NEW DATA ON THE RHYTHM OF PROTEIN SYNTHESIS IN PAROTID SALIVARY GLAND CELLS OF RATS FED AT 3-HOUR INTERVALS

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UDC 576.34:591.431.6

A definite rhythm of protein synthesis was found by scintillation counter analysis in cells of organ cultures of the parotid salivary gland of animals untrained for periodic feeding. The period of this rhythm was 50-60 min, corresponding to the period of fluctuation in protein synthesis in the glands of animals trained by feeding at 3-h intervals. The rhythm of fluctuations in protein content and the corresponding rhythm of protein synthesis thus revealed are endogenous rhythms of the parotid gland. An interval of 3 h between meals is a factor emphasizing the natural rhythm of the glands of various animals.

Previous investigations showed that periodic feeding of rats at 3-h intervals leads to the appearance of a definite rhythm (with a period of 50-60 min) of quantitative changes in protein secretion in the acinar cells of the parotid salivary glands [1-3, 5]. Two methods – interference microscopy and autoradiography – led to the same conclusion. The rhythm discovered experimentally is stable: in the acinar cells it persists for a short time (at least 2 h) without reinforcement and is found in the cells even when nervous regulation of the gland is disturbed in vivo. The characteristic structure of the rhythm during 3-h-interval feeding is also seen in cells of a surviving organ culture of the parotid gland 12-16 h after explantation of fragments.

Autocorrelation analysis of the fluctuations in the protein content undertaken earlier by the writers [3], together with the rapid formation of the rhythm, its stability within certain limits, and its persistence in vivo and in vitro are evidence that 3-h fluctuations in the protein content in the acinar cells are evidently a genetically determined property of the cells – an endogenous rhythm characteristic of the glandular cells of the parotid gland [3]. However, certain aspects of this phenomenon remain unexplained. No changes in protein content characteristic of a 3-h rhythm were detected in the parotid gland cells of animals untrained by periodic feeding [2]. Similar results were obtained by the writers when they investigated sections of glands taken from various untrained animals in vitro [1]. It was not clear whether the food stimulus acts as synchronizer of the activity of individual acinar cells of the same gland or of the gland as a whole in different animals.

The object of the present investigation was to study the rhythm of cell protein synthesis in an organ culture of the parotid gland of animals untrained and trained by periodic feeding at 3-h intervals. The rhythm was studied at all stages of the cycle in cells of the same animal. This investigation should clear up some of the uncertainties regarding the nature of the 3-h food rhythm of the parotid gland.

EXPERIMENTAL METHOD

Male Wistar rats weighing 120-150 g were used. After the animals had been starved for 20 h they were divided into two groups. Group 1 contained rats fed at 3-h intervals for 2.5 days and then decapitated. Group 2 contained untrained animals, i.e., animals fed only once. Just as in the first case, the animals were decapitated. Pieces of the parotid gland were quickly removed and weighed. The weighed fragments (from 2

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 4, pp. 95-98, April, 1973. Original article submitted September 26, 1972.

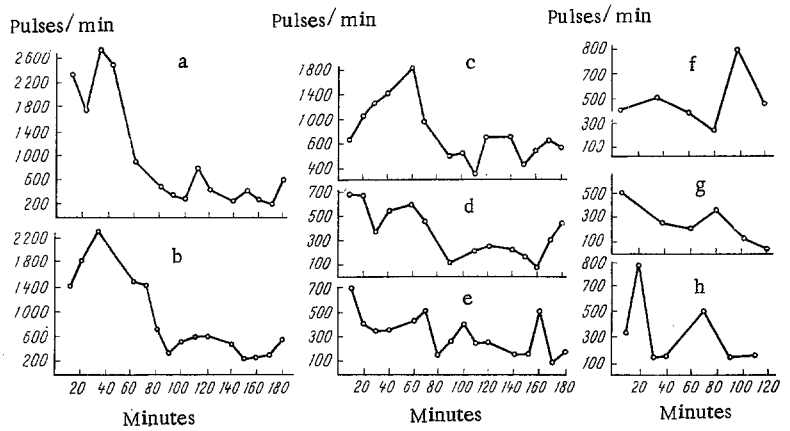

Fig. 1

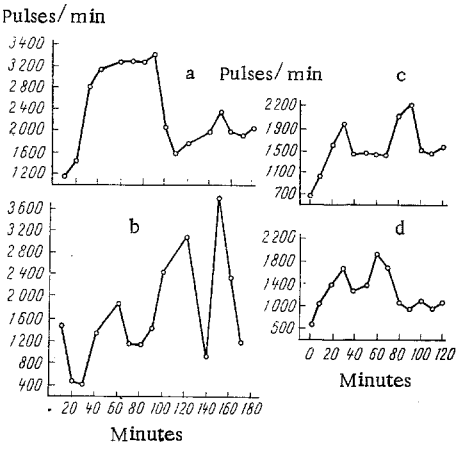

Fig. 2

Fig. 1. Changes in protein synthesis in parotid gland of trained and untrained rats on a 3-hourly food cycle, incubated briefly in medium No. 199. Each curve gives results of measurements on pieces of gland from the same animal. a, b) Trained animals; c-h) untrained animals; f, g, h) observations limited to 2 h. Abscissa, time after beginning of cycle (in min); ordinate, radioactivity of gland tissue (in pulses/g wet weight/min).

Fig. 2. Changes in protein synthesis in the parotid gland of untrained rats during organ cultivation. Each curve (a-d) is the result of measurements carried out on fragments of the gland from the same animal. Remainder of legend as in Fig. 1.

to 5.5 mg) were cut up into 10 to 12 pieces and incubated in medium no. 199 with the addition of 20% bovine serum, 70 μg vitamin C, and 4 mg glucose/ml medium. Cultivation was carried out in two ways. Pieces of the glands from the animals of group 1 and some animals of group 2 were incubated in medium no. 199 (with the additives) at 37°C for between 20 and 180 min. Pieces of the glands of the rest of the animals in group 2 were grown on HUFS membrane filters in the same medium no. 199 in Conway dishes for 12-16 h after explantation. The method of cultivating the parotid gland was described previously [4]. The intensity of incorporation of lysine-H^3 (Radiochemical Centre, Amersham, England; specific activity 20 Ci/mmole) in the parotid gland tissue was determined by measuring the radioactivity with a scintillation counter. For this purpose, a group of tissue fragments, 15 min before each investigation of the period of the food cycle, was transferred into medium no. 199 with the addition of lysine-H^3 (dose 20 μCi/ml), after which the pieces were washed with medium no. 199 containing an excess of unlabeled lysine, washed off with 5% TCA containing chlorine and, to remove the nucleic acids, hydrolyzed with 5% TCA at 90°C for 20 min. The supernatant was poured off and the residue washed with 96% ethanol, treated with hyamine, which disolved the residue, after which the toluene scintillator was added and radioactivity determined in pulses/min/g wet tissue. The counting efficiency was 25%. Puromycin and actidione (Serva, Heidelberg) in doses of 100 and 50 μg/ml, respectively, used as inhibitors of protein synthesis, verified the specificity of lysine-H^3 incorporation into the proteins of the parotid gland. Altogether 12 experiments were carried out. The rhythm was investigated at all stages of the feeding cycle in fragments from the same rat, i.e., ten to 12 fragments of the gland from the same animal were studied each time.

EXPERIMENTAL RESULTS

Curves a and b in Fig. 1 reflect changes in the radioactivity of the parotid gland of the same animal trained with periodic feeding at 3-h intervals. The results of an experiment after incubation of the gland for a short period in medium no. 199 are given in Fig. 1. The kinetics of the change in radioactivity of the glandular cells was indistinguishable from the fluctuations in protein content and 15-min amino acid labeling in vivo and in the organ culture determined by interferometry and autoradiography [1, 5]. As previous investigations showed, even after intensification of synthesis 15 min was not long enough for secretory

protein to be formed and liberated from the cell [5], so that the radioactivity of the cell after incubation for 15 min in medium no. 199 with lysine-H^3 characterizes the intensity of protein synthesis. Changes in the intensity of protein synthesis were fluctuating in character. The amplitude of these changes varied even in tests of the same animal. The properties of the curve at each moment were determined by successive coordination and interaction of separate biochemical rhythmic processes, and it was possibly for that reason that the curve was not strictly sinusoidal. However, the mean period of fluctuation in the intensity of protein synthesis, as in previous investigations in vivo and in vitro, when the animals were fed at 3-h intervals was 50-60 min. The decrease in amplitude of the intensity of protein synthesis in the second half of the 3-h feeding cycle after short-term incubation probably occurred because the conditions were less favorable than in organ cultivation. However, in these conditions also the period of the rhythm of protein synthesis was the same. The change in radioactivity in the parotid gland cells during short-term incubation was shown not to be random, but regular and rhythmic in character. Investigations of the parotid gland of the same untrained animal during short-term incubation (Fig. 1c-h) led to the conclusion that the rhythm of protein synthesis in this case is analogous to the rhythm of fluctuation in the protein content in animals trained by feeding at 3-h intervals.

Changes in the intensity of protein synthesis in the parotid gland of the untrained animals during organ cultivation are shown in Fig. 2. Each curve is the result of measurement of the radioactivity of fragments of gland from the same animal over a period of 3 h. From 14 to 16 h after explantation, rhythmic changes were clearly visible in the synthetic activity of the glandular cells with a mean period of fluctuation of 50-60 min. According to earlier observations, no rhythm of protein synthesis was detectable in untrained rats or in sections of the parotid gland of the same untrained animals [1, 2]. In the first case the explanation is that each point of the 3-h curve, when the previous method of investigation was used, was the mean for glands of several animals at the same stage. Glands of different animals, however, were in different phases of the secretory cycle relative to each other, and no regular rhythmic changes in protein were therefore observed. In the second case the material investigated by autoradiography was small in quantity and evidently insufficient to allow the conclusion to be drawn, as the present investigation showed. The glandular cells of the parotid glands evidently have their own natural rhythm of fluctuation in protein content, with a corresponding rhythm of protein synthesis. The mean period of the endogenous rhythm is 50-60 min. An interval of 3 h between meals is thus a factor synchronizing the natural rhythm of glands of different animals. As was stated above, the endogenous character of the rhythm of the parotid gland on a 3-h food cycle was also confirmed by autocorrelation analysis of fluctuations in the protein content in rats fed under different conditions, by the stability of the rhythm with a change in the feeding program of the rats within certain limits, by its steady persistence in vivo, and its detection in tissue culture [1]. A 3-h interval between meals is also perfectly natural for rats given a sufficient quantity of food.

Autoradiographic investigation of the 3-h rhythm showed that regular rhythmic changes in the protein content are connected with a change in the rate of synthesis and intracellular transport of proteins secreted by the cell [5]. The present investigation showed that these processes occur also in vitro. Information about them persisted for some time in vitro in the form of an after-reaction, thus permitting intercellular regulation of the rhythm.

It is clear from the literature that many tissues retain their typical endogenous rhythm observed in vivo, either circadian or paracircadian, when grown in tissue culture [6-10]. The natural rhythm of the parotid gland detected in the present experiments has a much shorter period. Possibly for rhythms unconnected with inevitable natural events (changes in the time of day, in temperature, the ebb and flow of the tide), but due to stimuli of a different order, are characterized by rhythms with a shorter than diurnal period. In the previous papers the hypothesis was put forward, and the grounds for it specified, that rhythmic changes in the protein content in glandular cells are essentially self-oscillation events [3]. If the time intervals between feeding of animals are changed so that the new time scale corresponds relatively to the natural scale, the period of fluctuation of the proteins in the cell will alter.

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