ANTIBODIES TO CALCIUM REGULATING HORMONES IN THE INFECTIOUS-ALLERGIC FORM OF BRONCHIAL ASTHMA

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An important role in the pathogenesis of bronchial asthma is played by Ca^{++} ions. Several hormones participate in the regulation of Ca^{++} metabolism. The most important of these are calcitonin, a thyroid gland hormone with a hypocalcemic effect, and parathormone and cortisol, hormones of the parathyroid and adrenal glands, respectively. These hormones participate in the resorption of bony tissue by eliminating Ca^{++} from it. If secretion of glucocorticoid hormones and, in particular, of cortisol is excessive, during treatment with glucocorticoid preparations a syndrome of calcium "depression" arises [3]. The authors showed previously [2] that antibodies to calcitonin appear. In all probability these bind the excess of the hormone, thus reducing its calcium-eliminating action.

Considering the effect of parathormone and cortisol on the blood Ca^{++} level, it was decided, in the investigation described below, to compare the time course of formation of antibodies to calcitonin, parathormone, and cortisol with the blood Ca^{++} level in patients with an infectious-allergic form of bronchial asthma during an exacerbation, and also after a 10-day course of combined treatment including the use of glucocorticoid therapy.

EXPERIMENTAL METHOD

Serum from patients with the infectious-allergic form of bronchial asthma during a flare-up (group 1) and after treatment with glucocorticoids (group 2) was used for investigation. Serum from healthy first-time blood donors served as the control.

The blood Ca^{++} concentration was determined by means of standard kits (from "Lachema," Czechoslovakia).

Antibodies to calcitonin, parathormone, and cortisol were determined by enzyme immunoassay, using the enzyme-labeled antibodies test (ELAT) [4].

Standard samples of calcitonin, parathormone, and cortisol, taken from kits for radioimmunoassay ("Mallinckrodt," West Germany; "CIS" France) were used as antigen. The conjugate for the test was prepared from the total globulin fraction isolated by means of ammonium sulfate from blood sera (N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow) against human γ-globulin, Protein was conjugated with enzyme (Mark A horseradish peroxidase, from "Biokhimreaktiv," USSR) by the periodate oxidation method [1]. The reaction was set up in polystyrene plates as follows: the antigens were fixed to the plates – calcitonin 20 pg/ml, parathormone 50 pg/ml, cortisol 7.8 μg/ml. Solutions were made up in 0.1 M carbonate-bicarbonate buffer, pH 9.6. Into each well of the plate 0.2 ml of a corresponding antigen was added and the plate was incubated overnight at 4°C. It was then thoroughly washed under a jet of tap water. All the wells were filled for 1.5-2 min with tap water containing 0.05% Tween-20, and again washed with a jet of water. Into all the wells 0.1 ml phosphate-salt buffer, pH 7.2, with 0.5% Tween-20 was poured, and titration was carried out with 0.1 ml of the experimental and control test sera in dilutions of 1:40 to 1:6400. The plates were incubated for 1 h at 37°C, after which they were again washed and 0.2 ml of the conjugate was added to all the wells in a dilution of 1:1000 in phosphate-salt buffer with Tween. After incubation for 1 h

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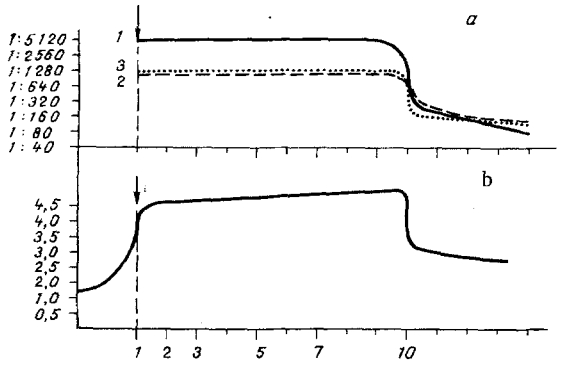

Fig. 1. Titer of antibodies (a) to calcitonin (1), parathormone (2), and cortisol (3) and blood Ca^{++} level (b) in acute stage of infectious-allergic form of bronchial asthma and after a 10-day course of combined treatment. Abscissa, time of investigation (in days); ordinate: a) dilution of serum; b) Ca^{++} concentration (in mM). Arrow indicates acute stage of disease.

at 37°C the plate was again washed. The substrate was 0.08% 5-aminosalicylic acid with 0.05% H_2O_2. The results of the test were read 1 h after addition of the substrate on a vertical-beam "Minireader P-590" spectrophotometer at 490 nm. The results obtained by analysis of plates containing patients' sera were compared with the corresponding readings for the blood donors' sera. Antibodies were discovered in the sera starting with a dilution of 1:100.

EXPERIMENTAL RESULTS

A high titer of antibodies to calcitonin (1:1280), parathormone (1:2560-1:5120), and cortisol (1:1280) was found in sera from patients with infectious-allergic bronchial asthma in the acute stage of the disease. The Ca^{++} concentration found in patients of this group was 4-5 mM (normal 2.25-2.75 mM).

The Ca^{++} level declined in the patients of group 2 after a 10-day course of combined treatment (with glucocorticoids), but still remained within normal limits (2.5-2.7 mM). Antibodies to calcitonin, parathormone, and cortisol were observed in the sera on average up to a dilution of 1:160, or they were absent altogether (Fig. 1).

Exacerbation of the infectious-allergic form of bronchial asthma is evidently accompanied by the sudden release of the hormones calcitonin, parathormone, and cortisol from the corresponding endocrine glands. The marked hypersecretory activity and the raised blood enzyme levels may probably lead to qualitatively new changes in the immune system, thus inducing antibody formation to calcitonin, parathormone, and cortisol. In the authors' view, the antibodies thus formed bind the excess of hormones in the blood and, perhaps, inhibit somehow the activity of the C-cells of the thyroid and parathyroid glands and adrenals [1, 5], thus restricting any further increase in hormone release.

The blood Ca^{++} level is regulated, not so much by the absolute concentration of each separate hormone, as by the ratio between their concentrations. It is probably their combined action which maintains the optimal blood level of Ca^{++}. In the acute period of the disease hypercalcemia was observed, possibly due to hypersecretion of parathormone and cortisol, and also perhaps the thyroid hormones and catecholamines, which have a catabolic action on bone tissue, raising the blood Ca^{++} level. The calcium-eliminating effect of calcitonin under these circumstances is evidently weak because of the low antibody titer. It can thus be tentatively suggested that changes in the relative blood levels of the hormones lead to hypercalcemia.

After the use of glucocorticoid preparations for the treatment of infectious-allergic bronchial asthma, the titer of antibodies to all the hormones fell to 1:160. In some cases the antibodies disappeared completely. Restoration of normal secretion of the hormones and their optimal ratio in the blood evidently leads to disappearance of the antibodies for lowering of their titer. At the same time, glucocorticoid preparations are known to lower the Ca^{++} level [1]. They evidently restore the normal ratio between the hormones, after which the antibody titer falls. The normal Ca^{++} level is simultaneously restored. The possible mechanism is the direct effect of the hormones on cells of the endocrine glands.

A longer course of glucocorticoid therapy of the infectious-allergic form of bronchial asthma will probably intensify development of the calcium "depression" syndrome, and when marked hypocalcemia is present, secondary osteoporosis may develop as a result of parathyroid gland stimulation.

Determination of antibodies to calcitonin, parathormone, and cortisol can thus be used to predict the course of bronchial asthma, and regulation of the levels of antibodies to these hormones may be a possible therapeutic measure for the treatment of this disease.

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