HOMEOSTATIC ROLE OF ANTICALCITONIN ANTIBODIES IN CALCIUM METABOLISM IN EXPERIMENTAL DIABETES MELLITUS

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UDC 616.379-008.64-092.9-07:616.152.41-092:616.153.962.4-097:616.154:577.175.446

KEY WORDS: diabetes mellitus, calcium, anticalcitonin antibodies, reaction of enzyme-labeled antibodies.

Calcium is essential for the triggering insulin secretion from β-cells of the pancreas [8, 9, 10]. In hypocalcemia insulin secretion is depressed, with the consequent development of hyperglycemia. Various workers have found a definite rule regarding changes in the blood calcium level and changes in the stages of their disease in patients with diabetes mellitus. For instance, in children with diabetic coma, the blood calcium and phosphorus levels were found to be low [7]. In the phase of compensation of diabetes, the serum calcium and phosphorus levels of nearly all patients were within normal limits [5]. In chronic diabetes, the blood calcium level was raised [1, 5]. Some authorities consider that the disturbance of phosphorus and calcium metabolism in diabetics is secondary and is connected with adaptive hyperparathyroidism [7]. At the same time, we know that the blood calcium level falls in the course of development of hyperglycemia under the influence of injected calcitonin in experiments on animals [3] and in healthy subjects receiving small doses of calcitonin [11, 13]. Glucose tolerance was reduced as also was insulin secretion by the pancreatic β-cells, but the blood calcitonin level itself was increased not only in diabetics, but also in newborn infants of diabetic mothers [2].

This suggested that calcitonin must be regarded as a diabetogenic factor.

Thus calcitonin, a hormone of the C-cells of the thyroid gland, has a marked hypocalcemic action. By inducing hypocalcemia, calcitonin tends to reduce insulin secretion by the pancreatic β-cells. This results in hyperglycemia. Meanwhile calcitonin activates gluconeogenesis and glycolysis. As a result of this combined action the blood sugar level rises.

In experimental (alloxan) diabetes in rats, accompanied by marked hyperglycemia, the present writers previously discovered the appearance of antibodies to calcitonin [4]. An excess of calcitonin in the blood evidently leads to qualitative changes in the immune system, resulting in the appearance of antibodies to this hormone. The appearance of antibodies to the hormone is evidently adaptive in character. Antibodies not only inhibit the thyroid C-cells, but most probably also bind an excess of the hormone in the blood, thus depressing its toxic effect.

The aim of this investigation was to study the concentrations of calcium and anticalcitonin antibodies in the course of development of experimental alloxan diabetes in rats.

EXPERIMENTAL METHOD

The test material consisted of blood serum from rats with experimental diabetes mellitus. To produce this condition alloxan was first recrystallized, then injected into animals (male rats weighing up to 300 g), previously deprived of food for 2 days, in the form of a 2% solution into the caudal vein in a dose of 4 mg alloxan/100 g body weight. The development of diabetes was observed 7-10 days later, on the basis of the rising blood sugar level. The blood glucose concentration was determined by the color reaction with orthotoluidine. The calcium concentration was determined by the color reaction with a solution of glyoxal-bis (kit from Lachema, Czechoslovakia).

Sera of the experimental rats were divided into two groups based on blood levels of anticalcitonin antibodies.

Department of Pathological Physiology, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 163-165, February, 1988. Original article submitted December 19, 1986.

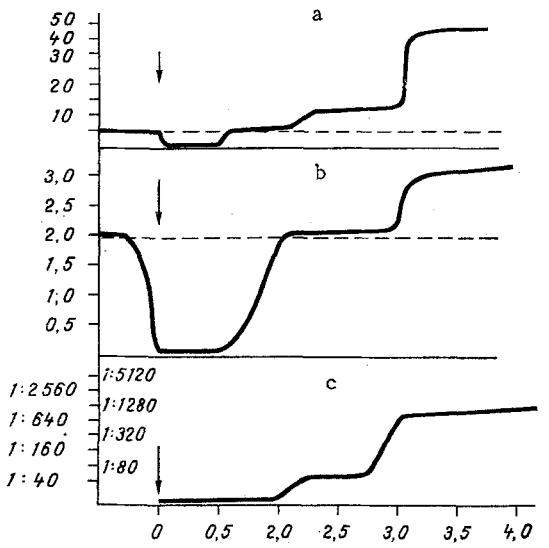

Fig. 1. Blood levels of sugar (a), calcium (b), and anticalcitonin antibodies (c) in course of experimental alloxan diabetes in rats. Abscissa, time (in msec); ordinate: a) sugar level (in mM), b) calcium level (in mM), c) antibody level (titer).

Anticalcitonin antibodies were determined by enzyme immunoassay by the enzyme-labeled antibodies test (ELAT), using a test system developed by ourselves [4].

Calcitonin isolated from normal rat thyroid glands by Stekol'nikov's method was used as the antigen.

EXPERIMENTAL RESULTS

Altogether 50 rats diabetic for between 2 weeks and 3.5 months were studied. In the experimental group of rats with diabetes for under 2 weeks the blood sugar level varied from hypoglycemia to the upper limits of normal, namely 6-8 mM (compared with the normal value of 4-7.7 mM; Fig. 1). However, the calcium level was sharply depressed to 0.23-0.5 mmole/liter (normal value 2.4-2.6 mM). Antibodies to calcitonin were not found. In the experimental groups with diabetes lasting 4-6 weeks or more the blood sugar concentration was above the optimal limits (10-12 mM) and the calcium concentration did not reach the lower limits of normal (1.8-2 mM). Antibodies to calcitonin either were observed in low concentrations or they were absent. Antibodies in the sera were detected starting with a dilution of 1:100 (Fig. 1a, b, c).

Antibodies to calcitonin appeared (titer 1:160) in individual animals with signs of marked hyperglycemia (15-16.8 mM).

In rats of the experimental group with diabetes lasting over 2.5-3.5 months, besides marked hyperglycemia (30-40-42 mM) hypercalcemia (from 2.8 to 3 mM) also developed. The titer of anticalcitonin antibodies in these cases was high (1:1260-1:6400).

In the early insulin-dependent form of diabetes (after 2 weeks of the experiment) hypocalcemia developed evidently on account of hypersecretory activity of the C-cells of the thyroid gland. The hypocalcemia observed in the present experiment could also have been the cause of inhibition of insulin secretion by pancreatic β-cells. The appearance of hyperglycemia in these experimental animals was probably connected with hypoinsulinemia also. It is perhaps pathogenetic changes of this character that take place in the acute period of the disease in patients with diabetic coma and also in newborn infants of diabetic mothers.

In experimental diabetes with a long course (more than 2.5-3.5 months) qualitative changes evidently take place in the immune system of rats in response to extremely high calcitonin secretion, and as a result, antibodies to calcitonin are formed.

The antibodies which appeared either somehow or other inhibit calcitonin secretion by the thyroid gland, or they bind calcitonin in the form of an antigen–antibody complex, which later is evidently eliminated from the body. We are inclined also to consider that the

binding of biologically active substances (histamine, serotonin), including calcitonin, observed by several workers [6], takes place through the formation of antibodies to those substances. In the present experiment, as a result of this action of antibodies, the calcium-excreting effect of calcitonin is evidently reduced. This hypothesis is supported by the rise of the blood calcium level in the presence of a high titer of anticalcitonin antibodies during chronic diabetes lasting longer than 3 months in rats. Similar changes may perhaps also take place in human patients with chronic diabetes.

The presence of anticalcitonin antibodies in association with hyperglycemia is probably one of the homeostatic factors that control insulin secretion through a change in the blood calcium level, and prevent the blood sugar from rising during a chronic course not only of experimental alloxan diabetes, but also evidently of human diabetics in the adaptation stage.

Determination of anticalcitonin antibodies can be used in the diagnosis of disturbance of electrolyte metabolism in diabetes mellitus, and regulation of the level of anticalcitonin antibodies can be used as a therapeutic measure for the correction of diabetic complications.

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