ROLE OF POLYAMINES IN THE REGULATION OF THE CIRCUMHORALIAN RHYTHM OF INTRACELLULAR PROTEIN SYNTHESIS

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Rhythms with a period of about 1 h have been found during the study of various biochemical phenomena including protein synthesis [2, 7]. In particular, in the rat parotid gland a circumhoralian rhythm of protein synthesis and a similar rhythm of activity of ornithine decarboxylase [8], the key enzyme in synthesis of intracellular polyamines (putrescine, spermidine, spermine), which participate in the regulation of protein biosynthesis [5, 9, 10], have been discovered. Fluctuations in activity of the enzyme and in the rate of protein synthesis had a similar period but did not coincide in phase [8].

These fluctuations suggested that polyamines participate in the regulation of the rhythm of protein synthesis. To test this hypothesis, the possibility of modifying the rhythm of protein synthesis in slices of parotid gland and in isolated liver cells in vitro was studied after addition of putrescine to the medium.

EXPERIMENTAL METHOD

Pieces isolated from one parotid gland of an adult rat were minced and placed on HUFS membrane filters in medium No. 199, to which 20% bovine serum, 70 mg vitamin C, and 4 mg glucose/ml were added. The slices were incubated in Conway dishes for 12-14 h. Isolated viable hepatocytes were obtained by the method in [11] in the writers' modification. The liver was perfused initially with calcium-free Hanks' solution (300 ml, 37°C) and then with a 0.05% solution of collagenase (80 ml, 37°C) at the rate of 10 ml/min (without recycling). The liver was removed 2-3 min after the end of perfusion, the capsule was stripped off, and the organ was cut into small pieces weighing 2-3 g, which were transferred to a 300-ml flask, and covered with 20 ml medium No. 199 aerated with a mixture of 95% air and 5% CO_2. The flasks containing liver tissue was placed on a magnetic mixer at 37°C for 10 min. The resulting suspension was passed through nylon filters and transferred to Petri dishes for preincubation for 15 min. The supernatant was then removed, the cells were resuspended in incubation medium containing 80% medium No. 199 and 20% bovine serum, and passed through nylon filters. Treatment of the liver in this way yielded about 90% of viable isolated cells. A suspension containing one million isolated hepatocytes in 1 ml medium was transferred to cover slips or slides (1 × 1 cm), kept in Petri dishes. The dishes were placed in a container aerated with the air mixture, and transferred to an incubator at 37°C. The hepatocytes were cultured for 2 or 3 days; the medium was changed 6 and 30 h after the beginning of culture. The rate of protein synthesis was determined by measuring the radioactivity of the proteins and of free ^{3}H-lysine or ^{3}H-leucine after pulse-labeling of the sections or isolated cells. The total radioactivity of the free labeled amino acid and protein fractions characterized the permeability of the cells for the labeled precursor. The velocity of protein synthesis was calculated as the ratio of incorporation into protein to total incorporation [4].

EXPERIMENTAL RESULTS

Polyamines, depending on their concentration, either inhibit or stimulate protein synthesis both in vivo and in vitro [6, 13, 14]. In preliminary experiments the effect of different doses of putrescine, with different

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TABLE 1

No. of sample	Sections of parotid gland										Isolated hepatocytes		
	control	incubation with putrescine, 10^{-3} M	control	incubation with putrescine, 10^{-3} M	control	incubation with putrescine, 10^{-5} M	control	incubation with putrescine, 10^{-7} M	control	incubation with putrescine, 10^{-7} M	control	incubation with putrescine, 10^{-7} M	incubation with putrescine, 10^{-8} M*
1	1323	1164	1162	918	1987	1538	1589	2387	1538	2320	1271	1025	1315
2	1462	1037	1186	1016	1759	1640	1342	2202	1640	2507	1240	1063	1325
3	1456	1197	1289	1172	1852	1660	1522	2057	1660	1877			
Average	1414	1132	1212	1032	1866	1612	1484	2215	1612	2234	1255	1044	1320

*Incubation with putrescine for 3 h; all others, 6 h.

durations of exposure, on slices of parotid gland and on isolated hepatocytes was studied (Table 1). The presence of putrescine in the medium in concentrations of 10^{-3} and 10^{-5} M for 6 h led to a decrease in the velocity of protein synthesis in the slices relative to the control. Putrescine in a dose of 10^{-7} M had the same action on isolated hepatocytes. The medium with putrescine was changed after 3 h; the half-life of putrescine is about 2 h [1]. In the medium with putrescine, just as in the control, the pH was adjusted to 7.2-7.4. These experiments confirm existing information on the inhibitory effect of polyamines on protein synthesis, and they showed that the same effect of isolated cells is achieved by the use of putrescine in a lower concentration than on slices. The presence of putrescine in lower concentrations, namely 10^{-7} M, for 6 h in medium with parotid gland slices and of 10^{-8} M for 3 h in the medium with isolated hepatocytes, did not inhibit protein synthesis in the gland cells.

To study the effect of polyamines on the rhythm of velocity of protein synthesis, in the experiments of series I some slices of parotid gland were transferred 6 h before the end of incubation to culture medium containing putrescine in a concentration of 10^{-7} M, the rest (control) to the same medium without addition of putrescine. The medium was changed after 3 h. In the experiments of series II some slides with isolated hepatocytes were transferred 3 h before the end of incubation to culture medium containing putrescine in a concentration of 10^{-8} M, the rest (control) to the same medium without addition of putrescine. The slices of parotid gland and slides with hepatocytes were transferred every 10 min to incubation medium containing 25 μCi ^{3}H-lysine and ^{3}H-leucine (Radiochemical Centre, Amersham, specific activity 20 Ci/mole) in 1 ml. After incubation for 10 min at 37°C the slices and slides were washed several times with cold medium containing an excess of unlabeled amino acid, treated with cold 5% perchloric acid, and covered with Hyamine. The radioactivity of the acid-soluble and protein fractions was determined on the SL-30 scintillation counter.

The mean period of fluctuations in the intensity of protein synthesis in slices of parotid gland was 40-60 min (Fig. 1a, c), similar to the period of the cyclic changes observed previously in the velocity of protein synthesis in slices of some glands: parotid, pancreas, and liver [3, 4]. The maximal values of the velocity of protein synthesis differs from minimal by a factor of 2.

The velocity of protein synthesis in isolated hepatocytes, just as in the slices, showed cyclic changes with a mean period of 40-60 min (Fig. 2a, b). The maximal values of the velocity of protein synthesis in these experiments differed from minimal by a factor of 1.5-1.9. Cyclic changes in the velocity of protein synthesis were discovered both in completely isolated hepatocytes (Fig. 2a) and in cells forming a monolayer in culture (Fig. 2b).

The velocity of protein synthesis in the presence and absence of putrescine was compared in slices of the same gland. Addition of putrescine to the culture fluid twice in the course of 6 h in a concentration of 10^{-7} M led to a decrease in the amplitude of cyclic changes in the velocity of protein synthesis in slices of the parotid gland by about half in one of the experiments (Fig. 1b). In another experiment no fluctuations whatsoever could be observed (Fig. 1d). During the action of putrescine in a concentration of 10^{-8} M on isolated hepatocytes, smoothing of the fluctuations in the velocity of protein synthesis was manifested to an even greater degree than in the slices (Fig. 2b).

Repetition of the rhythms of protein synthesis in slices of the parotid gland, pancreas, and liver incubated in vitro [3], is evidence of the existence of intracellular mechanisms of regulation of circumhoralian rhythms of protein synthesis. The rhythms of different tissues are evidently tuned by a common synchronizer,

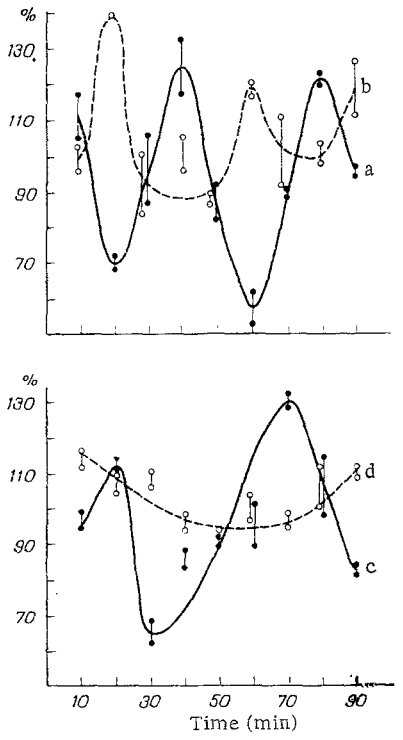

Fig. 1

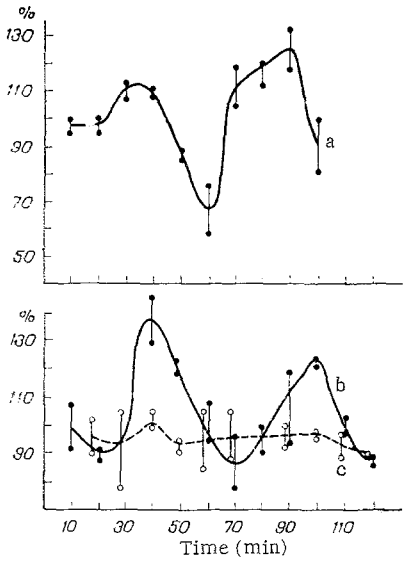

Fig. 2

Fig. 1. Effect of putrescine on rhythm of velocity of protein synthesis in slices of rat parotid gland during organ culture. Continuous lines, control; broken lines; putrescine 10^{-7} M. Each two curves (a and b, c and d) is the result of measurement on slices of the same gland. All curves obtained in experiments with ^{3}H-lycine. Abscissa, time after beginning of taking samples (in min); ordinate, protein synthesis (in percent of mean level); values of two separate samples given at each time point.

Fig. 2. Effect of putrescine on rhythm of velocity of protein synthesis in isolated hepatocytes cultured in vitro. Continuous lines, control; broken lines, putrescine 10^{-8} M. Curves b and c are the result of measurement of hepatocytes obtained from one liver. Curve a obtained in experiments with ^{3}H-leucine, curves b and c in experiments with ^{3}H-lysine. Remainder of legend as to Fig. 1.

but each of them is determined both in isolated sections and, as has just been shown, even in isolated cells. Consequently, the rhythm itself may be due to periodic changes in intercellular metabolism. Intensification of protein synthesis induced by an increase in physiological activity, by hormones, or by other factors as a rule takes place aginst the background of an increase in the concentration of polyamines, which are regulators of intracellular processes [1, 5, 12]. This increase takes place in most cases on account of an increase in activity of ornithine decarboxylase, which catalyzes the slowest stage of polyamine synthesis in eukaryotes, namely decarboxylation of L-ornithine with the formation of putrescine. In experiments with rat parotid gland slices, the writers previously found a circumhoralian rhythm of ornithine decarboxylase activity with a period equal to that of fluctuations in the velocity of protein synthesis, but preceding them in phase [8]. It is shown in the present communication that in experiments on parotid gland slices and on isolated hepatocytes the addition of the product of the reaction catalyzed by ornithine decarboxylase, namely putrescine, to the culture medium leads to smoothing of the fluctuations in the velocity of protein synthesis. These data are evidence in support of the participation of polyamines in the maintenance of the circumhoralian rhythm of protein synthesis.

The mechanism of this participation can be represented as follows. The velocity of protein synthesis, determined by the intracellular concentration of polyamines, undergoes cyclic changes, with a certain delay, in accordance with changes in the activity of the regulatory enzyme ornithine decarboxylase. An excess of exogenous putrescine modifies the rhythm of intracellular concentration of polyamines either directly or through a disturbance of the rhythm of ornithine decarboxylase activity. This leads to smoothing of the fluctuations in the velocity of protein synthesis.

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COLONY-FORMING ABILITY OF CELLS FROM DIFFERENT HEMATOPOIETIC ORGANS OF THE QUAIL EMBRYO

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KEY WORDS: colony-forming unit; hematopoietic stem cell; anlage of the heart; limb bud; yolk sac.

One of the most successful methods used to study the origin, dynamics of distribution, and quantitative aspects of the population of hematopoietic stem cells (HSC), their properties, and their relations with other tissue cells of the internal medium, is the method of splenic colonies, although it is applicable only to mice [7].

It is only recently that a method of cloning avian HSC in the bone marrow of lethally irradiated chicks has been developed [5]. The writers showed previously that a combination of this method with the method of natural cell markers [4] using sublethally irradiated chicks enabled the fate of both the donor's and the recipient's HSC, forming colonies in the bone marrow, to be studied [1].

In the present investigation colony-forming ability of yolk sac cells and of the quail embryo itself, at the stage corresponding to 48 h of incubation, and also of cells from the yolk sac, limb bud, and anlage of the heart of a quail embryo at the stage corresponding to 60 h of incubation.

EXPERIMENTAL METHOD

Hens of the Russian White breed and embryos of the Japanese quail Coturnix japonica L., of the Pharaoh breed, were used.

Full details of the experimental technique were described previously [1]. Chicks age 3 weeks, irradiated in a dose of 750 R (dose rate 50 R/min), received an injection of 0.6 ml of a suspension of embryonic quail cells 24 h after irradiation, into the marginal vein of the wing. Data on the sources of the cells of the suspension injected and the number of donor's cells injected into the irradiated chick are given in Table 1.

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