Nerve and Fibroblast Growth Factors as Modulators of Pancreatic β Cell Plasticity and Insulin Secretion

Marcia Hiriart MD PhD, Roman Vidaltamayo PhD and M. Carmen Sánchez-Soto MSc

Department of Biophysics, Institute of Cellular Physiology, National Autonomous University of Mexico, Mexico City, Mexico

Key words: insulin, diabetes mellitus, nerve growth factor, fibroblast growth factor, sodium channel, reverse hemolytic plaque assay

Abstract

Trophic factors such as nerve and fibroblast growth factors are important modulators of β cell physiology. These two factors induce the extension of neurite-like processes in primary cultures of adult rat β cells. Moreover, both NGF and FGF enhance glucose-induced insulin secretion. Since β cells synthesize NGF and pancreatic islet cells produce FGFs, it is possible that autocrine/paracrine interactions may be major regulators of insulin secretion, and impairment of these interactions could lead to pathological states such as diabetes mellitus.

IMAJ 2001;3:114-116

Insulin secretion by pancreatic β cells is necessary to maintain glucose homeostasis: when serum glucose levels increase, insulin secretion is stimulated. Insulin effects on glucose permeability and glycogen metabolism return glycemia to basal level. When insulin secretion and/or insulin actions on tissues are impaired, the pathological condition of diabetes mellitus ensues.

The regulation of insulin production and secretion is not completely understood. While glucose is the major insulin secretagogue, several hormones and neurotransmitters also affect β cell physiology. Some of these modulators arise from cells located within the islets, which leads to paracrine or autocrine interactions that appear to be of paramount importance for insulin secretion. Growth factors, such as fibroblast growth factor and nerve growth factor, are representative members of these β cell modulators that are coming under the spotlight in pancreas physiology.

Effects of NGF on β cell physiology

It has been demonstrated that islet cells are derived from an endodermal precursor [1,2]. However, β cells possess several neuron-like traits, such as electrical activity necessary for insulin secretion [3]; the lack of expression of the silencer NRSF/REST, a negative transcription factor that prevents the expression of neural genes in non-neural tissue [4]; and the expression TrkA, the high affinity receptor for NGF [5].

NGF = nerve growth factor
FGF = fibroblast growth factor

TrkA is expressed in the β cell-derived cell line RINm5F [6], in primary cultures of adult β cells [7], and in fetal and adult pancreas *in vivo* [5]. This receptor confers β cell sensitivity to NGF, and several aspects of the physiology of the cells are modulated by this growth factor.

First, β cell morphology is modified in the presence of NGF; the growth factor induces the extension of neurite-like processes that express Tau protein in primary cultures of β cells [8] and in the RINm5F cell line [6]. The development of these processes is enhanced by a permeable analog of cAMP, dibutyryl cAMP [8]. The relevance of this morphological plasticity is not known. However, the extension of processes could be reminiscent of the mechanisms that lead to islet formation during embryogenesis, since dispersed islet cells in culture tend to form large clusters and several neurite-like processes grow towards neighboring cells [8].

NGF also modulates β cell physiology. After 5–7 days in culture, NGF treatment increased rat β cell sodium current density [9] without affecting voltage dependence of activation and inactivation of the current, or cell capacitance, which is related to the size of the cell membrane. This increase in sodium current could be important for modulation of insulin secretion, since this ionic current was shown in the rat to be involved in the generation of action potentials that lead to an increase in intracellular calcium concentration, which promotes insulin secretion. On the other hand, NGF treatment for 5 days preserved the capability of β cells to secrete more insulin when exposed to 15.6 mM glucose than when exposed to 5.6 mM glucose. This property was lost in cells from the control group [8].

The maintenance of insulin secretion could be due to the effects on β cell ion currents or to the modulation of other different components of the exocytotic machinery in these cells. At least in the insulin-secreting cell line INS-1, NGF increases mitogen-activated protein kinase activity, which in turn is responsible for an increase in insulin secretion [10]. However, the possible targets of phosphorylation by this enzyme remain to be identified.

All these data reveal NGF to be an important modulator of β cell physiology, but in order to determine the relevance of NGF modulation for *in vivo* function, it was thought necessary to identify a pancreatic source of NGF to which β cells could be exposed. Recently, we found that β cells are positive for NGF immunostaining [Figure 1] and express the mRNA for this

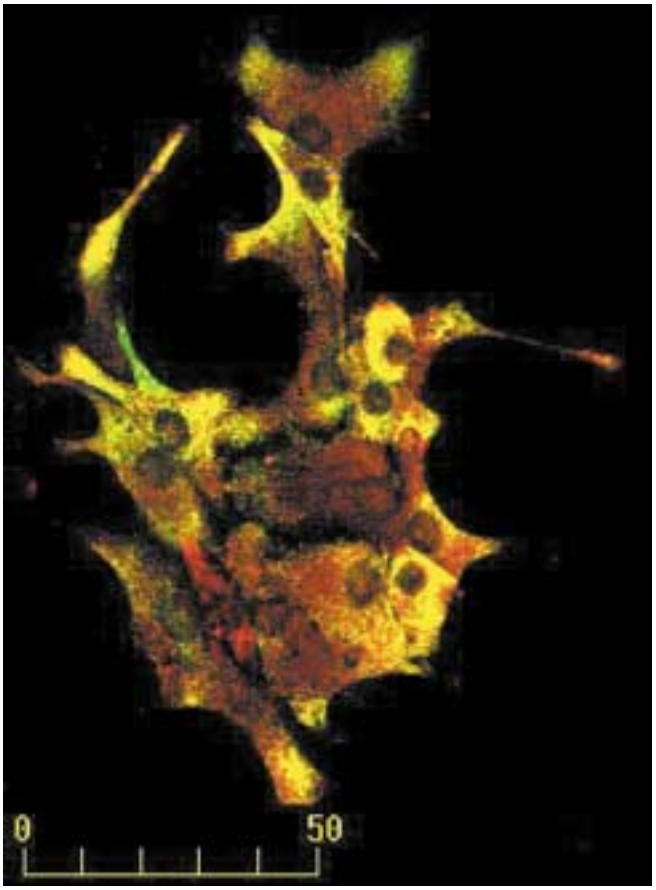

Figure 1. Pancreatic β cells express NGF
Confocal micrograph of β cells cultured for 11 days under treatment with NGF and dbcAMP and stained for insulin (green) and NGF (red). All insulin-producing cells are also labeled for NGF (yellow). In primary cultures of islet cells there is a small proportion (3–5%) of NGF-only labelled cells [7].

growth factor. Moreover, β cells also secrete NGF in response to glucose in a manner similar to that regulating insulin secretion [7].

The fact that β cells secrete NGF and are sensitive to it may imply that an autocrine/paracrine regulation loop exists in the islet. Moreover, it is possible that pancreatic NGF may be exported to the bloodstream where it functions as an endocrine messenger. This could correlate with the observation of decreased NGF serum levels observed in diabetic patients [11] and in animal models of diabetes [12].

Autocrine regulation may emerge as one of the major mechanisms leading to the correct function of β cells, since not only NGF but also insulin could induce and enhance insulin secretion [13]. These autocrine modulators could maintain β cells in a permissive state where conditions for secretion are optimal. In the case of NGF, this permissive state could be related not only to ion channel activity but also to the capability to metabolize glucose.

Effects of FGF on β cell physiology

The fibroblast growth factor family is a group of at least 18 homologous heparin-binding polypeptides that are potent regulators of cell proliferation, differentiation and function, depending upon the target cell. FGF signaling is mediated by high affinity tyrosine kinase receptors (FGFRs) and low affinity heparan-sulfate proteoglycan receptors that enhance ligand presentation to the FGFRs. Alterations in FGFs and their receptors have also been linked to several pathological conditions such as chronic pancreatitis and pancreatic cancers [14,15].

Acidic FGF (FGF-1) and basic FGF (FGF-2) are the prototypic FGF members and have been implicated in pancreatic islet embryogenesis [16]. Various types of FGFRs are expressed during pancreatic development, and it has been shown that FGF-2 induces the proliferation of pancreatic epithelial cells during embryonic life [17]. Moreover, in developing rat islets, immunoreactivity for FGF-1 was found in alpha cells, and immunoreactivity for FGF-2 was found throughout the islets, increasing with age. Both FGF-1 and FGF-2 were also located in ductal epithelium on days 10–14 of intrauterine development, coinciding with increased cell replication [18].

We previously found that a mixture of FGF induces morphological changes similar to those described for NGF. After 2 weeks in culture the percentage of adult rat β cells bearing neurite-like processes was 4.3-fold higher in cells treated with FGF compared to control cells. This effect was also potentiated by dibutyryl cAMP [8].

In this study, we analyzed the effects of FGF-1 and FGF-2 on insulin secretion and morphology of adult rat β cells. For this purpose islet cells were cultured as described previously [19]. Insulin secretion of single cells was measured with the reverse hemolytic plaque assay, as described previously [19].

Table 1 shows that both FGF-1 and 2 increase the percentage of β cells bearing neurite-like processes by nearly fourfold, and demonstrates the length of the processes. Figure 2 shows the effects on insulin secretion of challenging β cells, cultured for 5 days with FGF-1 and 2, for 1 hour with different glucose concentrations. The insulin secretion index was used to

Table 1. Morphological changes in cells induced by FGFs 1 and 2

Treatment	% of neurite-like bearing β cells	Neurite-like process length (m)
Control	10±6	37±6
FGF-1 (30 ng/ml)	42±10*	61±8*
FGF-2 (10 ng/ml)	42±11*	73±10*

After 12 days in culture in RPMI 1640 with FGF-1 (30 ng/ml) or FGF-2 (10 ng/ml), 10% fetal calf serum, antibiotics and 0.1% IBMX, β cells were identified with immunocytochemistry against insulin, as previously described [8]. A neurite-bearing cell is defined as a cell with one or more processes, at least twice the length of the perinuclear diameter.

* $P < 0.05$ significance level with respect to control cells (n = 4 different experiments by duplicate).

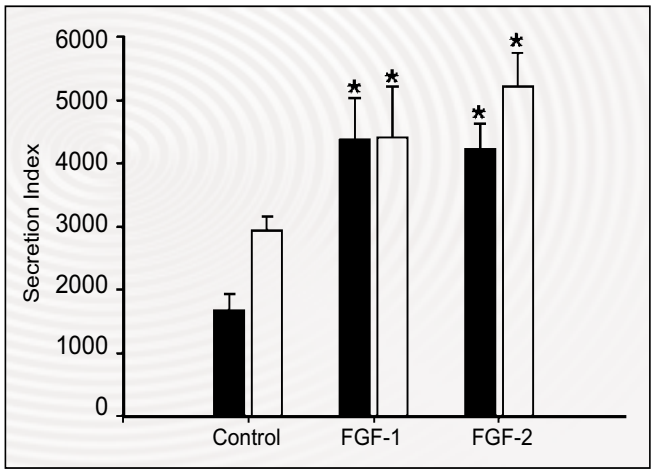

Figure 2. FGFs effects on insulin secretion
Islet cells were cultured for 5 days in RPMI 1640 with FGF 1 (30 ng/ml) or 2 (10 ng/ml), 10% of fetal calf serum, antibiotics and 0.1% IBMX. The cells were detached from culture dishes and mixed with an equal volume of protein A-coated sheep red blood cells, introduced to Cunningham chambers previously treated with poly-L-lysine to promote cell attachment, and incubated for one hour. The chambers were then rinsed and filled with Hanks balanced solution, which contained 5.6 (solid bars) or 15.6 mM glucose (open bars), and incubated for one hour in the presence of insulin antiserum (1:30 in HBSS). The monolayer was further incubated for 30 min with guinea-pig complement. Insulin released during the incubation time with the insulin antiserum was shown by the presence of hemolytic plaques around the secretory cells, which result from the complement-mediated lysis of red blood cells bearing insulin/anti-insulin complexes bound to protein A. We measured the size of plaques by projecting the image of the cell onto a monitor attached to a video camera and a Nikon Axiophot inverted microscope, with the aid of the JAVA video analysis software (Jandel Scientific, Version 1.40, Corte Madera, CA, USA). The plaque size was expressed as area, and the number of cells that formed plaques was counted. These results were expressed as percentage of insulin-secreting cells; at least 100 cells were counted per experimental condition. All experiments were carried out in duplicate ($n = 8$). $*P < 0.05$ vs. control; ANOVA followed by Fisher's LSD test.

measure the overall secretory activity of β cells under a given experimental condition, and was calculated by multiplying the average plaque area by the percentage of plaque-forming cells [20]. Both FGF-1 and FGF-2 increased insulin secretion in 5.6 and 15.6 mM glucose, compared to control cells. However, no difference between insulin secretion in both glucose concentrations was observed in cells treated with the FGFs.

It is possible that growth factor actions maintain β cells in optimal conditions to secrete insulin. For instance, the perturbation of the autocrine NGF loop may lead to impairment of β cell function and the onset of diabetes.

References

1. Gittes GK, Galante PE, Hanahan D, Rutter WJ, Debas HT. Lineage-specific morphogenesis in the developing pancreas: role of mesenchymal factors. *Development* 1996;122:439–47.
2. Fontaine J, le Douarin NM. Analysis of endoderm formation in the avian blastoderm by the use of quail-chick chimeras. The problem of the neuroectodermal origin of the cells of the APUD series. *J Embryol Exp Morphol* 1977;41:209–22.
3. Wollheim CB, Sharp WG. Regulation of insulin secretion by calcium [Review]. *Physiol Rev* 1981;61:914–73.
4. Atouf F, Czernichow P, Scharfmann R. Expression of neuronal traits in pancreatic beta cells. Implication of neuron-restrictive silencing factor/repressor element silencing transcription factor, a neuron-restrictive silencer. *J Biol Chem* 1997;272:1929–34.
5. Kanaka-Gantenbein C, Tazi A, Czernichow P, Scharfmann R. In vivo presence of the high affinity nerve growth factor receptor Trk-A in the rat pancreas: differential localization during pancreatic development. *Endocrinology* 1995;136:761–9.
6. Polak M, Scharfmann R, Seilheimer B, Eisenbarth G, Dressler D, Verma I-M, Potter H. Nerve growth factor induces neuron-like differentiation of an insulin-secreting pancreatic beta cell line. *Proc Natl Acad Sci USA* 1993;90:5781–5.
7. Rosenbaum T, Vidaltamayo R, Sanchez-Soto MC, Zentella A, Hiriart M. Pancreatic β cells synthesize and secrete nerve growth factor. *Proc Natl Acad Sci USA* 1998;95:7784–8.
8. Vidaltamayo R, Sɑnchez-Soto MC, Rosenbaum T, Martínez-Merlos MT, Hiriart M. Neuron-like phenotypic changes in pancreatic β-cells induced by NGF, FGF and dbAMPc. *Endocrine* 1996;4:19–26.
9. Rosenbaum T, Vidaltamayo R, Sɑnchez-Herrera D, Hiriart M. Nerve growth factor increases sodium current in pancreatic β cells. *J Memb Biol* 1996;153:53–8.
10. Frödin M, Sekine N, Roche E, Filloux C, Prentki M, Wollheim CB, Van Obberghen E. Glucose, other secretagogues, and nerve growth factor stimulate mitogen-activated protein kinase in the insulin-secreting β-cell line, INS-1. *J Biol Chem* 1995;270:7882–9.
11. Faradji V, Sotelo J. Low serum levels of nerve growth factor in diabetic neuropathy. *Acta Neurol Scand* 1990;81:402–6.
12. Fernyhough P, Diemel LT, Brewster WJ, Tomlinson DR. Altered neurotrophin mRNA levels in peripheral nerve and skeletal muscle of experimentally diabetic rats. *J Neurochem* 1995;64:1231–7.
13. Aspinwall CA, Lakey JR, Kennedy RT. Insulin-stimulated insulin secretion in single pancreatic beta cells *J Biol Chem* 1999;274:6360–5.
14. Kornmann M, Beger HG, Korc M. Role of fibroblast growth factors and their receptors in pancreatic cancer and chronic pancreatitis. *Pancreas* 1998;17:169–75.
15. Galzie Z, Kinsella AR, Smith JA. Fibroblast growth factors and their receptors [Review]. *Biochem Cell Biol* 1997;75:669–85.
16. Otonkoski T, Beattie GM, Rubin JS, Lopez AD, Baird A, Hayek A. Hepatocyte growth factor/scatter factor has insulinotropic activity in human fetal pancreatic cells. *Diabetes* 1994;43:947–53.
17. Le Bras S, Miralles F, Basmaciogullari A, Czernichow P, Scharfmann R. Fibroblast growth factor 2 promotes pancreatic epithelial cell proliferation via functional fibroblast growth factor receptors during embryonic life. *Diabetes* 1998, 47:1236–42.
18. Arany E, Hill DJ. Ontogeny of fibroblast growth factors in the early development of the rat endocrine pancreas. *Pediatr Res* 2000;48:389–403.
19. Sanchez-Soto MC, Larrieta ME, Vidaltamayo R, Hiriart M. Heterogeneity in glutamic acid decarboxylase expression among single rat pancreatic beta cells. *Diabetologia* 1999;42:1086–92.
20. Hiriart M, Ramirez-Medeles MC. Functional subpopulations of individual pancreatic B-cells in culture. *Endocrinology* 1991;128:3193–8.

Correspondence: Dr. M. Hiriart, Depto. Biofísica, Instituto de Fisiología Celular, UNAM, AP 70-253, Mexico D.F., 04510 Mexico. Phone: (52-5) 622-5665, Fax: (52-5) 622-5607, email: mhiriart@ifisiol.unam.mx