INVESTIGATION OF THE AMINO-ACID RESERVES IN PAROTID GLAND CELLS WITH RHYTHMIC FUNCTION

N. V. Nechaeva and V. I. Fateeva

UDC 612.313.015.348:612.398.192]-087.4

The dynamics of uptake of labeled amino acids by rhythmically functioning acinar cells of the rat parotid gland was studied. The amino-acid reserves in the parotid gland cells fluctuate regularly. However, the period of intake of the amino acids into the cell, unlike the periods of fluctuation of the intensity of protein synthesis, is independent of the animals' training program: In all the experiments the periods were the same and were equal to the endogenous period of the change in intensity of protein synthesis in the cells. The regular fluctuation of the total amino-acid reserves of the cell cannot be regarded as a direct regulator of synthesis of the protein assimilated by the cells during the experiment.

KEY WORDS: parotid salivary gland; protein synthesis; transport of amino acids through membranes; rhythmic activity of the cell.

A definite rhythm of quantiative changes in secretory protein with a mean period of 50-60 min has been found in the acinar cells of the parotid glands of intact rats by interference microscopy, autoradiography, and biochemical investigations [1, 3, 4, 6].

In the investigation described below a possible method of regulation of the rhythm at the level of membrane processes was studied by determining the dynamics of uptake of labeled amino acids by rhythmically functioning acinar cells.

EXPERIMENTAL METHOD

Male Wistar rats weighing 120-150 g were used. After starvation for 24 h the animals were divided into three groups. Group 1 included untrained rats, i.e., rats fed once only. Groups 2 and 3 contains rats fed at intervals of 3 h and 2 h respectively for 2.5 days. The animals were decapitated. Pieces of the parotid gland were cultured on membrane filters [5]. The reserves of labeled precursors in the gland cells were studied in explants of the parotid gland at various times of the digestive cycle. A group of these pieces was transferred to medium No. 199 without unlabeled lysine, but with the addition of lysine-H^3 (dose 10 μCi/ml and 15 μCi/ml) 5 min before each time of the digestive cycle investigated, and incubated at 37°C. The medium was then poured off, the pieces were washed 3 times with ice-cold medium No. 199 with excess of unlabeled lysine, and then treated with 1 ml 5% $HClO_4$ solution. After 2 h part of the extract was taken, neutralized with $KHCO_3$ to pH 6.0-8.0, and centrifuged for 5 min at 900 g. The residue was removed and the radioactivity of the acid-soluble fraction determined in a Bray scintillator on a type SL-30 scintillation counter. Meanwhile the intensity of incorporation of lysine-H^3 into proteins of the same pieces of the gland was determined by the method described earlier [2]. In both cases the radioactivity was calculated per milligram wet weight of tissue. Addition of these values gave the total radioactivity. The rhythm was investigated at all times of the digestive cycle in pieces from the same rat. In some experiments radioactivity of the acid-soluble fraction of the gland tissue only was determined. Parallel control experiments were carried out at 0°C. In this case the radioactivity of the acid-soluble

Laboratory of Cytology, Institute of Biology of Development, Academy of 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. 78, No. 9, pp. 47-49, September, 1974. Original article submitted November 12, 1973.

© 1975 Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $15.00.

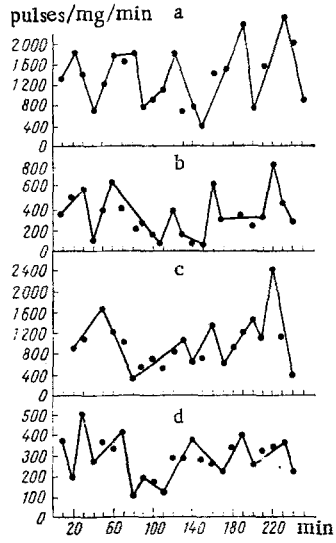

Fig. 1. Changes in incorporation of lysine-H^3 into proteins (b, d) of the gland and total reserves of that amino acid (a, c) in parotid gland cells of animals trained by feeding at intervals of 3 (a, b) and 2 h (c, d). Each curve shows results of measurements on pieces of gland from the same animal. Abscissa, time after beginning of cycle (in min); ordinate, radiography (pulses/mg wet weight/min).

fraction was reduced by 90-92%, evidence of a decrease in the uptake of free amino acids by the cell at a low temperature. The possibility of establishing the parameters of the rhythms of protein synthesis in the gland was tested by incubating pieces of the gland for 5 min in medium containing the isotope, instead of incubating for 15 min as in the previous experiments.

EXPERIMENTAL RESULTS

Fluctuations in the radioactivity of the acid-soluble fraction of the parotid gland were observed in untrained animals and in animals trained by short-term periodic feeding at intervals of 3 and 2 h. Each experiment gave the change in radioactivity of the acid-soluble fraction of the gland of the same animal at intervals of 10 min. Since the radioactivity of the free amino-acid reserves exceeded the intensity of incorporation of lysine-H^3 into protein, it is this parameter that essentially determines the fluctuations in the total reserves (Fig. 1) of this amino acid in the cell at a given moment of the digestive cycle. The magnitude of the total reserves presumably reflects the uptake of labeled amino acid by the tissue of the gland at the various times of the cycles studied. The changes in the radioactivity of this fraction are periodic in character. The amplitude of the changes in radioactivity of the free lysine-H^3, given at the same intensity of labeling, varied only slightly in the same animal. The periods of fluctuation of radioactivity of the acid-soluble fraction in cells of the same animal varied a little during the digestive cycle. However, the mean period of uptake of amino acid by the cells of the untrained animals, and also of animals trained by feeding at intervals of 2 and 3 h, was the same, namely 50-60 min. This period thus evidently does not depend on the feeding program of the animals.

Curves a and c (Fig. 1) reflect changes in the radioactivity of the total reserves of lysine-H^3 in the parotid gland cells of animals trained with food cycles of 3 and 2 h. Curves b and d show changes in the intensity of incorporation of lysine-H^3 into proteins of the same pieces of the gland, connected with changes in the intensity of protein synthesis in them [2]. The rhythm of protein synthesis, as has been shown previously, varies depending on the feeding pattern of the animals. The mean periods of the rhythm in digestive cycles of 2 and 3 h were 30-40 and 50-60 min, respectively, similar to those determined previously [2, 3, 6]. The amplitude of changes in the radioactivity of free lysine-H^3, given an identical intensity of labeling, varied only a little during the digestive cycle in the same animal. The reserves of the labeled amino acid in the cells, like the radioactivity of free lysine-H^3 in them, varied rhythmically in the digestive cycles of 2 and 3 h. However, the period of fluctuations of the total reserves of labeled amino acid in all the experiments was 50-60 min, corresponding to the endogenous period of the change in intensity of protein synthesis in the rat parotid gland [2, 3, 6]. As was shown previously, regular rhythmic changes in the protein content in the parotid gland cells are connected with changes in synthesis of the proteins secreted by the cell [6] and are independent of the time of year. Fluctuations in the intensity of protein synthesis, with a period that differs from the endogenous, arise as a variant of the endogenous rhythm. They can be found in the acinar cells only after certain forms of stimulation of the gland [3]. Information on the parameters of the rhythm is held in the acinar cells in vivo and in vitro as trace reactions [2, 3]. Intracellular regulation of the secretory rhythm has been shown to exist in the acinar cells of the parotid gland. The results of this investigation show that the amino-acid reserves in the parotid gland cells fluctuate rhythmically. This may be connected with changes in the permeability of the cell membrane. However, the period of uptake of amino acids by the cell in independent of the training program: in all experiments the mean periods were the same and were equal to the mean endogenous period of fluctuation in the intensity of protein synthesis in the cells. The rhythmic changes in the total amino-acid reserves of the cell, that are possibly a consequence of a change in the permeability of the cell membrane, cannot thus be regarded as a direct regulator of the rhythm of synthesis of protein assimilated by the cells during the experiment.

LITERATURE CITED

1. V. Ya. Brodskii and N. V. Nechaeva, Tsitologiya, No. 2, 221 (1971).
2. V. Ya. Brodskii and N. V. Nechaeva, Dokl. Akad. Nauk SSSR, 209, No. 3, 212 (1973).
3. V. Ya. Brodskii, N. V. Nechaeva, and V. I. Prilutskii, Tsitologiya, No. 2, 177 (1973).
4. N. V. Nechaeva and V. Ya. Brodskii, Tsitologiya, No. 1, 52 (1973).
5. N. V. Nechaeva, T. B. Aizenshtadt, and E. A. Luriya, Tsitologiya, No. 4, 465 (1970).
6. N. V. Nechaeva and V. I. Fateeva, Byull. Éksperim. Biol. i Med., No. 4, 95 (1973).