane), a product of the reaction catalyzed by ODC, to the culture medium smoothed out the fluctuations in the rate of protein synthesis in parotid gland slices and in a primary monolayer culture of hepatocytes [3]. Previously a circahoralian rhythm of polyamine concentration had been found in sea urchin blastomeres with a period that coincided with that of the rhythm of protein synthesis [7, 9]. It was postulated on the basis of data in the literature and our own results that polyamines and ODC, which regulate their concentration, are concerned in the control of the circahoralian rhythm of protein synthesis.

In the investigation described below, devoted to the study of the effect of 1,5-diaminopentane (cadaverine) and 1,3-diaminopropane on the rhythm of incorporation of [^{3}H]lysine into proteins in rat liver slices, the testing of this hypothesis was carried a stage further. The aliphatic diamines mentioned are noncompetitive inhibitors of ODC *in situ* and *in vitro* [6] but, unlike putrescine, which can also inhibit this enzyme, they evidently do not take part in polyamine metabolism at any other stage.

EXPERIMENTAL METHOD

Incorporation of [^{3}H]lysine into Wistar rat (males weighing 120-150 g) liver slices was studied by the method described previously [3]. Liver slices from the same animal were used in each experiment. The slices were incubated on membrane filters in Conway dishes for 12-14 h at 37°C in medium aerated with a mixture of 5% CO_2 and 95% air, containing 80% of medium 199, 20% bovine serum, 70 mg vitamin C, and 4 mg glucose (/ml medium). As was shown previously, stabilization of several metabolic parameters takes place in the tissue during this period [2]. Half of all the incubated slices were transferred to medium with the addition of cadaverine (concentration $5 \cdot 10^{-4}$ M) or diaminopropane (concentration 10^{-5} M) and the remaining slices were transferred to fresh medium without diamines. The control and experimental slices were incubated on Whatman 3 MM filters in Conway dishes. After a certain time the slices of each series were transferred simultaneously to medium containing $92.5 \cdot 10^7$ Bq of [^{3}H]lysine in 1 ml, incubated for 10 min at 37°C, washed with cold medium containing an excess of unlabeled lysine, treated with 5% $HClO_4$, and placed in hyamine. Radioactivity of the acid-soluble and protein fractions was determined on an SL-30 scintillation counter: Their total reflects the mean rate of penetration of [^{3}H]lysine into the slices during the corresponding 10-min interval, whereas the ratio of radioactivity of protein fraction to total radioactivity reflects the mean rate of incorporation of the precursor into protein, i.e., the rate of protein synthesis [1]. The dynamics of protein synthesis in the liver slices was studied from 110-120 min at intervals of 10 min.

Laboratory of Cytology, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 2, pp. 158-160, February, 1984. Original article submitted May 25, 1983.

 0007-4888/84/9702-0176$08.50

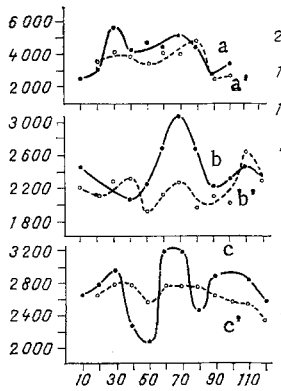

Fig. 1

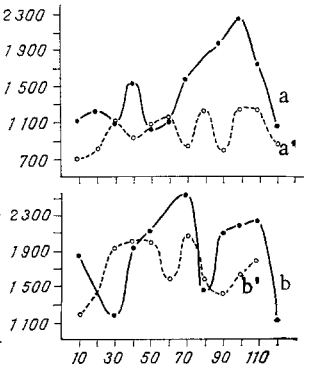

Fig. 2

Fig. 1. Effect of cadaverine on rhythm of rate of protein synthesis in rat liver slices during organ culture. Continuous lines — control, broken lines — cadaverine ($5 \cdot 10^{-4}$ M). Each two curves — a and a', b and b', c and c' — denote result of measurement with slices from one gland. Abscissa, time after beginning of taking samples (in min); ordinate, protein synthesis (cpm).

Fig. 2. Effect of 1,3-diaminopropane on rhythm of rate of protein synthesis in rat liver slices during organ culture. Continuous lines — control; broken lines — 1,3-diaminopropane (10^{-5} M). Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

There were five experiments. In the first experiment, 12-14 h after explantation of the liver slices in culture half of them (experimental) were transferred to medium containing cadaverine and the other half (control) to fresh medium. The rhythm of the rate of protein synthesis began to be studied after 30 min and continued for 2 h by the scheme described above, i.e., every 10 min slices were taken from the control and experimental series and the rate of protein synthesis in them was determined. Samples were incubated in medium with cadaverine for between 30 (sample 1) and 140 min (sample 12).

In the second experiment one-quarter of the slices were transferred 1 h before the study of the rate of protein synthesis began to medium containing cadaverine; at the same time the same number of slices was transferred to fresh medium. These media were changed again 30 min before the beginning of incubation of the experimental and control series of slices with labeled amino acid. These slices were used to determine the rate of protein synthesis at the 10th, 20th, 30th, 40th, 50th, and 60th minutes of the test period (first 6 points). The remaining slices, which also were divided into experimental and control series, were transferred to medium with or without cadaverine, just as in the first half of the experiment, 1 h before the study of the rhythm began, i.e., at the 70th minute. The media were again changed 30 min before the beginning of incubation of the experimental and control series. These slices were used to determine the rate of protein synthesis at the 70th, 80th, 90th, 100th, and 110th minutes (samples 7 to 12). In this experiment the difference between the length of stay of the various samples in medium with cadaverine was shorter than in the first experiment, namely from 60 to 110 min.

In the third experiment slices intended for determination of the rate of protein synthesis at the 10th, 20th, 30th, and 40th minutes were transferred to medium with cadaverine (experimental series) or to fresh medium 1 h before the study of the rhythm began; slices intended for measurement of the rate of incorporation of label at the 50th, 60th, 70th, and 80th minutes were transferred 30 min later, and the remaining slices after another 30 min. In this experiment the difference between the length of stay of the samples in medium with

cadaverine compared with experiment 2 was shorter by 20 min: the tissue was incubated in the presence of cadaverine for not less than 60 min and not more than 90 min.

The 4th and 5th experiments differed from the 3rd experiment only in the fact that diaminopropane was used instead of cadaverine.

The results obtained in these experiments are shown in Figs. 1 and 2. A circahoralian rhythm of protein synthesis with a mean period of 30-50 min was found in liver slices incubated in medium without diamines. The maximal values of the rate of synthesis differed from the minimal by 1.5-2.5 times. Addition of cadaverine or diaminopropane to the medium led to a decrease in amplitude of the fluctuations or even to complete suppression of the rhythm. The mean velocity of protein synthesis in two of the five experiments was reduced a little.

The period of fluctuations of the rate of protein synthesis in the control series corresponded to the period of regular fluctuations in the rate of protein synthesis discovered by the writers previously in liver slices [2], in a primary monolayer culture of hepatocytes [3], in slices of the parotid gland and pancreas, and in nerve cells [1, 2]. The effect of diaminopropane and cadaverine on the rhythm was similar to the action of putrescine — a product of the reaction catalyzed by ODC, on it: It also led to a decrease in amplitude of the rhythmic fluctuations of the rate of protein synthesis in parotid gland slices and in monolayer hepatocyte culture [3].

It is stated in the literature that aliphatic diamines, including diaminopropane, putrescine, and cadaverine, induce the formation of an endogenous noncompetitive protein inhibitor of ODC — so-called ODC-antienzyme, in the liver *in vivo* and *in vitro* [6]. It can be tentatively suggested that in our own experiments also the diamines affected the rhythm of protein synthesis by modifying polyamine metabolism on account of inhibition of the key enzyme of their biosynthesis. ODC has the fastest turnover of all proteins so far studied. Its half-life in the liver is 10-15 min [6]. With such a turnover rate ODC can participate effectively in the regulation of biological rhythms. As was stated previously, a circahoralian rhythm of ODC activity, differing from the rhythm of the rate of protein synthesis only in the phase of the waves, was found in parotid gland slices [5]. It has also been shown that formation of the rhythm of the rate of protein synthesis in the parotid gland coincided in time with morphological and physiological maturation of the gland and with a sharp increase in its ODC activity [4]. The facts described above, with data in the literature, are evidence that natural aliphatic polyamines and the enzymes of their biosynthesis participate in the regulation of circahoralian rhythms.

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