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Nerve Growth Factor Increases Sodium Current in Pancreatic β Cells

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Abstract. Nerve growth factor (NGF) induces neuritelike process outgrowth in cultured adult pancreatic β cells. DbcAMP partially mimics this effect on cell morphology, and both compounds act synergistically to promote neuritelike process outgrowth. To determine if NGF- and dbcAMP-induced differentiation was accompanied by changes in β cell electrical activity, we studied the macroscopic Na current of adult rat β cells identified with the reverse hemolytic plaque assay and cultured for one week with these factors.

After 5–7 days, β cells cultured in the presence of 2.5S NGF exhibited a 48% increase on the macroscopic Na current, which was due to an increase on Na current density. We did not observe changes on voltage dependence of current activation, nor on steady-state inactivation. Although dbcAMP also promotes changes on β cell morphology, it did not affect the Na current density.

Key words: NGF—Na channels—Pancreatic β-cells—cAMP—Reverse hemolytic plaque assay

Introduction

Nerve growth factor (NGF) regulates growth, survival and morphological plasticity of sensory and sympathetic neurons (*reviewed by* Levi-Montalcini, 1987). In other cell types, like chromaffin adrenal cells and rat pancreatic β cells, NGF induces the extension of neuritelike processes (Vidaltamayo, et al., 1996). This effect of NGF is potentiated by permeable analogues of cAMP (like dibutyril-cAMP) (Gunning et al., 1981; Pacheco-Cano et al., 1990; Vidaltamayo et al., 1996).

Moreover, concomitantly to the morphological changes induced in different cell types, in the rat pheochromocytoma cell line PC12, NGF also promotes changes in cell excitability, enabling them to generate action potentials (Dichter, Tischler & Greene, 1977). In these cells, NGF induces an increase both on the Na-peak current and the Na channel density (Mandel et al., 1988; Pollock et al., 1990). These effects are also observed in primary cultures of rat chromaffin cells (Islas-Suárez et al., 1994).

Pancreatic β-cells have Na channels that are important in electrical activity and insulin secretion stimulated by high glucose concentrations (Hiriart & Matteson, 1988; Pressel & Misler, 1990). This current can be identified by its rapid activation and inactivation during a sustained depolarization and by its sensitivity to the Na-channel blocker tetrodotoxin (TTX) (Hiriart & Matteson, 1988; Plant, 1988; Pressel & Misler, 1990).

Since sodium channel modulation and activity are important features in neuronallike differentiation and in β cell physiology, we decided to investigate if NGF and dbcAMP treatment has an effect on Na current of pancreatic β cells, identified with the reverse hemolytic plaque assay.

Materials and Methods

Materials were obtained from the following sources: Insulin antibody from Biodesign International, (Kennebunkport, ME); collagenase class IV from Worthington (Freehall, NJ); fetal calf serum, antibiotics and guinea pig complement from Gibco (Grand Island, NY); tetrodotoxin from Calbiochem (La Jolla, CA); all salts, protein A, RPMI-1640, glucose, dbcAMP and NGF 2.5S from Sigma Chemical (St. Louis, MO).

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 Publication No. 85-23, revised, 1985).

Young adult male Wistar rats (200–250 g) were obtained from the

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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 (Hiriart & Ramírez-Medeles, 1991). Briefly, pancreas were separated from the acinar tissue by collagenase digestion and a Ficoll gradient centrifugation. Islet cells were then dissociated with trypsin (2.5 mg/ml in Spinner salt solution). Single cells were cultured for 5–7 days in RPMI-1640 supplemented with 5% fetal calf serum, 200 U/ml penicillin G, 200 μg/ml streptomycin and 0.5 μg/ml amphotericin B. Experimental cells were treated for 5–7 days with either NGF 2.5S (50 ng/ml) or dbcAMP (5 mM), control cells did not receive any treatment.

Reverse Hemolytic Plaque Assay

To identify insulin-secreting cells, a reverse hemolytic plaque assay (RHPA) was performed as described by Hiriart and Ramírez-Medeles (1991). We chose to evaluate the initial change of the cells under the different treatments after 5 to 7 days in culture because phenotypical changes were mild at this time. In the RHPA it is necessary to detach cells from the Petri dishes to mix them with red blood cells and seed them together on Cunningham chambers. Thus, using cells after one week of culture obviated mechanical injury of β cells with long neuritic processes, which are observed in cultures on subsequent days.

Previous to the assay, sheep red blood cells (SRBC) were coupled to Staphylococcal protein-A using chromium chloride. These SRBCs were incubated with islet cells, harvested from culture dishes by incubating with dissociation cell solution, in the presence of glucose (20.6 mM) and insulin antibody for 1 hr. Following this incubation, guinea pig complement was added to induce the lysis of the SRBC. β Cells were identified as those surrounded by a lysis halo.

Electrophysiological Recordings and Analysis

Whole-cell patch-clamp recordings (Hamill et al., 1981), were made 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 3MΩ. Electrode tips were coated with Sylgard (Dow Corning).

In each cell, the capacity transient of the pipette was canceled 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.

Capacity transients were canceled 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.

The pulse protocol used for the analysis of the peak I_{Na} consisted in applying depolarizing test pulses of 15 msec, from −30 to +60 mV in 10 mV steps, from a holding potential of −80 mV.

Na current activation curves were obtained by converting the peak-current values to conductances. We used the equation:

$$g_{Na} = I_{Na}/(V_m - E_{Na})$$

where I_{Na} is the peak sodium current, V_m the command pulse potential and E_{Na} is the reversal potential observed in the *I-V* relationships obtained from cells where 15 mM NaCl was included in the internal solution, that in average was +43 mV (varied from +42 to +45 mV). Conductance values were normalized and fitted to a Boltzmann relation:

$$g/g_{max} = \{1 + \exp [-(V - Va_{1/2})/ka]\}^{-1}$$

where g is the Na peak conductance, g_{max} the maximal Na conductance $Va_{1/2}$ the midpoint of the activation curve and ka the activation steepness factor.

For the steady-state I_{Na} inactivation curve, a two-pulse protocol was used. A 35-msec prepulse, starting at −120 to −30 mV with 10 mV steps, was used to inactivate Na channels. This pulse was followed by a test pulse at +10 mV, the peak current from the test pulse was plotted as a function of the prepulse potential, and a steady-state inactivation curve was obtained by normalizing the current values and fitting data with a Boltzmann equation:

$$I/I_{max} = \{1 + \exp [(V - Vi_{1/2})/ki]\}^{-1}$$

where I is the peak current, I_{max} is the peak current from a prepulse to −120 mV, V is the prepulse potential, $Vi_{1/2}$ is the half-inactivation value and ki is the inactivation steepness parameter.

Recording Solutions

External Solution (mM)	Internal Solution (mM)
130 NaC1	120 CsAsp
5 KC1	10 CsC1
5 $CaC1_2$	10 BAPTA
2 $MgC1_2$	10 HEPES
10 HEPES	5 CsF
10 Glucose	2 ATP-Mg

In 15 experiments, 15 mM NaCl was added to the internal solution in order to obtain the reversal potential of the Na current.

Statistical Analyses

All data are reported as the mean ± SEM; *n* denotes the number of cells studied. The statistical significance was obtained with the one way ANOVA test using the Number Cruncher Statistical System (Dr. J. L. Hintze, Kaysville, UT, 1983).

Results

Na Channel Density Increases in NGF-treated Adult β Cells

Figure 1*A* and *B* illustrates families of whole-cell currents recorded from control and β-cells treated with NGF (50 ng/ml) for 5–7 days, respectively, in response to depolarizing voltage steps. β-cells were identified with a RHPA. In both conditions, the I_{Na} rapidly activates to a peak and then inactivates during maintained depolarization.

The peak current-voltage relationships for I_{Na} in control cells and NGF-treated cells are shown in Fig. 1*C*. In both cases, currents activated near −30 mV and reached a maximum around +10 mV. Maximum Na current in NGF-treated cells was, in average, 48% ($P < 0.01$) larger than in control cells.

In 15 cells, 15 mM NaCl was included in the internal solution. In this condition, the currents reversed direc-

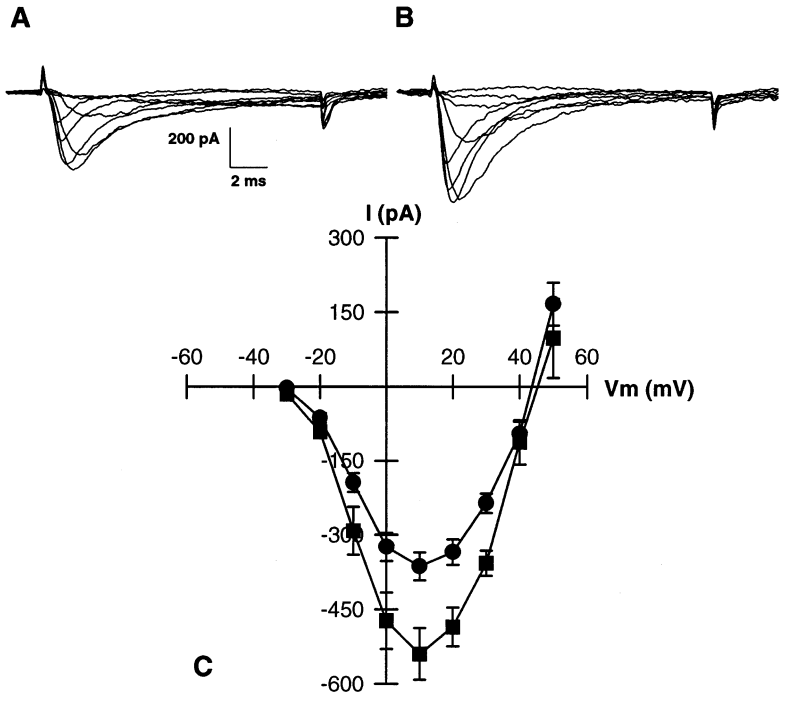

Fig. 1. Comparison of Na currents and Na current-voltage relationships between control and β cells treated during one week with NGF (50 ng/ml). (*A*) Control β cells, (*B*) NGF treated β-cells. Representative Na current families, which were recorded during 10-msec steps to different voltages, from a holding potential of −80 mV (*see* Materials and Methods). (*C*) The mean-peak Na current was plotted as a function of voltage, under control conditions (circles), $n = 28$ and in NGF-treated cells (squares), $n = 27$. Error bars represent SE. In both conditions, the last two points were obtained from experiments with 15 mM NaCl in the internal solution ($n = 15$), hence the reversal potential is around +43 mV.

tion from inward to outward at about +43 mV (Fig. 1*C*), which is close to the calculated sodium Nernst potential ($E_{Na} = +47$ mV, at 20°C).

When 5 mM $CoCl_2$ was added to the external solution to block Ca currents, the peak Na current amplitude was not altered by the treatment (*data not shown*). Moreover, the currents were completely blocked by 100 nM TTX in both control and NGF-treated β cells (*data not shown*), as previously observed in rat β-cells (Hiriart & Matteson, 1988).

The increase in sodium current amplitude in β cells exposed to NGF does not result from changes on the voltage dependence of current activation, nor on the steady-state inactivation of the current, as shown in Figs. 2 and 3.

In Fig. 2, the voltage dependence of peak sodium conductances was calculated and used to construct a Boltzmann relationship (as described in Materials and Methods), for control and NGF-treated β-cells. NGF-treatment did not modify the midpoint of activation ($Va_{1/2}$), nor the steepness *ka* of the curves (Table 1).

Figure 3 shows the steady-state fraction of noninactivated Na channels plotted as a function of membrane potential. In both conditions, half-inactivation ($Vi_{1/2}$) was around −60 mV and the inactivation was practically complete at voltages positive to −40 mV. The steepness parameter *ki* did not change with NGF treatment (Table 1).

The increase in Na current could not be attributed to a difference in cell surface area, average cell capacitance

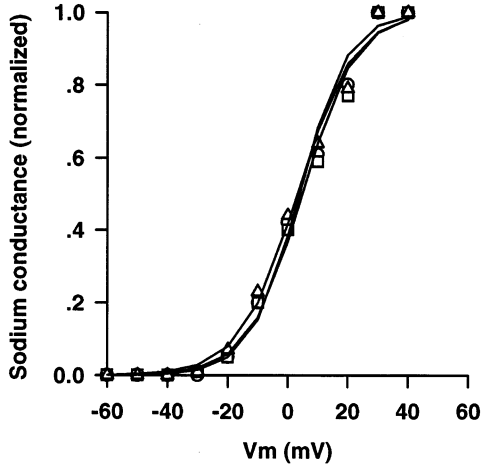

Fig. 2. Voltage dependence of Na current activation. At each voltage the Na conductance was calculated, as described in Materials and Methods, and plotted as a function of voltage. Experimental data are plotted as symbols: control β-cells (circles), NGF-treated β-cells (squares) and dbcAMP-treated β-cells (triangles); and the smooth curve represents a Boltzmann fit to the data (*see* Materials and Methods and Table 2).

in NGF-treated cells increased by 6.8% and in dbc-AMP treated cells, average cell capacitance increased by 9%, although these increments were not statistically significant (Table 2). Moreover, in Fig. 5 individual data from control, NGF- and dbcAMP-treated β cells were pooled, it is clear that there is no direct relationship between cell

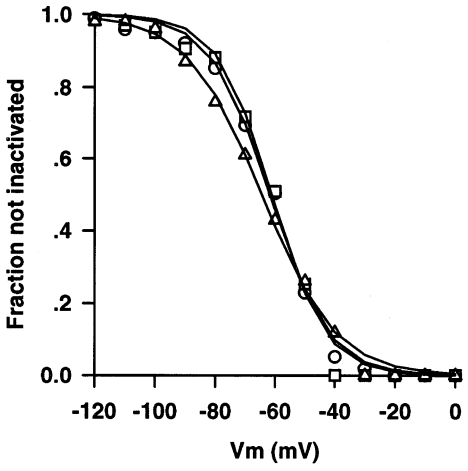

Fig. 3. Voltage dependence of Na current inactivation. A 35-msec prepulse was used to inactivate Na channels to a steady-state level. The Na currents recorded during a test pulse to +10 mV were used to determine the fraction of noninactivated channels. The Na current during the test pulse was normalized and plotted as a function of the prepulse voltage. Experimental data are plotted as symbols: control β-cells (circles), NGF-treated β-cells (squares) and dbcAMP-treated β-cells (triangles); and the smooth curve represents a Boltzmann fit to the data (*see* Materials and Methods and Table 2).

Table 1. Effects of NGF and dbcAMP on β-cell peak Na current

Treatment	Capacitance (pF)	Density (pA/pF)	Peak current (pA)
Control	8.8 ± 0.4	−44.7 ± 4.5	−363.7 ± 2.8
NGF	9.4 ± 0.3	−58.1 ± 5.7†	−539.5 ± 5.2*
dbcAMP	9.6 ± 0.6	−47.3 ± 6.7	−419.4 ± 4.5

* $P < 0.01$, † $P < 0.05$.

Table 2. Boltzmann parameters of β-cell sodium currents

	$V_{a1/2}$	k_a	$V_{i1/2}$	k_i
Control	4.04 ± 1.0	9.12 ± 0.2	−62.57 ± 2.3	10.49 ± 0.2
NGF	4.45 ± 1.2	8.95 ± 0.4	−62.52 ± 2.3	9.93 ± 0.6
dbcAMP	3.03 ± 1.1	9.62 ± 0.3	−60.64 ± 2.8	9.08 ± 1.4

size (that is closely related to capacitance) and sodium channel density. Together, these data indicate that NGF increased β-cell Na current density by approximately 30% ($P < 0.05$).

Effect of dbcAMP on Na Current of β Cells

Since dbcAMP also induced the outgrowth of neuritelike processes in β cells, we also tested the effect of this agent on the Na peak current of these cells. Figure 4 shows families of whole-cell Na currents for a control cell (*A*) and a dbcAMP-treated cell (*B*).

The peak current-voltage relationships of Na currents for control cells and dbcAMP-treated cells are shown in Fig. 4*C*. Both for control and dbcAMP-treated cells, the currents activate around −30 mV and reach a maximum near +10 mV. Nevertheless, no significant effects of dbcAMP could be observed on the peak Na current, nor on voltage dependence activation or steady-state inactivation of β cells (Tables 1 and 2; Figs. 2, 3 and 4).

Discussion

This study represents the first description of the effects of NGF on the electrical properties of a nonneural-crest-derived cell type. We observed that NGF treatment increases Na channel density in pancreatic β-cells.

Adult and fetal pancreatic β-cells, as well as insulin-producing cell lines like RINm5F and INS-1, express both types of NGF receptors; high affinity Trk-A and low affinity $p75^{NGFR}$ (Kanaka-Gatenbein et al., 1995*a,b*, and Scharfmann et al., 1993). We have observed that cultured rat β-cells undergo neuronallike phenotypic changes in response to NGF and dbcAMP (Vidaltamayo et al., 1995), in a way similar to what has been observed in chromaffin cells and PC12 cells (Greene & Tischler, 1976, Schubert & Whitlock, 1977; Heidenmann et al., 1985 and Pacheco Cano et al., 1990).

We evaluated the effect of culturing islet cells for five to seven days in the presence of NGF or dbcAMP, on Na current of β cells identified with the reverse hemolytic plaque assay.

We observed that NGF treatment in culture induces, on the average, an increase of 48% on the Na peak current. This increment on Na current may be due to an increase in the number of Na channels in the membrane of β cells, since the membrane capacitance of NGF-treated cells and control cells is not significantly different, these data represent a 30% increase on the Na current density.

It has been well documented that phenotypical responses to NGF by chromaffin cells and PC12 cells, involve an increased synthesis and expression of functional Na channels (Islas-Suárez et al., 1994; Mandel et al., 1988; Pollock et al., 1990). This is what probably happens in our model, since it is not likely that NGF induces the expression of a different type of Na channel in β cells, because the voltage dependence of Na current, the kinetics of activation and the steady-state inactivation and TTX sensitivity, show no differences between control and experimental cells.

This increase in Na channel density in β cells could be important for the NGF-induced sprouting of the neuritelike process, since it has been shown that Na channels

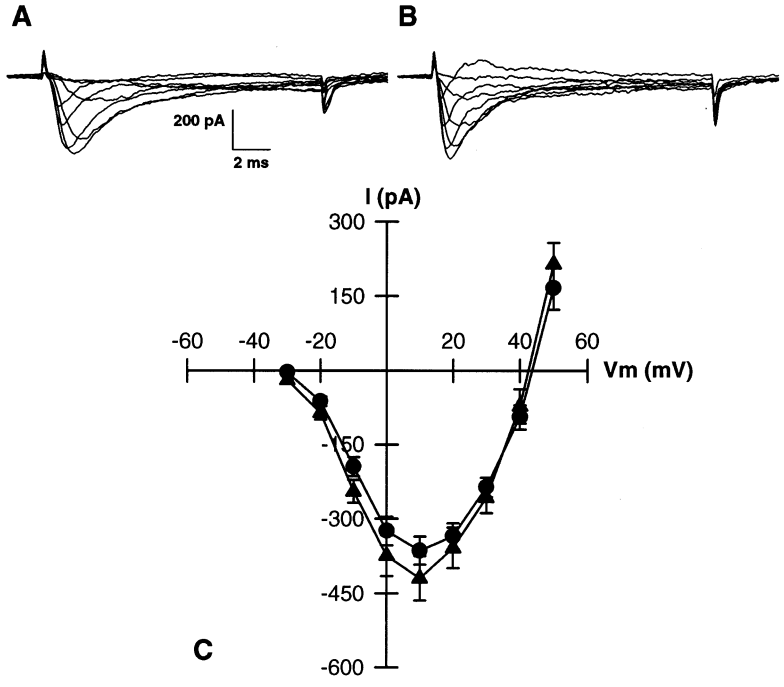

Fig. 4. Comparison of Na currents and Na current-voltage relationships between control and β cells treated during one week with dbcAMP (5 mM). (*A*) Control β cells, (*B*) dbcAMP-treated β-cells. Na current families were recorded during 10-msec steps to different voltages, from a holding potential of −80 mV (*see* Materials and Methods). (*C*) The peak Na current was plotted as a function of voltage, under control conditions (circles), $n = 28$, and in dbcAMP-treated cells (triangles) $n = 21$. Error bars represent SE. In both conditions, the last two points were obtained from experiments with 15 mM NaCl in the internal solution ($n = 15$), hence the reversal potential is around +43 mV.

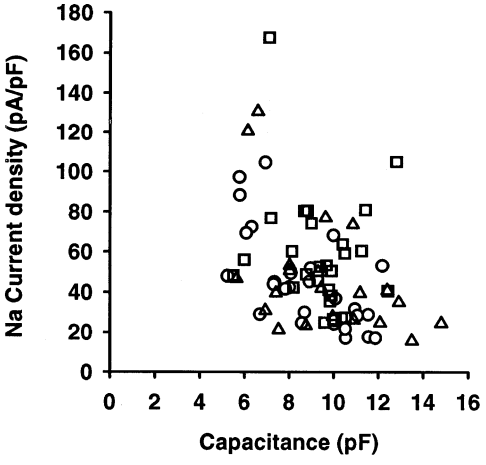

Fig. 5. Relationship between peak Na current density and cell capacitance. Individual peak Na current densities at 10 mV were plotted against the respective cell capacitance. Circles denote control cells, triangles denote NGF-treated cells and squares denote dbcAMP-treated cells.

could be associated with cytoskeletal elements, providing a potential anchoring mechanism for different membrane regions (*reviewed by* Mandel, 1992).

Although there is a synergistic effect between NGF and dbcAMP on β-cell process outgrowth (Vidaltamayo et al., 1996), there is no direct effect of dbcAMP treatment on Na current density, nor on the Na current behavior. Similar results have also been observed in PC12 cells (Pollock et al., 1990).

We have previously shown that after one week in culture, NGF enables β cells to secrete more insulin in the presence of 20.6 mM glucose than in the presence of 5.6 mM, a property that is lost in control cells (Vidaltamayo et al., 1996). This could be due in part to the effect of NGF on β cell Na current.

It has been alleged that Na current does not contribute significantly to β cell excitability (Ashcroft & Rorsman, 1989). However several data support that Na current is important for β cell physiology:

-Na current contributes to the generation of action potentials during the initial phases of secretagogue-induced depolarization (Pressel & Misler, 1990).

-TTX inhibits up to 57% of high glucose-stimulated insulin secretion (Hiriart & Matteson, 1988) and also carbachol-induced insulin secretion in 5.6 mM glucose (Hiriart & Ramírez-Mendeles, 1993).

-Finally, the fact that the NGF-treated β cells secrete insulin more efficiently than control cells (Vidaltamayo et al., 1986) could be explained by a faster and stronger depolarization due to the presence of more Na channels on the β-cell membrane.

Finally, it is important to note that since NGF has been implicated in islet morphogenesis (Kanaka-Gatenbein et al., 1995*b*), it is also possible that NGF increases β-cell excitability during development by modulating Na channel expression.

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