DEPENDENCE OF BIOSYNTHETIC ACTIVITY OF PARATHYROCYTES ON STATE OF PARATHYROID FUNCTION

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UDC 616.447-008.6-07:616.447-018.1-073.171-073.916

KEY WORDS: parathyroid glands, protein synthesis, autoradiography, morphometry.

The problem of the morphometric equivalents of functional activity of the parathyroid gland epithelium has not yet been adequately studied. We know that the average size of the parathyrocytes may characterize the functional state of the gland cells: they become smaller during hypofunction and larger during hyperfunction of the gland [5, 8, 9]. However, to assess the reliability of this morphometric parameter, quantitative comparisons have to be made of the average size of the parathyrocytes and the intensity of biosynthetic processes taking place in them within a wide range of possible variations of function of the parathyroid glands. This problem is not discussed in the literature. The aim of the investigation described below was to undertake such an analysis.

EXPERIMENTAL METHOD

Experiments were carried out on 31 male albino rats weighing 170-200 g. Parathyroid hypofunction was achieved: a) by intraperitoneal injections of a 10% solution of calcium gluconate in a dose of 1.0 ml twice a day for 4 days [3] — four rats; b) keeping the animals for 10 days on a diet with a high content of calcium and vitamin D_2 [11], for which purpose four rats were given a 3.5% solution of calcium chloride for drinking and a daily injection by gastric tube of 10,000 IU of vitamin D_2. Parathyroid hyperfunction was achieved: a) by intraperitoneal injections of a 2.5% solution of trilon B in a dose of 1.0 ml twice a day for 4 days [3] — three rats; b) by keeping the animals for 10 days on a diet with a high content of inorganic phosphates [9, 12], for which purpose the rats were given an aqueous solution of mono- and disubstituted calcium phosphates in the ratio of 8:1, pH 6.8-6.9, to drink; the average inorganic phosphorus concentration was 10 mg/ml — eight rats; c) by subtotal (75-90%) resection of the parathyroid glands, 30 days after the operation [2] — seven rats. The control consisted of five intact animals. Biosynthetic activity of the parathyrocytes was assessed by giving a single injection of ^{3}H-leucine (specific activity 90 Ci/mmole) in a dose of 2.5 μCi/g intraperitoneally, 25 min before sacrifice [4, 10]. This essential amino acid is one most frequently found in the composition of the parathormone molecule [7]; according to the results of an autoradiographic investigation of the parathyroid glands during pulse labeling with ^{3}H-leucine about 85% of the isotope is involved in hormonal synthesis [6]. The rats were killed with ether vapor. Paraffin sections 4 μ thick were coated with type M emulsion and exposed for 11 days at 4°C; after development, they were stained with hematoxylin and eosin. Using the stereologic "fields" method, the mean areas of cross-section of the parathyrocytes were determined with the aid of an ocular attachment with 100 equidistant points [1]. The number of grains of silver was counted in each animal simultaneously in 75 randomly chosen squares of the ocular attachment (the area of each square is 65 μ^2). To evaluate synthetic processes in the cells the mean number of grains was counted per unit area of section (100 μ^2) and per parathyrocyte (allowing for the mean area of section of the gland cells). The background level in the series was 6-8% of the basic labeling level. The results were subjected to statistical analysis by methods of correlation and regression analysis and the significance of differences was judged by Student's test.

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0007-4888/89/0001-0115$12.50

EXPERIMENTAL RESULTS

Inhibition of parathyroid gland function led to a decrease in the average area of cross-section of the parathyrocytes to 49.4 ± 1.3 μ^2 (58.8 ± 1.2 μ^2 in the control; $p<0.01$) and a decrease of 47% in the average number of grains calculated per gland cell (to 5.7 ± 0.3 from 10.8 ± 0.5 in the control; $p<0.01$). The average areas of cross-section of the parathyrocytes during hyperfunction of the glands were greater in all the animals than in the control. Stimulation of the glands by injection of trilon B or by a phosphate-rich diet led to moderate hypertrophy — on average to 70.0 ± 1.7 μ^2 ($p<0.05$ compared with the control). The most marked increase in the average size of the cells (80-100 μ^2) was observed after subtotal resection of the parathyroid glands (on average to 95.3 ± 3.5 μ^2; $p>0.01$ compared with the control). The number of grains of silver calculated per parathyrocyte was on average 30% higher during parathyroid hyperfunction than in the control (13.7 ± 0.5; $p<0.05$). Close positive correlation was found between the average area of cross-section of the cells and the number of grains calculated per parathyrocyte ($r_{xy} = +0.85$; $\eta_{yx} = +0.98$; $p<0.01$). The corresponding statistical estimations of this dependence showed it to be nonlinear in character. The number of grains calculated per cell increased most strongly within the interval from 45 to 65 μ^2, it stabilized between 65 and 80 μ^2, and rose gradually between 80 and 110 μ^2.

Changes in biosynthetic activity of the cells during hypo- and hyperfunction of the glands within the range from 45 to 75 μ^2 (+25% from the control level) are linked mainly with differences in the intensity of incorporation of labeled leucine into the parenchyma of the gland. This is shown by the similar character of the change in the average number of grains of silver calculated per cell and per unit area of cross section ($r_{xy} = +0.94$; $p<0.01$). Variations in the number of grains per unit area of cross section which were found were strictly specific for parathyrocytes; similar calculations for follicular thyrocytes in the same sections showed no significant changes in this parameter in the experimental groups compared with the control ($p>0.05$). After subtotal resection of the parathyroid glands the intensity of incorporation of leucine per unit area of section stabilized at the control level, and a progressive increase in the progressive increase in the number of grains calculated per parathyrocyte within the 80-110 μ^2 interval took place entirely on account of marked hypertrophy of the cells (no correlation could be found between the number of grains calculated per cell and per unit area of section within this interval, $r_{xy} = +0.25$; $p>0.05$).

The results of this investigation are evidence that the intensity of incorporation of ^{3}H-leucine into the parenchyma of the parathyroid glands is closely connected with parathyrocyte function. This is confirmed by data obtained by various workers [6, 10] on the informativeness of autoradiographic analysis of incorporation of labeled amino acids for the study of parathormone biosynthesis. The presence of close positive correlation between the intensity of synthetic processes in the cells and the average area of cross section of the parathyrocytes suggests that the latter is an informative parameter, characterizing objectively the functional state of the parathyroid epithelium. Analysis of the character of this correlation can provide a firm basis for the functional interpretation of the results of morphometric investigation of the parathyroid glands.

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