PHYSIOLOGY

Adaptation to Hypoxia Stimulates the Pancreatic Islet Apparatus in Intact and Diabetic Rats

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Using immunocytochemical and immunoradiometric assays, it is found that intermittent exposure to hypoxia stimulates insulin synthesis and secretion by pancreatic beta cells and activates *de novo* formation of these cells in the acinar tissue of both intact rats and rats with streptozotocin-induced diabetes mellitus, as well as inhibiting the destruction of Langerhans islets in the latter animals.

Key Words: *hypoxia; diabetes mellitus; beta cells*

Adaptation to hypoxia has been shown to produce a wide range of protective effects on human and animal organs and tissues [6]. Our previous studies demonstrated that adaptation to hypoxia stimulates the function of beta cells of the Langerhans islets [4], while other workers have reported beneficial effects from a sojourn at high altitudes on the course of diabetes mellitus [7,8]. However, the mechanism of these effects remains undisclosed.

The purpose of the present study was to examine the influence of adaptation to hypoxia on the pancreatic islet apparatus in animals with diabetes mellitus.

MATERIALS AND METHODS

A total of 80 Wistar rats of both sexes (body weight 200-230 g) were used. They were divided into four groups, 10 males and 10 females in each. Group 1 rats were left intact and served as controls. Rats of group 2 were conditioned to progressive hypoxia in a pressure chamber 6 h per day for 21 days, the air pressure corresponding to an altitude of 1 km on day 1, 2 km on day 2, 3 km on day 3, 4 km on day 4, 5 km on day 5, and 6 km on days 6 through 21. In groups 3 and 4, diabetes mellitus was induced with streptozotocin [5], after which group 4 rats were exposed to hypoxia as described above, starting on day 15 of the disease. In all four groups, animals were sacrificed at the same time of day, after a 16-hour fast, to take blood samples and remove the pancreas. In the blood samples, insulin and C peptide concentrations were measured by radioimmunoassays (using RIO-INS-PG-^{125}I [Belarus] and RIA-MAT C-Peptid II [BYK-SANGTEC Diagnostica] kits) and glucose concentrations, by the orthotoluidine test. Glucose tolerance was tested by the standard procedure [1]. For quantitative immunocytochemical determination of insulin in serial sections from various parts of the pancreas, monoclonal antibodies (Amersham) and a computerized spectrophotometric system based on the LYuMAM-I2 cytofluorimeter (LOMO, Russia) were used, as previously described [5]. From 200 to 400 beta cells were examined in micropreparations for each group, using the VIDAS-2.5 computer system for digital image analysis (Zeiss-Kontron Elektronik) hooked up via a COHU 4722 video camera with an Axioskop

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microscope (Zeiss). Copies of the micropreparations were obtained on a CP 100E video printer (Mitsubishi). The results were subjected to statistical analysis.

RESULTS

The figures presented in Table 1 reflect the changes in pancreatic endocrine function in the different groups. In the hypoxia-conditioned rats, the greatest difference from the control animals was recorded for the blood concentration of C peptide (0.354±0.009 pmol/liter *vs.* 0.169±0.0033 pmol/liter in the controls; $p<0.05$); insulin was at the control level in the blood and significantly higher in the beta cells. Microscopic examination of acinar tissue samples revealed large numbers of solitary cells giving a positive reaction with the anti-insulin monoclonal antibodies (Fig. 1, cf. *b* and *c* against *a*); this indicates that the activity of beta cells was increased and that newly formed insulin-secreting cells were present, derived either from the epithelium of the excretory ducts [2] or from the so-called acinar islet cells, which combine structural and functional features of exocrine and endocrine cells [3].

In group 3, changes typical of diabetes mellitus were recorded (Table 1), such as hyperglycemia, impaired glucose tolerance, Langerhans islet destruction (Fig. 1, *d*), degenerative changes in beta cells, and lowered insulin levels in these cells and in the blood. Acinar tissue samples from these rats contained small numbers of solitary beta cells. We had observed *de novo* formation of beta cells in the acinar tissue of diabetic rats in a previous study [5]. Since beta cells have also been reported to form *de novo* in patients with

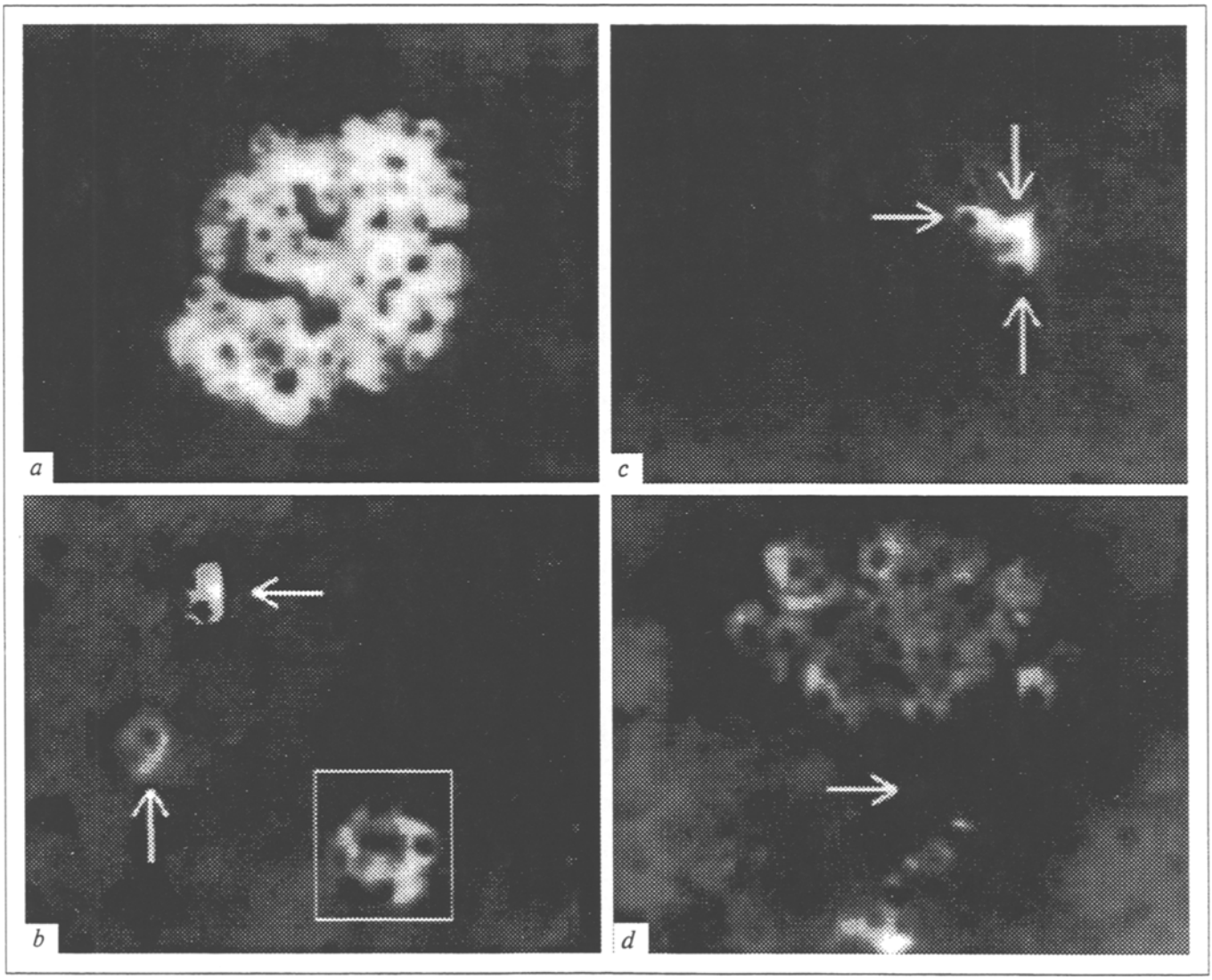

Fig. 1. Pancreatic beta cells from rats, as demonstrated by indirect immunofluorescence assays with monoclonal antibodies to insulin. ×400. *a*) beta cells of a Langerhans islet from an intact rat; *b*) solitary beta cells in acinar tissue (arrowed) near a Langerhans islet (boxed); *c*) individual beta cells in acinar tissue (arrowed); *d*) partial destruction of a Langerhans islet (arrowed); undamaged beta cells show reduced fluorescence intensity.

TABLE 1. Glucose and Insulin Levels in the Blood Serum and Endocrine Cells of Langerhans Islets ($M \pm m$)

Rats	Glucose, mmol/liter	Insulin	
		serum, μU/ml	beta cells, arbitrary μU
Controls	4.62±0.24 4.90±0.11	34.7±3.6 31.5±4.9	1560.7±21.5 1585.7±16.7
Hypoxia-conditioned (for 21 days)	3.52±0.29** 4.00±0.17***	32.6±6.2 30.4±4.7	1648.4±27.6* 1670.7±17.7***
Diabetic for 15 days	6.37±0.35*** 6.09±0.52*	15.4±4.2** 13.8±3.7**	727.9±16.3*** 879.2±16.6***
Diabetic for 35 days	8.78±0.59*** 7.75±0.88*	11.3±3.8*** 12.3±1.1**	618.7±12.9*** 758.5±12.9***
Diabetic and hypoxia-conditioned	7.59±0.46*** 6.06±0.56*	30.4±4.1 26.4±2.3	1298.4±11.2*** 1259.8±9.8***

Note. Figures above and below the lines are glucose and insulin levels in male and female rats, respectively. *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with controls.

insulin-dependent diabetes mellitus [9], this phenomenon may be regarded as one of the mechanisms compensating for the insulin deficiency in diabetes.

The adaptation to hypoxia reduced glycemia and altered glucose tolerance in the diabetic rats. Insulin concentrations in their sera reached levels close to those in the controls and their pancreases contained much smaller numbers of islets with signs of destruction, while their beta cells had considerably higher insulin concentrations and, moreover, newly formed insulin-secreting cells were present in the pancreatic acinar tissue. These findings indicate that the adaptation to hypoxia mitigated the disease.

Thus, adaptation to hypoxia exerts favorable effects on the pancreatic islet apparatus in diabetic rats, one of the most important determinants of these effects being, in our view, the *de novo* formation of beta cells. Such adaptation may therefore be considered a potentially promising method of treating this disease.

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