EFFECT OF FIBROBLAST AND NERVE GROWTH FACTORS ON PROLIFERATION AND FUNCTION OF CELLS OF ISOLATED ISLETS OF LANGERHANS

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Growth of mammalian cells in culture depends on the composition of the nutrient medium and the presence of serum, which acts as the source of various hormones and macromolecular peptide growth factors. Fibroblast growth factor (FGF) is widely represented in many tissues, including in the pituitary gland, brain, liver, spleen, and thymus [10], and is considered to stimulate proliferation of various cell lines of entodermal and mesodermal origin [14]. Nerve growth factor (NGF), discovered in the submandibular salivary gland of mice, in the prostate of guinea pigs, and in bovine semen, is an absolutely essential component for differentiation and growth of adrenergic and sensory neurons and ganglia [5].

The mitogenic role of these factors has been studied quite fully for several tumor cells [3, 4]. Their activating role on growth of untransformed cell cultures, including those of epithelial kidney cells, ovarian granulosa cells, and thyroid gland cells in culture, has also been established [2, 11, 13]. However, it must be assumed that the spectrum of target cells for these growth factors is much wider and includes as yet unidentified systems.

In the investigation described below the action of FGF and NGF on proliferation and insulin-secretory activity of cultures of islets of Langerhans was studied. The grounds for this investigation were entodermal embryogenesis of the insular apparatus and the presence of many nerve endings in the islets of Langerhans. The importance of this trend is also due to the search for preparations capable of increasing the mass of insulin-secreting cells in vitro with a view to their subsequent transplantation, in order to correct hyperglycemic states in diabetes mellitus.

EXPERIMENTAL METHOD

Experiments to study the possible growth-stimulating effects of FGF and NGF in cell cultures were carried out in the presence of a reduced concentration of serum in the incubation medium (3 and 1%, respectively). The experimental approaches to the study of proliferative activity in cell cultures were developed by the writers previously [1].

Pancreatic islets were isolated from the pancreas of newborn Wistar or Lewis 1W rats by fractionated treatment of the tissue with collagenase (type IV, 170 U/mg; Biochemical Corporation, USA) at the rate of 1 mg to 1 ml of Hanks' solution, and cultured for 3-5 days in medium

TABLE 1. Effect of FGF (0.1 ng/ml) on Insulin Secretion and Concentration in Islets of Langerhans (M ± m)

Test parameter, pmoles/islet	Control	FGF
Insulin in medium	5,93±0,58 (12)	6,46±0,83 (12)
Insulin in islets	7,06±0,35 (17)	7,53±0,76 (12)

Legend. Number of observations given in parentheses.

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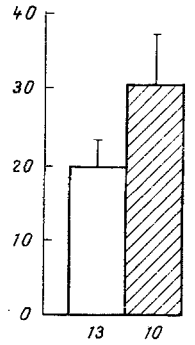

Fig. 1. Effect of FGF on incorporation of ^{3}H-thymidine into DNA of islets of Langerhans from rat pancreas in culture. Abscissa, number of observations; ordinate, incorporation of thymidine into DNA (in cpm/islet). Unshaded column — control, shaded — FGF (100 μg/ml). *$p < 0.01$ compared with control.

199 containing 10 mM glucose and inactivated human serum or embryonic calf serum, in an atmosphere of 97% air + 3% CO_2 at 37°C, with the addition of growth factors. FGF (from Serva, West Germany) was used in a dose of 0.1 ng/ml, and NRF (also from Serva) in doses of 0.1, 10, and 1000 ng/ml.

Methyl-^{3}H-thymidine (specific radioactivity 96 mBq/ml) was added to the medium 16-18 h before the end of culture, after which the islets were harvested in groups of five and washed in Hanks' solution and then twice in distilled water. The washed islets were transferred into plastic test tubes containing 0.25 ml water and fragmented ultrasound. Aliquots of the homogenates (0.1 ml) were withdrawn for estimation of the DNA concentration [9] and for measurements of radioactivity [15].

The intensity of insulin secretion was estimated by radioimmunoassay [7], determining the concentration of the hormone in the incubation medium. The intracellular insulin concentration was determined after homogenization of the islets in acid ethanol [8]. The final results were expressed in picomoles per islet during the period of incubation.

The action of NGF on insulin secretion also was investigated after completion of the main experiment, for which purpose the islets were washed for 30 min to remove culture medium at 37°C in Krebs-Ringer-bicarbonate buffer containing 5 mM glucose and 20 mM HEPES, after which they were transferred into buffer of the same composition for subsequent incubation for 2 h.

EXPERIMENTAL RESULTS

FGF (0.1 ng/ml) did not change the rate of insulin secretion in the islets during culture with preparation for 5 days (Table 1) but it stimulated incorporation of ^{3}H-thymidine into DNA (Fig. 1).

NGF did not change the intensity of proliferation or insulin secretion in the pancreatic islets during culture with the preparation for 3 days (Table 2).

The results (Table 3) show that islets of Langerhans cultured in the presence of NGF also preserved their ability to secrete insulin adequately and in the postcultural period in response to rising concentrations of glucose and isobutylmethylxanthine in the medium. NGF in a dose of 1000 ng/ml, incidentally, significantly ($p < 0.05$) increased insulin secretion only if the glucose concentration in the medium was 5 mM, i.e., under conditions when the basal rate of hormone secretion was minimal.

The investigations are evidence that of the two growth factors only FGF can stimulate DNA synthesis in cells of the islets of Langerhans, without changing the intensity of secretory processes. The results do not identify the type of cells whose proliferation activates FGF in the total cell population of the islets of Langerhans. The answer to this question can be obtained only by studying the cellular composition of the islets with the aid of immunohistochemical and autoradiographic methods of investigation.

FGF is not the only serum factor to active proliferation of pancreatic cells. Similar properties also are possessed by insulin-like growth factor [12] and epidermal growth factor [6].

The results confirm the hypothesis accepted nowadays by most investigators, that replication of pancreatic islet cells is controlled by complex interaction between several hormones and growth factors. Analysis of the action of each of these factors by the use of cul-

TABLE 2. Effect of NGF on Secretion and Intracellular Concentration of Insulin and Incorporation of ^{3}H-Thymidine into DNA of Islets of Langerhans in Culture (M ± m)

Parameter studied	Control	NGF, ng/ml		
		0,1	10	1000
Insulin in islets, pmoles/islet	4,04±0,38	3,50±0,32	3,44±0,36	3,55±0,39
Insulin in islets, pmoles/islet	3,11±0,21	3,57±0,44	3,27±0,44	3,11±0,18
Incorporation of ^{3}H-thymidine into DNA, cpm/islet	36,48±8,46	36,28±6,41	35,28±6,34	39,83±9,96

Legend. Here and in Table 3 mean values from 7-8 parallel determinations in group are given.

TABLE 3. Insulin Secretion by Pancreatic Islets (in pmoles/islet) under the Influence of NGF (M ± m)

Glucose concentration in medium, mM	Control	NGF, ng/ml		
		0,1	10	1000
1,5	0,24±0,004	0,015±0,003	0,032±0,008	0,035±0,011
5,0	0,009±0,002	0,019±0,004	0,025±0,012	0,025±0,006**
10,0	0,133±0,023**	0,142±0,023	0,138±0,024	0,150±0,034
20,0	0,351±0,054**	0,417±0,045	0,358±0,037	0,373±0,039
10,0 + 0,1 mM isobutylmethylxanthine	0,968±0,125*	0,866±0,105	0,895±0,050	0,329±0,101

Legend. *p < 0.01, **p < 0.05 compared with values obtained using 1.5 mM glucose.

tures of isolated islets of Langerhans can shed light on the fine mechanisms of control of growth and differentiation of the pancreatic islet cells in vivo.

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