MORPHOLOGY AND PATHOMORPHOLOGY

Immunoreactivity of Neuron-Specific Enolase (NSE) in Human Pancreas in Health and Type 1 Diabetes Mellitus

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 6, pp. 704-708, June, 2010
Original article submitted March 20, 2009

The role of neuron-specific enolase (glycolytic enzyme; marker of nerve fibers and Langerhans islet in human pancreas) in the development of type 1 diabetes mellitus was studied in autopsy specimens from 6 adult patients. Autopsied specimens of the pancreas from 7 subjects without carbohydrate metabolism disorders served as the control. Autopsied specimens of the pancreas from a child with the clinical diagnosis of type 1 diabetes mellitus, a child without carbohydrate metabolism disorders, and from 7 human fetuses of 15-40 weeks gestation were also studied. In control specimens, the neuron-specific enolase was detected in the pancreatic nerve fibers and Langerhans islets. Studies of pancreatic tissue specimens from adult patients with type 1 diabetes mellitus showed no immunopositive reaction to neuron-specific enolase in insulin-negative specimens. A possible mechanism of type 1 diabetes mellitus development is suggested.

Key Words: *pancreas; diabetes mellitus; neuron-specific enolase*

Neuron-specific enolase (NSE) revealed in 1968 by B. Moore and R. Perez in cattle brain, was the first enzymatic neurospecific protein discovered [2]. It is a glycolytic enzyme (2-phospho-D-glycerate hydrolase) from the enolase family. In humans enolases exist as variants of dimers of three immunologically different subunits (α, β, and γ). The enolase α-subunit is found in various tissues, β-subunit is present only in the heart and striated muscles, and γ-enolase is found in nervous and neuroendocrine cells. NSE is now used as a marker of nervous and neuroendocrine differentiation in normal tissues and in tumors, including pancreatic tumors (insulinoma) [3,11]. Enzyme activity correlates with clinical status and is used for disease monitoring and prognosis. Despite active use of NSE for the diagnosis of pancreatic diseases, its role in the development of diabetes mellitus remains virtually not studied.

In order to evaluate the role of this nervous system marker and a marker of islet cells, we carried out an immunohistochemical study of human pancreas in health and during the development of type 1 diabetes mellitus (DM1).

MATERIALS AND METHODS

Autopsied specimens of the pancreas from 6 patients with the clinical diagnosis of DM1 were analyzed. Autopsy specimens of the pancreas from 7 subjects with-

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out carbohydrate metabolism disorders served as the control. In addition, pancreatic tissue specimens from a child with the clinical diagnosis of DM1 and from a child without carbohydrate metabolism disorders were studied. All specimens were fixed in buffered formalin. In addition, specimens of the pancreas from 7 human fetuses (weeks 15-40 of gestation) were studied. The specimens were fixed (24 h) in Bouin's fixative and stored in 70% ethanol. Specimens of the pancreas were embedded in paraffin and 10-μ sections were prepared. Endocrine cells and nerve elements were detected by immunohistochemical methods.

For immunohistochemical reaction, the deparaffinized and hydrated sections were treated with 3% H_2O_2 for 10 min in order to block endogenous peroxidase. The sections were incubated at ambient temperature (45 min) with antibodies to insulin (mouse monoclonal antibodies 1:1000-1:2000, Sigma), glucagon (mouse monoclonal antibodies, 1:1000-1:2000, Sigma), and NSE (mouse monoclonal, Ab-1 (Clone E27), ready to use, Thermo Scientific). These antigens were detected by Ultra Vision Detection System Anti-polyvalent, HRP/DAB (Lab Vision Corp.) or Ultra Vision LP Detection System Anti-polyvalent, HRP polymer & DAB Plus Chromogen (Lab Vision Corp.).

RESULTS

Reactions to insulin and glucagon were positive in the Langerhans islets of control specimens of human pancreas (Fig. 1, *a*, *c*). The NSE was detected in Langerhans islet cells (Fig. 1, *b*) and in nerve fibers in all pancreatic specimens from subjects without carbohydrate metabolism disorders. The density of the islets distribution in a visual field and in the specimens from human fetuses and from children in the control was

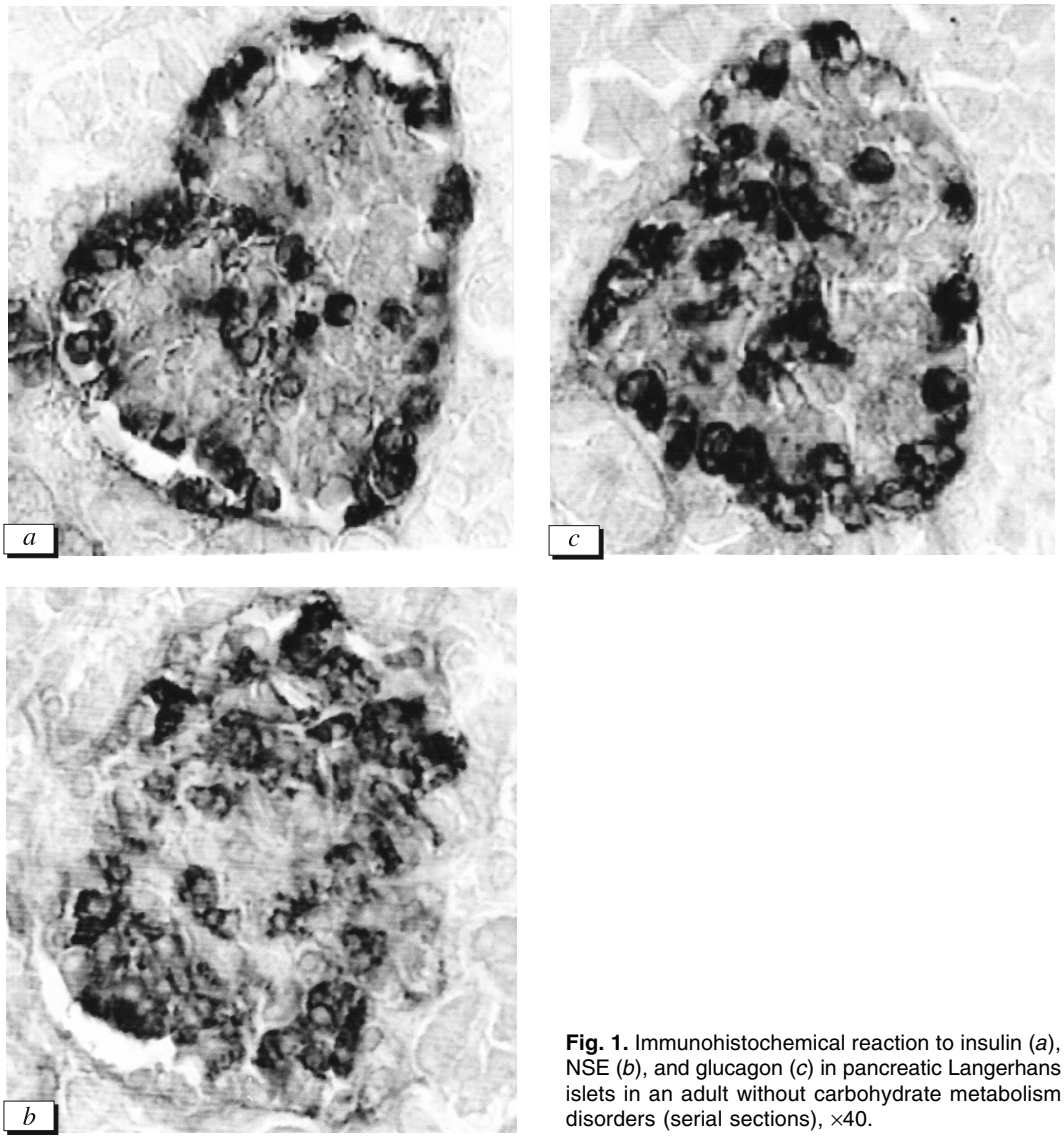

Fig. 1. Immunohistochemical reaction to insulin (*a*), NSE (*b*), and glucagon (*c*) in pancreatic Langerhans islets in an adult without carbohydrate metabolism disorders (serial sections), ×40.

significantly higher than that in adults, and the pancreatic tissue was more intensely innervated (large and fine fibers were seen); in addition, in some cases the islets were enveloped in a fine membrane with positive reaction to NSE (Fig. 2).

Labeling with antibodies to insulin revealed no insulin-containing cells in Langerhans islets in 2 DM1 patients. Detection of glucagon-containing cells showed that α-cells constituted the bulk of the islet in these 2 cases (Fig. 3, *a*). No reaction to NSE was detected in islet cells; in pancreatic nerve elements the reaction was seen only in large fibers (Fig. 3, *b*). Partial replacement of the endocrine tissue with connective tissue was found in one case. In addition, residual insulin secretion was detected, in the presence of partially retained β-cells in the islets, and a slight reaction to NSE. In 3 cases, the reactions to insulin, glucagon, and NSE were positive. Insulin was retained in some islets in a child with the clinical diagnosis of DM1, while the bulk of endocrine cells in Langerhans islets was glucagon-secreting. In this case NSE was also found in the islets completely without insulin secretion. In addition, the number of nerve fibers was significantly lower in this specimen in comparison with tissue specimen from a child without carbohydrate metabolism disorders.

The development of DM1 is assumed to be associated with destruction of pancreatic β-cells, which usually leads to absolute insulin insufficiency [1]. The absence of immunohistochemical reaction to insulin in pancreatic Langerhans islets during the development of severe DM1 is a characteristic phenomenon, described for experimental animals [9] and humans [12]. It is also assumed that the development of this type of diabetes is associated with destruction of β-cells, and the bulk of the islet is constituted by glucagon-secreting cells. It was also reported that the counts of somatostatin cells and PP cells are not reduced in this disease [8].

In this study we observed no immunohistochemical reaction to NSE in the islets of insulin-negative adults. Previous data [3,11] suggest that NSE is primarily characteristic of β-cells of pancreatic islets and can serve as an immunohistochemical marker of DM1 development in adults.

Cases with residual secretion of insulin and hence, retained β-cells during even many years after disease

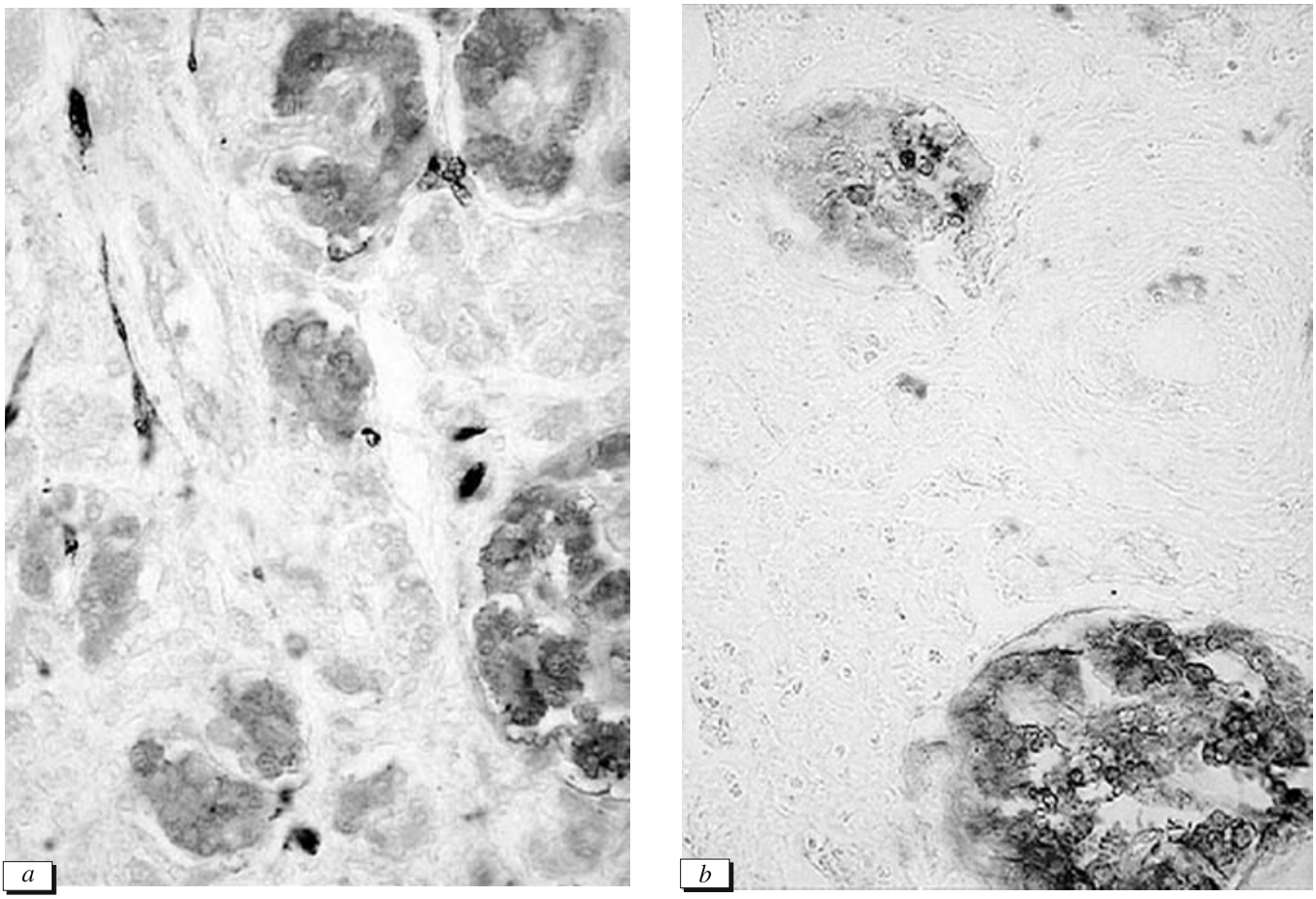

Fig. 2. Immunohistochemical reaction to NSE in pancreatic Langerhans islets in specimens from a fetus (*a*) and from an adult without carbohydrate metabolism disorders (*b*), ×40.

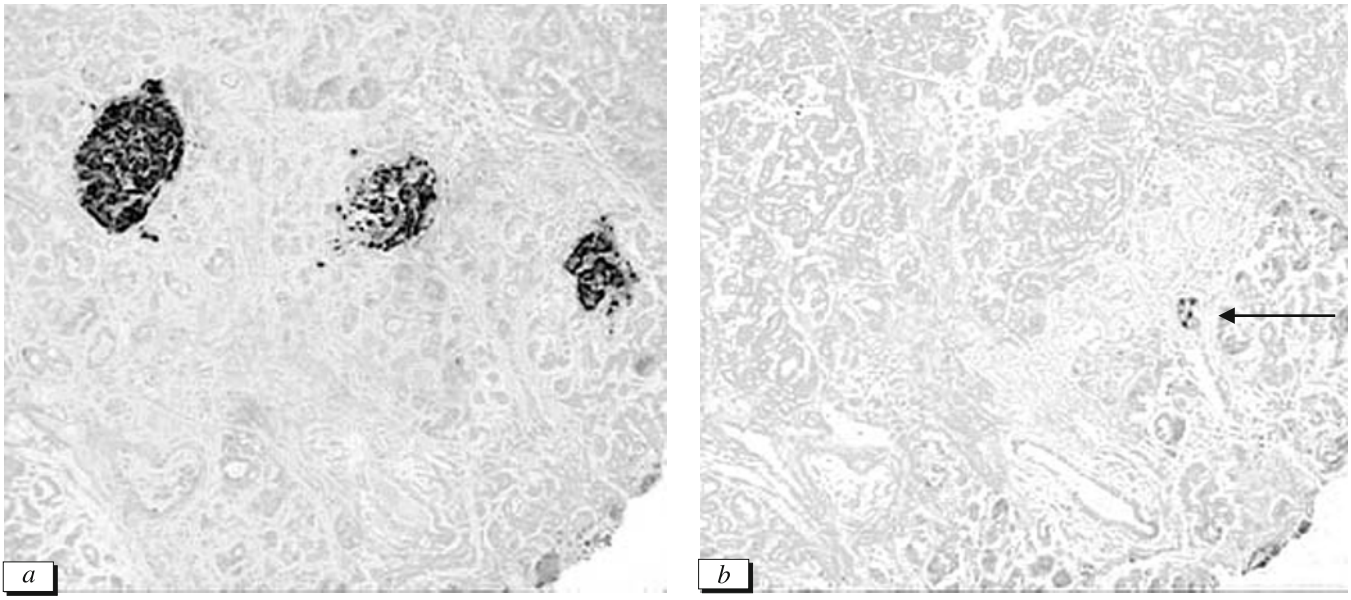

Fig. 3. Immunohistochemical reactions to glucagon (*a*) and NSE (*b*) in pancreatic Langerhans islets in severe DM1 (serial sections), ×10. Arrow shows a nerve fiber.

manifestation were described [5]. In the present study, the reaction to insulin and NSE was retained to this or that measure in Langerhans islets of 4 patients with DM1 (according to case histories). This fact also confirms the hypothesis on correlation between insulin and NSE levels in pancreatic β-cells of adults.

Study of the pancreatic nervous elements showed that the reaction to NSE in nerve fibers virtually disappeared in comparison with the normal status in 2 completely insulin-negative cases. We previously showed [4] that DM1 is associated with a decrease in the number of fine nerve fibers immunoreactive to peripherin.

It is known that DM1 is associated with involvement of the peripheral nervous system. According to the traditional approach to the mechanism of neuropathy development, hyperglycemia causes nerve injuries [1]. However, some authors think that the chain of pathological processes in diabetes is initiated by disorders in the work of nerve endings in the pancreas [6,13-15]. We previously described a possible mechanism of this disorders [4]. Now we somewhat more accurately describe the possible mechanisms of DM1 development.

By the end of 1980s, a concept of autoimmune pathogenesis of DM1 was developed. According to this concept, autoimmune aggression against β-cells causes their total death [1]. Within the framework of autoimmune nature of DM1 we suggest the following sequence of events in the development of this disease. Stage 1 consists in autoimmunization of the CNS with proteins, specifically, with NSE. This event can be caused by long-term stress, mechanical injury, and aftereffects of infections or chemical intoxication. CNS and peripheral nervous system are much better protected than Langerhans islets. As a result, T cells attack β-cells producing proteins characteristic of the nervous system.

The pathological processes can be accelerated due to glycolysis disorders in β-cells without NSE or in the presence of its low concentrations and hence, reduced production of insulin. Glycolysis is the main pathway of glucose utilization in animals and humans [2]. The β-cells are glucose sensors: insulin is secreted in response to an increase in blood glucose concentration in order to restore the initial concentration glucose. In turn, insulin release by β-cells in response to an increase of glucose concentration is realized by the following mechanism. Glucose is subjected to glycolysis in the cell and is then oxidized in the respiratory cycle with the formation of ATP, this eventually leading to a drastic increase in calcium ion concentration in the cell and to release of pre-synthesized insulin [10].

However, glycolysis in β-cells differs from glycolysis in other cells by the stage of 2-phospho-D-glyceric acid conversion into phosphoenol pyruvate catalyzed by NSE. Glycolysis processes in a β-cell are disordered (and hence, insulin production is reduced) without NSE or if its concentration is low, which can eventually lead to cell death. This hypothesis is confirmed by the data on a significant increase in the level of autoantibodies to NSE in DM1 patients [7].

On the other hand, the results of immunohistochemical analysis of the pancreas in a child with the clinical diagnosis of DM1 indicate that NSE can be expressed not only in insulin-secreting cells of Langerhans islets and hence, that other mechanisms of DM1 development can exist.

The study was supported by the specialized foundation for monitoring the task-force capital for research support in biology and medicine (Basic).

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