J. Membrane Biol. 186, 177–184 (2002)
DOI: 10.1007/s00232-001-0143-9

The Journal of
Membrane
Biology

Nerve Growth Factor Increases L-Type Calcium Current in Pancreatic β Cells in Culture

T. Rosenbaum, D.T. Castañares, H.E. López-Valdés, M. Hiriart
Dept. Biophysics, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, UNAM, Circuito Exterior, Ciudad Universitaria, A.P. 70-243 Coyoacán, Mexico City, D.F. 04510, Mexico

Received: 25 August 2001/Revised: 13 December 2001

Abstract. We analyzed the effect of culturing adult rat β cells with NGF2.5 S for 5 to 7 days on macroscopic barium current (I_{Ba}), and determined the role of Na and Ca channels on neurite-like process extension induced by NGF and dbcAMP, and by KCl depolarization. After five days in culture with 2.5S NGF, β cells exhibit a 102% increase in I_{Ba} density. This effect is on L-type calcium channels because most of the current is blocked by nifedipine. The application of NGF for 5 minutes to the cells deprived of the trophic factor for 24 hr further increases I_{Ba} current by 91%. These results suggest that the trophic factor regulates I_{Ba} by two different mechanisms, a) an increase in channel density and b) a rapid modulation of the channels already present in the membrane. Finally, we found that ion-channel activity modifies the growth of neurite-like processes. After 2 weeks in culture with high KCl, almost 14% of β cells extend neurite-like processes and the most impressive effect is observed in the presence of KCl, NGF, and dbcAMP simultaneously, where nearly 60% of the cells extend neurite-like processes. Tetrodotoxin and nifedipine reduce the morphological changes induced by these agents.

Key words: Growth factor — Calcium channels — Neurite-like — Potassium-depolarization — TTX — Nifedipine

Introduction

Nerve growth factor (NGF) regulates the survival and differentiation of specific populations of neurons during embryonic development and provides trophic support for particular types of neurons in the adult (Levi-Montalcini, 1987; Bradshaw et al., 1993). NGF also has trophic actions in cells of the endocrine and immune systems (Otten, Ehrhard & Peck, 1989; Patterson & Childs, 1994).

Pancreatic β cells show phenotypic changes in response to NGF. We have previously shown that after two weeks in culture, barely 3% of β cells extend neurite-like processes spontaneously but when treated with NGF, almost 30% of them extend this type of processes. Moreover, in the presence of the permeable analogue of cAMP, dibutyryl cAMP (dbcAMP), and NGF together, the percentage of β cells bearing neurite-like process is increased to 50% (Vidaltamayo et al., 1996). In addition, ultrastructural morphometric analysis of these cells has demonstrated a substantial increase in insulin and GABA content in granules and endoplasmic reticulum in response to NGF and dbcAMP (González del Pliego et al., 2001). Neurite-like outgrowth in response to NGF has also been observed in the insulinoma cell-line RINm5F (Polak et al., 1993).

Morphological changes in β cells are accompanied by physiological changes; among them we find that NGF increases sodium current by 48% in adult β cells cultured for 5 to 7 days in the presence of the factor (Rosenbaum et al., 1996). Moreover, a short exposure of β cells to NGF increases insulin secretion and barium current (Rosenbaum, Sánchez-Soto & Hiriart, 2001).

We have also demonstrated that adult rat pancreatic β cells synthesize and secrete NGF in response to increasing extracellular glucose concentrations, and to potassium-induced depolarization (Rosenbaum et al., 1998). Pancreatic β cells also express the high-affinity NGF receptor Trk A (Scharfmann et al., 1993; Kanaka-Gantenbein et al., 1995). The fact that β cells secrete and respond to NGF implicates an autocrine and/or paracrine role for NGF in the pancreas.

In the present study, we analyze the effect on I_{Ba} of culturing β cells with the biologically active

Correspondence to: M. Hiriart

β-subunit of NGF (NGF2.5S) for 5 to 7 days. We assess the role of ionic channels on β cell morphology in order to provide an insight into how the activity of these channels affects the induction of neurite-like processes by NGF. Isolated islet cells were cultured with a depolarizing KCl concentration and it was determined whether this could induce cytoplasmic neurite-like process growth in β cells and if sodium and calcium channel blockers could inhibit the development of the processes in cells cultured with KCl, NGF, and dbcAMP.

Materials and Methods

Reagents were obtained from the following sources: collagenase type IV from Worthington (Freehold, NJ); 2.5S NGF, bovine serum albumin (BSA), HEPES, poly-L-lysine, RPMI-1640 salts, Spinner-Eagle's salts, 3-3′-diaminobenzidine tetrahydrochloride, tissue culture dishes (Corning, Cat. No 25000-35), dibutyryl-cAMP (dbcAMP) and all salts for electrophysiological recordings from Sigma (St. Louis, MO); fetal bovine serum (FBS), Hank's balanced salt solution (HBSS) and penicillin-streptomycin solutions from GIBCO (Grand Island, NY); nifedipine and tetrodotoxin (TTX) from Calbiochem (La Jolla, CA).

Pancreatic β-cell culture

Animal care was performed according to the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications No. 85-23, revised 1985). Young adult male Wistar rats (200–250 g) were obtained from the local animal facility, maintained in a 14-hr light (06:00–20 hr), 10-hr dark cycle, and allowed free access to standard laboratory rat chow and tap water. Pancreatic β cells were obtained following the technique previously described (Sánchez-Soto et al., 1999). Briefly, clean pancreatic islets were isolated and separated from the acinar tissue by collagenase digestion and centrifugation in a Ficoll gradient. Dissociation of the cells was achieved by incubation in a shaker bath, for 10 min, at 37°C in calcium-free Spinner solution, with 15.6 mM glucose, 0.5% BSA, and 0.01% trypsin, followed by mechanical disruption. Single cells used in electrophysiological experiments were cultured for 5 to 7 days in RPMI-1640 medium, supplemented with 5% fetal calf serum, 200 U/ml penicillin G, 200 μg/ml streptomycin and 0.5 μg/ml amphotericin B, in control conditions or with NGF 2.5S (50 ng/ml). In other experiments, single islet cells were cultured under the conditions above described for 12 days with NGF 2.5S (50 ng/ml) and dbcAMP (5 mM) and/or KCl (40 mM), in control conditions, or with TTX (100 nM), and/or nifedipine (5 μM). These compounds were present through out the time of culture.

Electrophysiological Recordings and Analysis

The whole-cell patch-clamp configuration (Hamill et al., 1981), was used to record I_{Ba} at a temperature of 20–22°C using an Axopatch 200 amplifier (Axon Instruments, Foster City, CA). Patch electrodes were pulled from capillary tubes Kimax-51 (Kimble Products) and had a resistance of 1.5 to 3 MΩ. Electrode tips were coated with Sylgard (Dow Corning). In each cell, the capacity transient of the pipette was cancelled before accessing the cell and total cell capacitance was determined by digital integration of capacitive transients with pulses of 10 mV, from a holding potential of −80 mV.

Table 1. Boltzmann parameters

Parameter	Control	NGF-treated cells
$V_{a1/2}$	−18.5 ± 1.9	−16 ± 2
k_a	5.0 ± 2	8.6 ± 1.1

$V_{a1/2}$ depicts half voltage of activation and k_a depicts the activation constant. Data are the mean ± SEM of cells.

Capacity transients were cancelled and series resistance was compensated using the internal voltage-clamp circuitry. Remaining linear capacity transients as well as leakage currents were subtracted by a P/2 procedure (Armstrong & Bezanilla, 1974). The pulse protocol used for the analysis of the peak I_{Ba} consisted of applying depolarizing test pulses of 15 msec, from −40 to +50 in 10-mV steps, from a holding potential of −80 mV. Nifedipine (5 μM) was used to block the L-type calcium currents. This concentration of the blocker was used because it produces fast maximal block of L-type channels without affecting other calcium channels (Hillyard et al., 1992; Kasai & Neher, 1992). The blocker was present all the time during the electrophysiological recordings. Boltzmann parameters where obtained using the following equation:

$$g/g_{max} = \{1 + exp[V - V_{a1/2}/ka]\} - 1$$

where g is the peak Ba conductance, g_{max} is the peak maximum Ba conductance, $V_{a1/2}$ is the mid-point of activation and k_a is the slope.

Recording Solutions

The ionic compositions of the solutions used in the experiments are shown in Table 1. Outward currents were eliminated by using Cs and TEA in the pipette solution. TTX 100 nM was always present in the external solution to ensure block of sodium currents. Ba was used as a charge carrier. External Solution (mM): 130 NaCl, 10 $BaCl_2$, 5 KCl, 2 $MgCl_2$, HEPES, 10 glucose. Internal Solution (mM): 120 CsAsp, 10 CsCl, 5 CsF, 2 ATP-Mg, 10 HEPES, 2.5 BAPTA.

Immunocytochemistry

After 12 days in culture, β cells were identified with immunocytochemistry (ICC) against insulin. Briefly, cells were fixed overnight, at 4°C, in a solution of 4% paraformaldehyde prepared in a 0.1 M sodium phosphate buffer (pH 7.4), then washed with 0.1 M Tris buffer solution (pH 7.4), permeabilized for 30 min with 0.3% of Triton X-100 in Tris buffer, and incubated for 48 hr at 4°C with the primary antiserum. The dilution of the insulin antiserum was 1:4000. The ICC to detect insulin was performed with a secondary peroxidase-antiperoxidase antibody and 3,3′-diaminobenzidine as final substrate, to form a brown reaction product. Morphological changes were evaluated by measuring the percentage of neurite-bearing cells. A neurite-bearing cell is defined as a cell with one or more processes with a length of at least 10 μm. When a cell exhibited more than one process, the length of the longest process was considered in the analysis. The length of the neurite-like processes was measured by projecting the image of the cell on 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).

During culture, cells tended to form clusters; the neurite-like processes were measured only in isolated β cells, or in the cells from the periphery of a cluster that could be clearly distinguished from the rest. At least 150 cells were measured per culture dish. At any

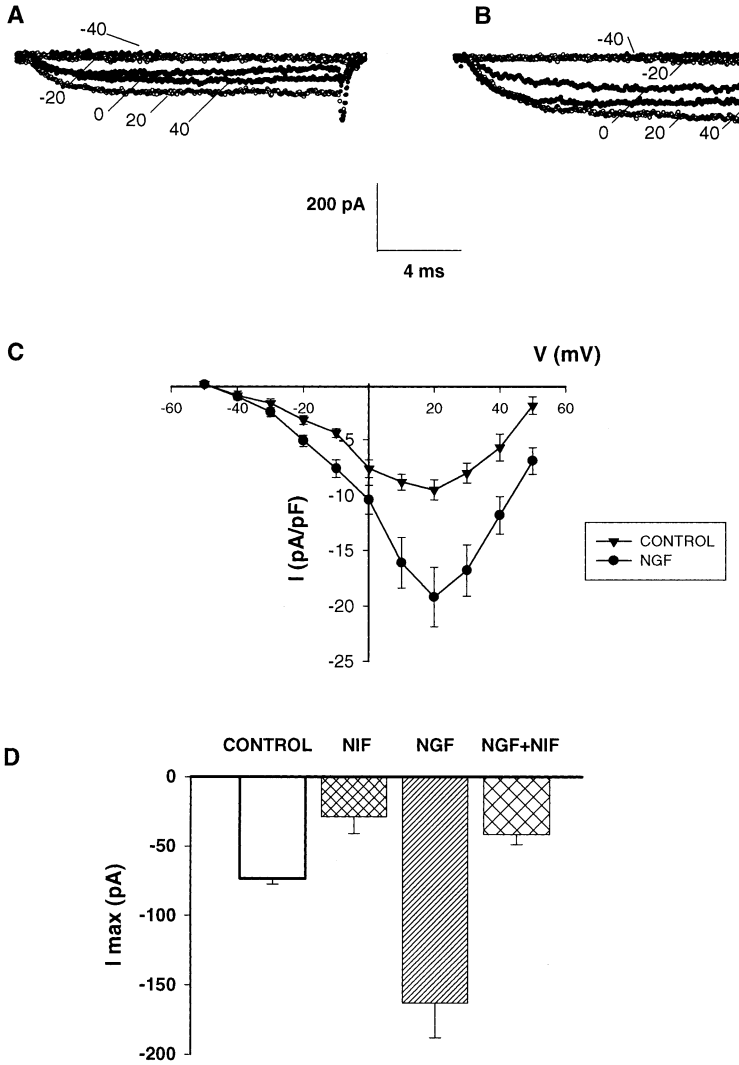

Fig. 1. Comparison of Ba currents and Ba current-voltage relationships between control and β cells cultured with NGF (50 ng/ml) for 5–7 days. (*A*) Control β cells, (*B*) NGF-treated β cells. Representative Ca-current families, which were recorded during 15-msec steps to different voltages, from a holding potential (HP) of −80 mV. (*C*) The mean Ba current density was plotted as a function of voltage in cells under control conditions (*triangles*, $n = 12$) and in NGF-treated cells (*circles*, $n = 15$). Error bars represent SEM. *Denotes significance level with respect to control cells of ($P < 0.01$. (*D*) Effect of nifedipine (5 μM) on averaged whole-cell maximum Ba current at +20 mV; control, $n = 9$; nifedipine (NIF), $n = 11$; NGF, $n = 12$; NGF + nifedipine (NGF+NIF), $n = 8$.

given condition, a minimum of four separate cultures was evaluated.

Statistical Analysis

All data are reported as the mean ± SEM; n denotes the number of cells studied or experiments performed. The statistical significance was obtained with the one-way ANOVA test, followed by Fisher's multiple range test using the Number Cruncher Statistical System (NCSS, 4.2; Dr. J.L. Hintze, Kaysville, UT, 1983).

Results

NGF Increases I_{Ba} in β Cells

Cells were cultured for five days in control conditions and with NGF (50 ng/ml). Figure 1 (*A* and *B*) illustrates families of whole-cell I_{Ba} of control and NGF-treated cells and Fig. 1*C* shows the mean I_{Ba} densities plotted as a function of voltage. In both cases, maintained inward currents activate around −30 mV, reach a maximum around +20 mV, and do not inactivate during the 15 msec depolarizing pulse. Maximum Ba current density in cells exposed to NGF was, in average, 102% larger than in control cells.

To determine whether NGF induced changes in the activation kinetics of the current we calculated the voltage dependence of peak barium conductance and used it to construct a Boltzmann relationship (as described in Methods), for control and NGF-treated β cells. NGF exposure does not change the mid-point of activation or the slope of the activation curve of I_{Ba} (Table 1).

To assess if NGF was acting on L-type calcium channels, we used the Ca-channel blocker nifedipine (5 μM). Figure 1*D* shows that nifedipine blocked around 60% of maximum I_{Ba} in control cells and 75% in NGF-treated cells, without modifying I/V parameters (*not shown*). In other cellular systems, NGF induces de novo synthesis of calcium channels (Levine et al., 1995; Cavalie et al., 1994). Nevertheless, it is also possible that NGF could be inducing its

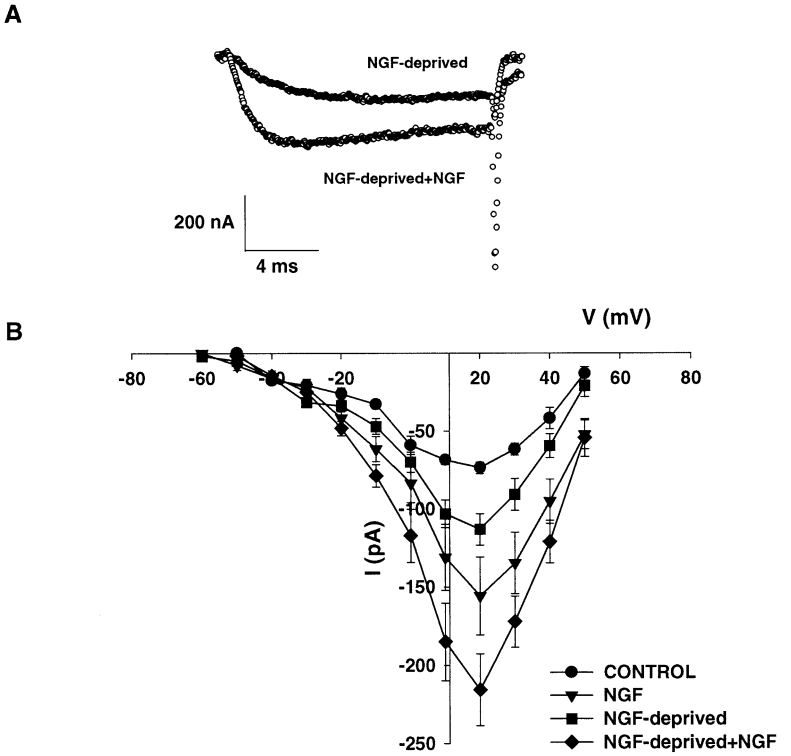

Fig. 2. (A) Example of I_{Ba} elicited by a 15-msec pulse to +20 mV, from a HP of −80 mV, recorded from an NGF-deprived cell and from an NGF-deprived cell exposed for 5 min to NGF. (*B*) Mean I_{Ba} was plotted as a function of voltage, in cells under control conditions (*circles*, $n = 12$), in NGF-treated cells (*triangles*, $n = 15$), in cells deprived of NGF for 24 hr (*squares*, $n = 18$) and in NGF-deprived cells exposed to NGF for 5 min ($n = 13$).

Table 2. Effects of NGF on β-cell I_{Ba}

Treatment	Capacitance (pF)	Maximum I_{Ba} (pA)	I_{Ba} Density (pA/pF)	*n*
Control	8.2 ± 0.6	−73.5 ± 3.7	9.5 ± 0.9	12
NGF	8.1 ± 0.6	−155.7 ± 25*	19.2 ± 2.7*	15
NGF-deprived	8.0 ± 0.7	−113 ± 10*	15.6 ± 1.8*	18
NGF-deprived + NGF	7.7 ± 0.5	−216 ± 23*$^{+}$	28.0 ± 2.1*$^{+}$	13

*$P < 0.01$ with respect to control cells, $^{+}P < 0.01$ with respect to NGF and NGF-deprived cells.

effects on calcium currents by promoting the phosphorylation of these channels, as has been described in other systems (Liu & Sperelakis, 1997). To investigate this possibility, we cultured β cells for 4 days in the presence of NGF and then deprived them of the growth factor for the next 24 hr to promote the separation of the ligand from the receptor and to eliminate NGF-mediated phosphorylation of the channels.

Figure 2*B* show the Ba I/V relationship of β cells cultured in the different culture conditions. A decrease of nearly 20% in I_{Ba} density of NGF-deprived cells compared to cells cultured continuously with NGF can be observed. This decrease could be a reflection of calcium-channel dephosphorylation. However, this is not statistically significant compared to cells cultured continuously with NGF (Table 2). These data suggest that the mechanism used by NGF to increase I_{Ba} is by promoting de novo synthesis of calcium channels.

We have recently described that a 5-min exposure to NGF of β cells cultured for one day without exogenous NGF increases I_{Ba} current density by 32% (Rosenbaum, Sánchez-Soto & Hiriart, 2001). However, this effect is achieved through a different mechanism, which seems to involve an increase in L-type calcium channels available for voltage activation. To further confirm the possibility that NGF induces the synthesis de novo of these channels, we exposed NGF-deprived cells to a 5-min NGF-pulse. The rationale was that, if NGF is inducing an increase of the number of channels in the membrane of β cells, then the exposure of these to a short pulse of the factor could further increase I_{Ba}.

Figure 2*A* shows the maximum I_{Ba}, elicited by a pulse to +20 mV in an NGF-deprived cell and in an NGF-deprived cell exposed for 5 min to NGF. In Fig. 2*B* it is clear that a 5-min exposure to NGF in NGF-deprived cells further increases mean I_{Ba} current by 91% compared to NGF-deprived cells. Table 2

Table 3. Effects of NGF + dbcAMP, KCl, TTX and nifedipine on β-cell morphology

Treatment	% of neurite-like extension-bearing β cells	Ionic-channels blockers	% of neurite-like extension-bearing β cells
Control	0.6 ± 0.4	—	—
NGF + dbcAMP (NA)	26.5 ± 3.0*	+ TTX (T)	11.7 ± 1.6*
		+ Nifedipine (F)	12.3 ± 2.6*
		+ TF	6.7 ± 1.6*
KCl (K)	14.0 ± 2.6*	+ TTX (T)	6.9 ± 3.5**
		+ Nifedipine (F)	0*
		+ TF	0*

Data are the mean ± SEM of 4 different experiments. *Denotes significance level with respect to its own control, $P < 0.01$; **$P < 0.05$.

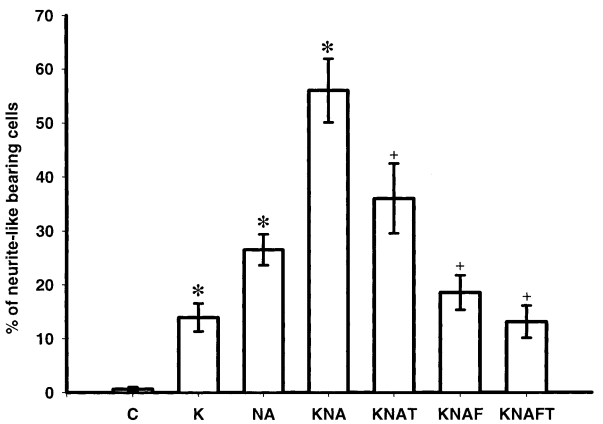

Fig. 3. Effect of KCl (K), NGF + dbcAMP (NA), nifedipine (F) and TTX (T) on the percentage of neurite-like bearing β cells. Bars represent mean ± SEM of 4 separate experiments. *Denotes significance level with respect to cells cultured in control conditions of $P < 0.01$; + Denotes significance level with respect to cells cultured with KCl + NGF + dbcAMP (KNA) of $P < 0.01$.

shows the mean current density at +20 mV of cells cultured in all conditions. These observations suggest that NGF is acting on calcium channels trough two different mechanisms: a long-term effect that could involve de novo synthesis of channels and an acute effect that modifies their activity (i.e., channel phosphorylation).

Sodium and Calcium Currents are Important for the Induction of Neurite-like Process Growth by KCl, NGF, and dbcAMP

We have previously described that NGF and/or dbcAMP induce morphological changes in β cells (Vidaltamayo, et al., 1996). To examine the role of sodium and calcium channels in the induction of neurite-like process growth by NGF, we analyzed two morphological parameters: the percentage of neurite-bearing cells and the length of the neurites.

After two weeks in culture, NGF and dbcAMP induce extension of neurite-like processes in 27% of β cells (Fig. 3 and Table 3). In this condition, TTX and nifedipine decrease cells bearing neurite-like processes by nearly 55%, compared to cells cultured with NGF and dbcAMP (Table 3). Figure 3 and Table 3 show that KCl (40 mM) in the culture medium increases the percentage of β cells bearing neurite-like processes to 14%, compared to control medium where less than 1% extend neurite-like processes. This effect is completely blocked by nifedipine, while TTX induces a 50% decrease (Table 3). In addition, KCl increases the percentage of cells bearing neurite-like processes induced by NGF and dbcAMP by twofold, with respect to cells cultured with NGF and dbcAMP alone (Fig. 3 and Table 4). It is noteworthy that the neurite-like processes are 55% longer than those of cells cultured only with NGF and dbcAMP, and 150% longer than in cells cultured only with KCl (Table 4). In this culture condition, TTX decreases the percentage of cells bearing neurite-like processes by 36%, nifedipine by 67% and the presence of both ionic channel blockers decreases this percentage by 77%, compared to the respective control cells (Fig. 3). Photographs of cells grown under various culture conditions are shown in Fig. 4.

Discussion

We observed that β cells cultured for 5–7 days with NGF show an increase of 102% I_{Ba} density compared to control cells. This increment seems to be due to an increase in the number of L-type Ca channels in the membrane of β cells (de novo synthesis), because it is blocked with nifedipine and membrane capacitances of NGF-treated cells and control cells are not significantly different.

This suggestion is also supported by the observation that cells deprived of the factor for 24 hr nearly retain the increase in I_{Ba} density observed in cells cultured continuously for 5–7 days with NGF. After 24 hr of NGF-deprivation, it is unlikely that the increase observed in I_{Ba} density is due to other phenomena such as channel phosphorylation. Moreover, the addition of a 5-min NGF-pulse to these cells further increases I_{Ba}, suggesting that there are more channels on the membrane surface, which can be

Table 4. Effect of NGF + dbcAMP and KCl on neurite-like processes of β cells

Treatment	Number of neurite-like processes/cell	Length of neurite-like processes (μm)
Control	0.9 ± 0.4	20.2 ± 12.3
NGF + dbcAMP (NA)	5.1 ± 0.8*	91.4 ± 9.4*
KCl (K)	3.3 ± 0.5*	54.6 ± 7.6*
NAK	5.5 ± 0.9*	141.6 ± 9.3*+

Data are the mean ± SE of 4 different experiments. *Denotes significance level with respect to its own control, $P < 0.01$; +Denotes significance level with respect to all the other treatments, $P < 0.01$.

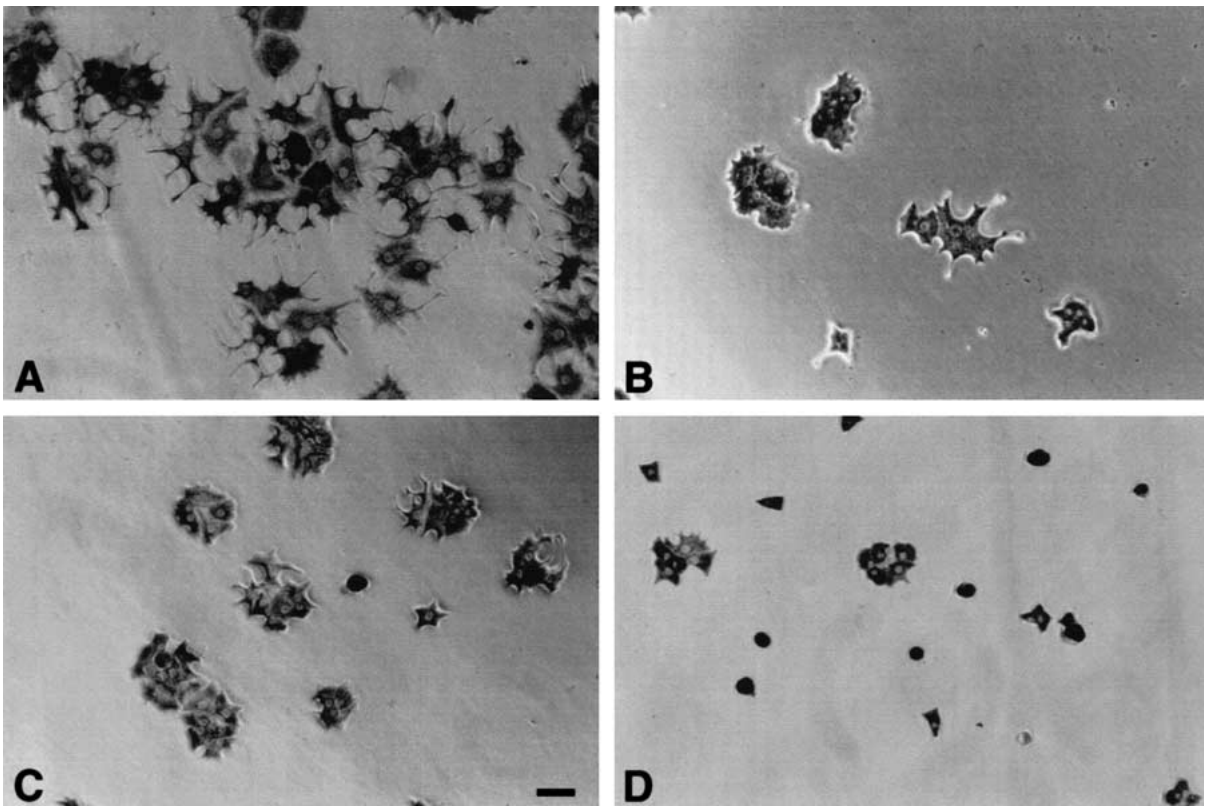

Fig. 4. Effect of KCl on NGF- and dbcAMP-induced neurite outgrowth. Cells were cultured for two weeks with the respective treatments, and then fixed and immunostained for insulin (*see* Methods). (*A*) Cells cultured with KCl (40 mM), NGF (50 ng/ml) and dbcAMP (5 mM). (*B*) Cells cultured with KCl, NGF, dbcAMP and 100 nM TTX. (*C*) Cells cultured with KCl, NGF, dbcAMP and nifedipine 5 μM. (*D*) Cells cultured with KCl, NGF, dbcAMP, TTX and nifedipine. Bar = 0.20 μm.

stimulated by a short exposure to NGF. We do acknowledge that changes in the I_{Ba} could also be explained by steady-state recruitment of already present channel-containing vesicles into the plasma membrane. However, we think that the possibility of recruitment of previously inactive channels to the membrane is unlikely since this process has not been observed widely, but only in skeletal muscle cells (Strong et al., 1987; and DeRiemer et al., 1985).

Whereas it is also possible that NGF could promote an increase in open probability of L-type calcium channels, there are no lines of evidence that point to changes in single-channel conductance. In skeletal muscle cells it has been demonstrated that phosphorylation of calcium channels by PKC (a downstream element of Trk A activation) promotes changes in open probability but does not modify the conductance of these channels (Rios & Pizarro, 1991). Although the data in this study do not address directly the involvement of PKC in the NGF-induced effects on calcium current density, the acute effects of this factor on these channels suggest that this is a possibility. Further experiments will have to be performed to assess the role of protein kinases on NGF-mediated effects.

Previous results show that the physiological changes described above are accompanied by changes on β-cell morphology (Vidaltamayo et al., 1996). In order to identify and study the role of the channels involved in neurite-like process extension, sodium- and calcium-channel blockers were used. In addition,

experiments to determine the effects of depolarization, using KCl (40 mM), on the development of these neurites, were carried out.

We observed that induction of neurite outgrowth by NGF and dbcAMP are significantly diminished by the addition of TTX and nifedipine. This result demonstrates that the activity of sodium and calcium channels plays an important role in β-cell morphology.

We also observed that KCl alone can induce neurite-like sprouting by pancreatic β cells. Depolarization-induced neurite outgrowth is also dependent upon the activity of ionic channels, because TTX decreased, and nifedipine completely inhibited extension of neurite-like processes. This suggests that calcium influx trough L-type calcium channels is essential in the development of neurite-like processes in β cells. A similar effect has been observed in PC12 cells, where KCl induces the growth of neurites (Hillborn, Rane & Pollock, 1997). It has been suggested that KCl-induced neurite outgrowth involves the metabolism of inositol phosphates and phosphatidic acid (Traynor, 1984).

Since pancreatic β cells secrete NGF in response to KCl depolarization (Rosenbaum, et al., 1998), it is also possible that endogenous NGF could be playing an autocrine role. It has been observed in PC12 cells that depolarization can synergize with subthreshold activation of NGF receptors to induce neurite growth through a calcium and a CaM kinase-dependent signal transduction pathway (Solem, McMahon & Messing, 1995). In addition, KCl is capable of increasing the percentage of neurite-bearing cells when added together with NGF and dbcAMP, compared to cells cultured only with NGF and dbcAMP. These observations further confirm the hypothesis that the activity of ion channels is essential for the promotion of neurite outgrowth by growth factors.

In summary, the data presented here support the idea that NGF induces an increase in the number of L-type calcium channels in the plasma membrane of pancreatic β cells, and that calcium and sodium channels play a pivotal role in the morphological changes induced by the growth factor.

This work was supported by grants from: Dirección General de Asuntos del Personal Académico, Universidad Nacional Autónoma de México (DGAPA, UNAM); Consejo Nacional de Ciencia y Tecnología (CONACyT), and Fundacíon Miguel Alemán, A.C., México. We thank Carmen Sánchez for expert technical assistance, Dr. Leon Islas for proofreading and discussion of the manuscript, Victor Navarro for actively participating in some experiments and Dr. Román Vidaltamayo for help in preparation of figures.

References

Armstrong, C.M., Bezanilla, F. 1974. Charge movement associated with the opening and closing of the activation gates of Na channels. *J. Gen. Physiol.* **63:**533–552

Bradshaw, R.A., Blundell, T.L., Lapatto, R., McDonald, N.Q., Murray-Rust, J. 1993. Nerve growth factor revisited. *Trends Biochem. Sci.* **18:**48–52

Cavalie, A., Berninger, E., Haas, C.A., García, D.E., Lindholm, D., Lux, H.D. 1994. Constitutive upregulation of calcium channel currents in rat pheochromocytoma cells: role of c-fos and c-jun. *J. Physiol.* **479:**11–27

DeRiemer, S.A., Strong, J.A., Albert, K.A., Greengard, P. Kaczmarek, L.K. 1985. Enhancement of calcium current in *Aplysia* neurones by phorbol ester and protein kinase C. *Nature* **313:**313–316

González del Pliego, M., Aguirre-Benítez, E., Sánchez-Soto, M.C., Larrieta M.E., Velázquez-Carranza, A., Vidaltamayo, R., Meza-Mora, M., Zepeda-Rodríguez, A., Hernández-Falcón J., Hiriart, M. 2001. Ultrastructural changes in pancreatic beta cells treated with NGF and dbcAMP. *Cell Tiss. Res.* **305:**365–378

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* **391:**85–100

Hilborn, M.D., Rane, S.G., Pollock, J.D. 1997. EGF in combination with depolarization or cAMP produces morphological but not physiological differentiation in PC12 cells. *J. Neurosci. Res.* **47:**16–26

Hillyard, D.R., Monje, V.D., Mintz, I.M., Bean, B.P., Nadasdi, L., Ramachandran, J., Miljanich, G., Azimi-Zoonooz, A., McIn tosh, J.M., Cruz, L.J., Imperial, J.S., Olivera, B.M. 1992. A new conus peptide ligand for mammalian presynaptic Ca^{2+} channels. *Neuron* **9:**69–77

Kanaka-Gantenbein, C., Dicou, E., Czernichow, P., Scharfmann, R. 1995. Presence of nerve growth factor and its receptors in an in vitro model of islet cell development: implication in normal islet morphogenesis. *Endocrinology* **136:**3154–3162

Kasai, H, Neher, E. 1992. Dihydropyridine-sensitive and ω-conotoxin-sensitive calcium channels in a mammalian neuroblastoma-glioma cell line. *J. Physiol.* **448:**161–168

Levi-Montalcini, R. 1987. The nerve growth factor thirty-five years later. *EMBO J.* **6:**1145–1154

Levine, E.S., Dreyfus, C.F., Black, I.B., Plummer, M.R. 1995. Differential effects of NGF and BDNF on voltage-gated calcium currents in embryonic basal forebrain neurons. *J. Neurosci.* **15:**3084–3091

Liu, H., Sperelakis, N. 1997. Tyrosine kinases modulate the activity of single L-type calcium channels in vascular smooth muscle cells from rat portal vein. *Can. J. Physiol. Pharmacol.* **75:**1063–1068

Otten, U., Ehrhard, P., Peck, R. 1989. Nerve growth factor induces growth and differentiation of human B lymphocytes. *Proc. Natl. Acad. Sci. USA* **86**:10059–10063

Patterson, J.C., Childs, G.V. 1994. Nerve growth factor in the anterior pituitary: regulation of secretion. *Endocrinology* **135:** 1697–1704

Polak, M., Scharfmann, R., Seilheimer, B., Eisenbarth, G., Dressier, D., Verma, I., Potter, H. 1993. Nerve growth factor induces neuron-like differentiation of an insulin-secreting pancreatic β-cell line. *Proc. Natl. Acad. Sci. USA* **90:**5781–5785

Rios, E., Pizarro, G. 1991. Voltage sensor of excitation-contraction coupling in skeletal muscle. *Physiol. Rev.* **71:**849–908

Rosenbaum, T., Vidaltamayo, R., Sánchez-Herrera, D., Hiriart, M. 1996. Nerve growth factor increases sodium current in pancreatic β cells. *J. Membrane. Biol.* **153:**53–58

Rosenbaum, T., Vidaltamayo, R., Sánchez-Soto, M.C., Zentella, A., Hiriart, M. 1998. Pancreatic β cells synthesize and secrete nerve growth factor. *Proc. Natl. Acad. Sci.* **95:**7784–7788

Rosenbaum, T., Sánchez-Soto, M.C., Hiriart, M. 2001. Nerve growth factor increases insulin secretion and barium current in pancreatic beta-cells. *Diabetes* **50:**1755–1762

Sanchez-Soto, M.C., Larrieta M.E., Vidaltamayo, R., Hiriart, M. 1999. Heterogeneity in glutamic acid decarboxylase expression among single rat pancreatic beta cells. *Diabetologia* **42:**1086–1092

Scharfmann, R., Tazi, A., Polak, M., Kanaka-Gantenbein, C., Czernichow, P. 1993. Expression of functional nerve growth factor receptors in pancreatic β-cell lines and fetal rat islets in primary culture. *Diabetes* **42:**1829–1836

Solem, M., McMahon, T., Messing, R.O. 1995. Depolarization-induced neurite outgrowth in PC12 cells requires permissive, low level NGF receptor stimulation and activation of calcium/calmodulin-dependent protein kinase. *J. Neurosci.* **15:**5966–5975

Strong, J.A., Fox, A.P., Tsien, R.W., Kaczmarek, L.K. 1987. Stimulation of protein kinase C recruits covert calcium channels in *Aplysia* bag cell neurons. *Nature* **325:**714–717

Traynor, A.E. 1984. The relationship between neurite extension and phospholipid metabolism in PC12 cells. *Brain Res.* **316:** 205–210

Vidaltamayo, R., Sánchez-Soto, M.C., Rosenbaum, T., Martínez-Merlos, T., Hiriart, M. 1996. Neuron-like phenotypic changes in pancreatic β-cells induced by NGF, FGF, and dbcAMP. *Endocrine* **4:**19–26