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Differential Regulation of Neuronal Sodium Channel Expression by Endogenous and Exogenous Tyrosine Kinase Receptors Expressed in Rat Pheochromocytoma Cells

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The biological activity of growth factors that act through receptor tyrosine kinases (RTKs) can differ dramatically, depending on both the properties of the RTKs and the cellular environment in which the RTKs are expressed. To determine the ability of different RTKs to elicit *ras*-independent responses central to neuronal differentiation, we analyzed voltage-dependent sodium (Na) channel expression in rat pheochromocytoma (PC12) cells after activation of a variety of endogenously and exogenously expressed RTKs. In PC12 cells expressing trkB (Ip et al., 1993), the increase in Na current density caused by brain-derived neurotrophic factor (BDNF) was similar to that observed upon activation of endogenous trkA by NGF. BDNF also increased type II Na channel mRNA expression, as did neurotrophin-3 in PC12 cells expressing trkC (Tsoulfas et al., 1993). In contrast, insulin did not increase type II Na channel mRNA expression or Na current density in PC12 cells, while epidermal growth factor (EGF) elicited small, yet reproducible increases in type II Na channel mRNA and Na current density when compared to NGF, even upon coexpression of an EGF receptor/p75 receptor chimera (Yan et al., 1991). Finally, in PC12 cells expressing β-platelet–derived growth factor (PDGF) receptors (Heasley and Johnson, 1992), PDGF increased type II Na channel mRNA and Na current density to the same extent as NGF. The results show the capabilities of these RTKs in eliciting Na channel expression and the specificity arising due to differences in their intrinsic properties.

[Key words: neuronal differentiation, neurotrophic factors, growth factors, sodium channels, gene expression, patch clamping]

Neuronal survival, growth, and differentiation depends upon a variety of growth factors acting through specific receptor tyrosine kinases (RTKs) (for reviews, see Barde, 1989; Wagner and Kostyk, 1991; Chao, 1992a). Among these factors, NGF has been extensively studied (for reviews, see Levi-Montalcini, 1987; Bothwell, 1991; Gage et al., 1991), with the product of the proto-oncogene trkA, either alone or in conjunction with a 75 kDa membrane spanning protein (p75), serving as its receptor (for discussion, see Chao, 1992a; Meakin and Shooter, 1992). While recent discovery of other members of the trk RTK family, such as trkB and trkC (for review, see Chao, 1992a), as well as other NGF-related neurotrophins, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) (for review, see Thoenen, 1991), have emphasized the importance of neurotrophins in neuronal survival and differentiation (for reviews, see Barde, 1989; Gage et al., 1991; Wagner and Kostyk, 1991), other factors also appear to contribute to these processes. For example, epidermal growth factor (EGF) enhances not only neuronal proliferation, but *in vitro* survival and neurite outgrowth of neocortical neurons (Walicke and Baird, 1988). Furthermore, insulin and its receptor are present in the nervous system, and together can promote neuronal viability, neurite outgrowth, and the differentiation of neuroblastoma cells (Bhat, 1983; Recio-Pinto et al., 1986; for discussion, see Saltiel and Decker, 1991). Finally, both forms of platelet-derived growth factor (PDGF) and PDGF receptor are expressed throughout the mammalian nervous system (Sasahara et al., 1991; Yeh et al., 1991; Eccleston et al., 1993) and PDGF enhances survival, neurite outgrowth, and differentiation of rat brain neurons *in vitro*, suggesting a neurotrophic or neuroregulatory role (Smits et al., 1991). Despite evidence suggesting these factors play a role in the nervous system, however, the extent to which they govern cellular events associated with a neuronal phenotype and the mechanisms by which they do so have not been clearly defined.

A key aspect of neuronal differentiation that has not been examined in detail with regard to the actions of growth factors and their receptors is the induction of voltage-dependent sodium (Na) channels, which play a central role in the production and propagation of action potentials (Hodgkin and Huxley, 1952). The influence of growth factors on Na channel expression, as evidenced by the NGF induction of Na channel expression in rat pheochromocytoma (PC12) cells (Dichter et al., 1977; Mandel et al., 1988; Fanger et al., 1993), provides not only an avenue for identifying steps crucial to the ability of growth factors to induce neuronal differentiation (for discussion, see Chao, 1992b), but also a means of understanding processes fundamental to nervous system development. In particular, as opposed to many of the characterized responses of PC12 cells to NGF, induction of Na channel expression by NGF is independent of *ras* activity (D'Arcangelo and Haleguoa, 1993; Fanger et al., 1993), making analysis of Na channel induction useful for understanding the nature of this apparently biologically important (Borasio et al.,

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1993), yet less understood facet of growth factor–mediated differentiation.

As an approach to understanding regulation of neuronal Na channel expression by growth factors and their receptors, we have analyzed Na channel expression in PC12 cells. In response to NGF, PC12 cells acquire characteristics of sympathetic neurons during a transformation that includes cessation of cell division, neurite extension, and increased Na channel expression (for reviews, see Greene and Tischler, 1982; Halegoua et al., 1991). PC12 cells express the type II Na channel α-subunit gene (Mandel et al., 1988; Fanger et al., 1993), one of the four α-subunit genes (type I, II, IIa, and III) expressed in the brain that have been shown to encode functional Na channels (Noda et al., 1986; Auld et al., 1988; Suzuki et al., 1988). Mechanisms governing neuronal Na channel expression are largely unknown, although transcriptional, translational, and posttranslational events all appear to play a role (Wollner et al., 1988; Scheinman et al., 1989; Maue et al., 1990; Ginty et al., 1992) and may be important in determining the distinct pattern of type II α-subunit expression during development (Beckh et al., 1989; for review, see Mandel, 1992). In the present studies, PC12 cells provided a common neuronal cellular context in which to determine the relative abilities of various endogenously and exogenously expressed RTKs to induce type II Na channel expression, with the results highlighting the specificity arising from differences in their intrinsic properties.

Materials and Methods

Cell culture. Cells were maintained in a humidified CO_2 environment in DMEM (Dulbecco's Modified Eagle's Medium) containing 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO Laboratories, Grand Island, NY). The culture medium for the 6-15 and 6-24 sublines, and the wild-type PC12 cells from which they were generated (obtained courtesy of D. Kaplan, NCI–Frederick Cancer Center, Frederick, MD), contained 10% fetal bovine serum (FBS) and 5% heat inactivated horse serum (HS) (GIBCO Laboratories, Grand Island, NY). The medium for the trkB/PC12 cells and the wild-type cells they were generated from (obtained courtesy of G. Yancopoulos, Regeneron Pharmaceuticals, Tarrytown, NY) contained 6% FBS and 6% HS. For the PC-PDGFR-102, PC-PDGFR-111, and PC-NEO-102 cells (obtained courtesy of L. Heasley, Univ. Colorado School of Medicine, Denver, CO) the medium contained 5% plasma-derived FBS and 5% plasma-derived HS (Cocalico Biologicals, Reamstown, PA). Medium for the 6-15, 6-24, trkB/PC12, PC-PDGFR-102, PC-PDGFR-111, and PC-NEO-102 cells contained 250 μg/ml G418 (GIBCO Laboratories, Grand Island, NY). Cells were plated and maintained on 150 mm (ODC assays), 100 mm (RNA analysis), or 35 mm (electrophysiology) tissue culture dishes (Falcon Labware, Becton Dickinson, Lincoln Park, NJ). In cases where 100 ng/ml 7S NGF, 100 ng/ml EGF, 30 ng/ml PDGF (Upstate Biotechnology, Plattsburg, NY), 100 ng/ml BDNF (Regeneron Pharmaceuticals, Tarrytown, NY), or 300 nM insulin (Sigma Chemical Co., St. Louis, MO) was included, fresh growth factor was added every other day when the media was changed.

Ornithine decarboxylase assay. Cells were plated at ~60% confluency 2 d prior to the assay. Cells were washed twice with DMEM containing 1% HS and treated for 5 hr in DMEM containing 1% HS and the indicated growth factors. Cells were then rinsed with ice cold PBS (130 mM NaCl, 10 mM Na_2HPO_4, 10 mM NaH_2PO_4, pH 7.2), harvested in PBS by trituration, pelleted by brief centrifugation, and then resuspended and lysed by sonication in homogenizing buffer (50 mM NaH_2PO_4, 0.1 mM EDTA, 5 mM dithiothreitol, and 40 mM pyridoxal phosphate, pH 7.2). Cellular debris was removed by centrifugation and the protein concentration of the supernatant adjusted to ~1 mg/ml. Ornithine decarboxylase activity in the supernatant was assayed by the method of Djurhuus (1981), with the conversion of ^{3}H-ornithine (Amersham, Arlington Heights, IL) to ^{3}H-putrescine assayed at 0, 20, 40, 60, and 80 min by spotting an aliquot of the assay mixture on to P81 ion-exchange paper (Whatman, Hillsboro, OR) and washing in 0.1 M NH_4OH; pH 11.3. Specific activities were determined from slopes calculated by linear regression.

RNA isolation and analysis. Total cellular RNA was isolated by the method of Chirgwin et al. (1979). Northern blot analysis and preparation of ^{32}P-UTP–labeled cRNA probes specific for Na channel and cyclophilin mRNA were done as in our previous studies (Ginty et al., 1992; Fanger et al., 1993). A random primed cDNA probe for fos mRNA was generated using ^{32}P-CTP (Amersham, Arlington Heights, IL), a commercially available kit (Bethesda Research Laboratories, Grand Island, NY), and a fos cDNA (courtesy of J. Belasco and M. Greenberg, Harvard Medical School, Boston, MA). RNase protection analysis was performed essentially as described by Hod (1992). The Na channel type specific cRNA probe and cyclophilin specific cRNA probe were generated using ^{32}P-UTP and hybridized with RNA as described in our previous studies (Fanger et al., 1993), with the exception that the samples were digested with RNase for 24 hr at 6°C before being separated on a 6% acrylamide gel and exposed to Kodak XAR film for 24 hr at −80°C. The NIH IMAGE program was used for densitometric analysis of the autoradiographic signals representing the Na channel and cyclophilin mRNAs.

Electrophysiological recording and analysis. Whole-cell patch-clamp analysis of Na current density was done as in our earlier studies (Ginty et al., 1992; Fanger et al., 1993). Culture medium was replaced with a saline solution (150 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 6 mM HEPES, 6 mM glucose, pH 7.2), and recordings were made using a List EPC-7 patch-clamp amplifier (Medical Systems Corp.) and patch electrodes (3–7 MΩ) filled with an internal solution designed to minimize the contribution of other voltage-activated currents (140 mM CsCl, 10 mM EGTA, 10 mM HEPES). Electronic compensation was used to reduce the effective series resistance and the time constant of membrane charging, and to provide measurements of access resistance and cell membrane capacitance. Series resistance compensation of 50–70% was routinely used and reduced estimated series resistance errors to 5 mV or less. Voltage commands were applied and current measurements were made using an Atari computer-based acquisition system (Instrutech Corp., Elmont, NY). Cells were held at −80 mV and every 3 sec the membrane potential stepped through a 40 msec prepulse to −120 mV, followed by a 20 msec depolarizing test pulse to a potential between −60 mV and +30 mV. Current signals were low pass filtered at 10 kHz, digitized at 20 kHz for storage, and digitally filtered at 2 kHz during analysis. Linear leakage currents and capacity transients were subtracted with scaled pulse (P/4) routines. The maximum Na current amplitude elicited, along with the measurement of cell membrane capacitance, was used to calculate Na current density. Statistical significance was determined using a two-tailed Student's t test.

Results

Type II Na channel expression in PC12 cells can be regulated through different trk *tyrosine kinase receptors*

The discovery of other NGF-related neurotrophins, such as BDNF and NT-3 (Liebrock et al., 1989; Hohn et al., 1990; Maisonpierre et al., 1990; for review, see Thoenen, 1991), in addition to the discovery of other members of the trk tyrosine kinase receptor family, such as trkB and trkC (Klein et al., 1989; Lamballe et al., 1991; Middlemas et al., 1991; Tsoulfas et al., 1993; for review, see Chao, 1992a), has led to considerable interest in the biological capabilities of these neurotrophins and receptors. To determine if the activation of trkB or trkC receptors can cause changes in neuronal Na channel expression, we assayed the effects of BDNF and NT-3 on Na channel expression in PC12 sublines stably expressing either trkB or trkC receptors (Ip et al., 1993; Tsoulfas et al., 1993). We also analyzed Na channel expression in two PC12 sublines in which trkA has been stably overexpressed (Hempstead et al., 1992). Using c-fos induction and neurite outgrowth as indications of the general responsiveness of the cells to the NGF, BDNF, or NT-3 treatments (Fig. 1), the cells used in our analysis responded as expected based on previous studies (Hempstead et al., 1992; Ip et al., 1993; Tsoulfas et al., 1993). As predicted for cells that do not express trkB or trkC, wild-type PC12 cells did not respond to

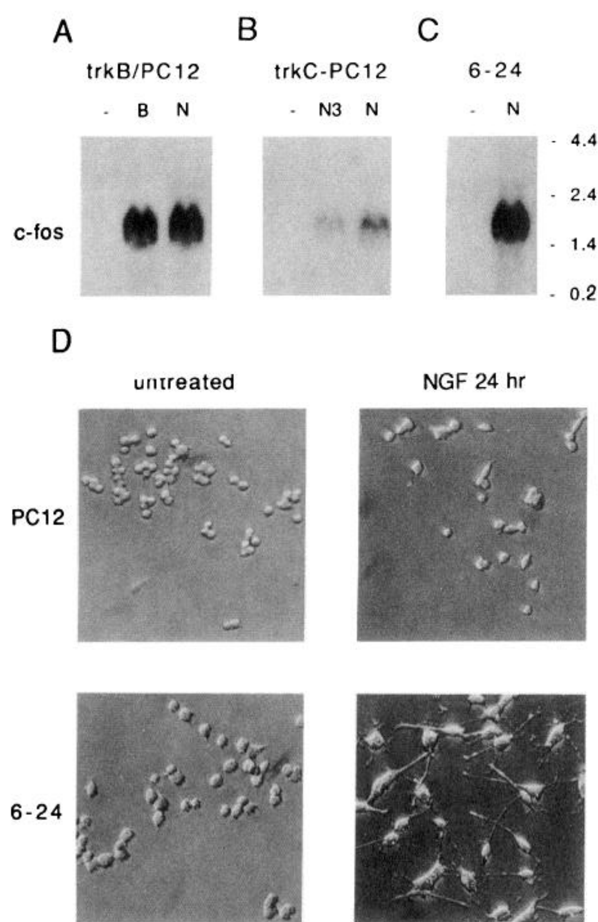

Figure 1. Neurotrophin-mediated responses of PC12 sublines stably expressing members of the trk family of tyrosine kinase receptors. In *A–C*, Northern blot hybridization was used to analyze c-fos mRNA levels in 20 μg samples of RNA from cells maintained in the absence or presence of neurotrophins for 30 min (except trkC-PC12 cells, which were treated for 60 min). The c-fos signals are shown after 12 hr exposure to film. The position and molecular sizes (kb) of a commercially available RNA ladder (Bethesda Research Laboratories, Grand Island, NY) are shown on the *right*. In *A*, RNA samples were from trkB/PC12 cells either untreated (−) or treated with 100 ng/ml BDNF (*B*) or 100 ng/ml NGF (*N*). In *B*, RNA samples were from trkC-PC12 cells either untreated (−) or treated with 100 ng/ml NT-3 (*N3*) or 100 ng/ml NGF (*N*). In *C*, RNA samples were from 6–24 cells either untreated (−) or treated with 100 ng/ml NGF (*N*). In *D*, photographs illustrate the accelerated neurite outgrowth from 6–24 cells, as compared to the wild-type PC12 cells, when treated for 1 d with 100 ng/ml NGF. Cell bodies in the untreated PC12 cells are approximately 15 μm in diameter.

BDNF or NT-3 with either an increase in c-fos mRNA or neurite outgrowth (data not shown). In contrast, the trkB-expressing PC12 cells responded to either NGF or BDNF with an increase in c-fos mRNA expression (Fig. 1*A*), flattening of the cell body, and extensive neurite outgrowth (data not shown). Similarly, NT-3 caused increases in c-fos mRNA expression (Fig. 1*B*) and neurite outgrowth (data not shown) in the PC12 cells stably expressing trkC. In PC12 cells overexpressing trkA, the increases in c-fos mRNA (Fig. 1*C*) and the accelerated neurite outgrowth in response to NGF (Fig. 1*D*) were consistent with the previous characterization of these cells (Hempstead et al., 1992).

To determine whether activation of trkB or trkC could increase type II Na channel gene expression, RNAse protection assays were used to analyze RNA from PC12 sublines treated with either NGF, BDNF, or NT-3 (Fig. 2), using previously developed procedures and a ^{32}P-labeled probe specific for type II Na channel mRNA (Fanger et al., 1993). Given that cyclophilin mRNA is constitutively expressed in PC12 cells and unaffected by growth factor treatment (Machida et al., 1989), a radiolabeled probe specific for cyclophilin mRNA was also included in the assays and the cyclophilin mRNA signal used as an internal control for variations in RNA loading. In wild-type PC12 cells, treatment with BDNF for 7 d failed to increase type II Na channel mRNA expression, while exposure to NGF for 7 d caused a threefold increase (Fig. 2*A*). In contrast, treatment of the trkB-expressing PC12 cells with BDNF for 7 d caused a 2.3-fold increase in type II Na channel mRNA (Fig. 2*A*). In PC12 cells expressing trkC receptors, there was an increase in type II Na channel mRNA expression after 4 d treatment with either NGF (2.9-fold) or NT-3 (2.0-fold) (Fig. 2*B*). The trkB- and trkC-mediated increases in type II Na channel mRNA expression appeared to be due to specific signaling capabilities of these receptors and not simply the consequence overexpressing a tyrosine kinase receptor, since overexpression of trkA in PC12 cells did not appreciably alter the NGF-mediated induction of type II Na channel mRNA when compared to that occurring in wild-type cells. In both trkA-overexpressing sublines that were analyzed (6-15 and 6-24), the time course of the type II Na channel mRNA induction was not accelerated, nor was the extent of induction enhanced. In fact, the induction of type II Na channel mRNA was relatively modest (though within the range normally observed), perhaps due to slightly elevated levels of type II Na channel mRNA in these cells in the absence of NGF (Fig. 2*C*).

Whole-cell patch-clamp analysis was used to determine if any of these changes in Na channel gene expression were accompanied by changes in functional Na channel expression. Using previously developed procedures (Fanger et al., 1993), cells grown on tissue culture plastic in the presence or absence of growth factors for 7 d were held at −80 mV, the membrane potential prepulsed to −120 mV, and the responses to step depolarizations between −60 mV and +30 mV measured in order to determine the peak Na current (Fig. 3). Cell membrane capacitance was determined as an estimate of cell size, and from the measurements of peak Na current and cell membrane capacitance, the Na current density was estimated. As expected for the wild-type PC12 cells, exposure to NGF for 7 d caused the proportion of cells with appreciable Na currents (>50 pA) to increase (from 10/26 to 23/23) and caused significant ($p < 0.01$) increases in the magnitude of the Na currents and the average Na current density (Fig. 3*A*,*C*), while exposure to BDNF for 7 d had no effect (Fig. 3*A*,*C*). In contrast, BDNF had a dramatic effect on the trkB-expressing PC12 cells. In these cells, the proportion of BDNF-treated cells with appreciable Na currents (20/20) was similar to that observed in response to NGF (22/22), and there were significant ($p < 0.01$) increases in the magnitude of the Na currents, average cell size, and average Na current density (Fig. 3*B*,*C*). Although the increase in Na current density in response to BDNF was less than observed in the cells in response to NGF (Fig. 3*C*), the difference was not statistically significant, and when coupled with the changes in type II Na channel mRNA expression (Fig. 2*A*), the ~3-fold increase in Na current density in the BDNF-treated cells indicates that activation of trkB can result in increases in Na channel expression.

The effect of NGF on functional Na channel expression was

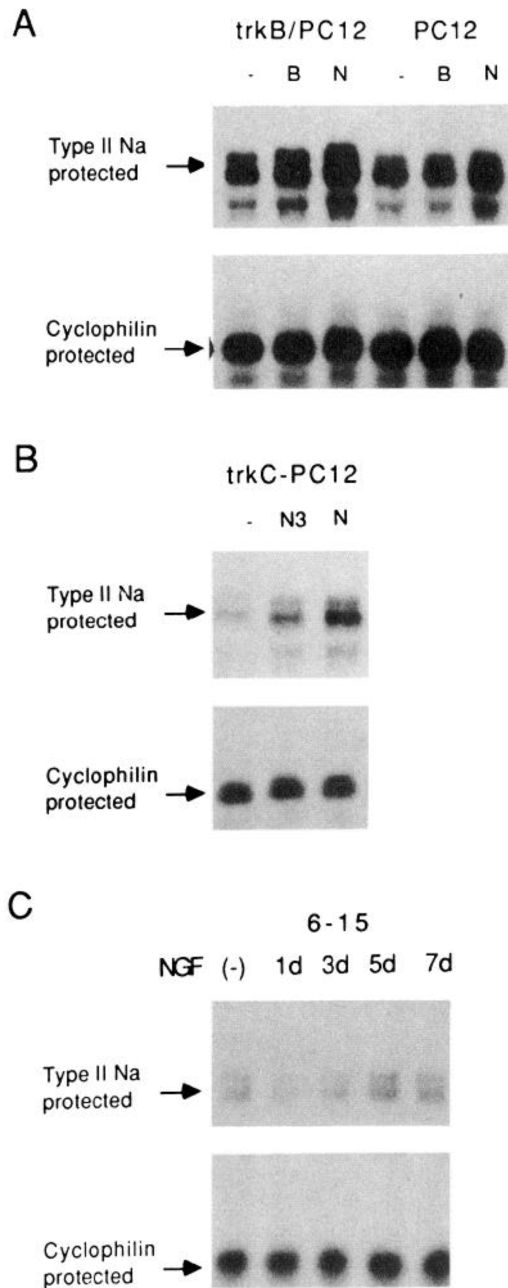

Figure 2. Neurotrophin-mediated induction of type II Na channel mRNA in PC12 sublines stably expressing members of the trk family of tyrosine kinase receptors. In *A–C*, RNase protection assays were used to analyze type II Na channel and cyclophilin mRNA levels in 20 μg samples of RNA from cells maintained in the absence or presence of neurotrophins. Probes specific for type II Na channel mRNA and for cyclophilin mRNA were used, with the signals representing cyclophilin mRNA, which is constitutively expressed in PC12 cells and unaffected by growth factor treatment, used as an internal control for variations in RNA loading (see Materials and Methods). Only the signals representing the protected mRNA fragments are shown. Signals representing Na channel mRNA are shown after 24 hr exposure to film; signals representing cyclophilin mRNA are shown after 30 min exposure to film. In *A*, RNA samples were from trkB/PC12 cells or wild-type PC12 cells either untreated (−) or treated for 7 d with 100 ng/ml BDNF (*B*) or 100 ng/ml NGF (*N*). In *B*, RNA samples were from trkC-PC12 cells either untreated (−) or treated for 4 d with 100 ng/ml NT-3 (*N3*) or 100 ng/ml NGF (*N*). In *C*, RNA samples were from 6–15 cells either untreated (−) or treated with 100 ng/ml NGF for 1–7 d.

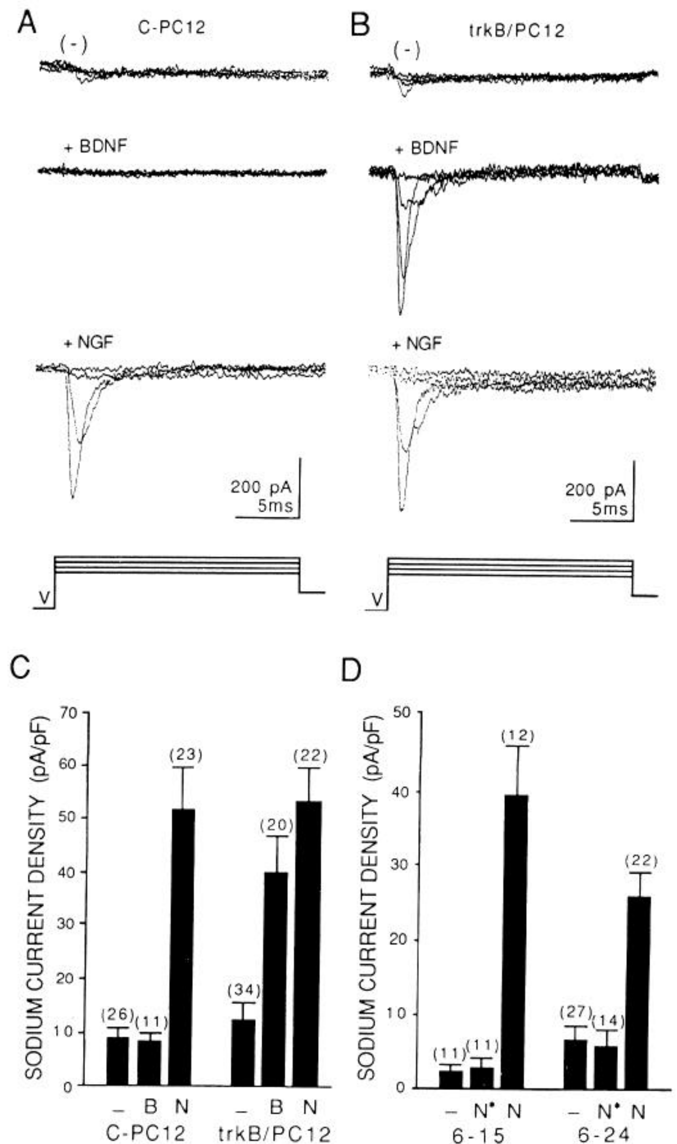

Figure 3. Neurotrophin-mediated increases in Na current density in PC12 sublines stably expressing members of the trk family of tyrosine kinase receptors. In *A* and *B* are representative recordings of voltage-dependent Na currents in wild-type PC12 cells (*C-PC12*) and trkB/PC12 cells that were either untreated, or treated for 7 d with 100 ng/ml BDNF or 100 ng/ml NGF. Superimposed current records illustrate the response to depolarizing pulses to −40, −30, −20, and −10 mV, from a prepulse potential of −120 mV and holding potential of −80 mV. *C*, Average Na current density in wild-type PC12 cells (*C-PC12*) and trkB/PC12 cells that were either untreated (−) or treated for 7 d with 100 ng/ml BDNF (*B*) or 100 ng/ml NGF (*N*). *D*, Average Na current density in the 6–15 and 6–24 sublines of PC12 cells when either untreated (−) or treated with 100 ng/ml NGF for 1 d (*N**) or 7 d (*N*). In *C* and *D*, the number of cells in each group is in parentheses above the bars. Error bars represent the SEM. Cell membrane capacitance (mean ± SEM) was 9.2 ± 0.5 pF for untreated, 8.4 ± 0.6 pF for BDNF-treated, and 22.1 ± 1.2 pF for NGF-treated wild-type PC12 cells, 9.8 ± 0.6 pF for untreated, 15.0 ± 1.0 pF for BDNF-treated, and 18.3 ± 1.0 pF for NGF-treated trkB/PC12 cells, 7.6 ± 0.6 pF for untreated, 38.7 ± 7.4 pF for NGF-treated (1 d), and 27.8 ± 1.6 pF for NGF-treated (7 d) 6–15 cells, 12.3 ± 0.5 pF for untreated, 32.4 ± 4.6 pF for NGF-treated (1 d), and 54.1 ± 9.4 pF for NGF-treated (7 d) 6–24 cells.

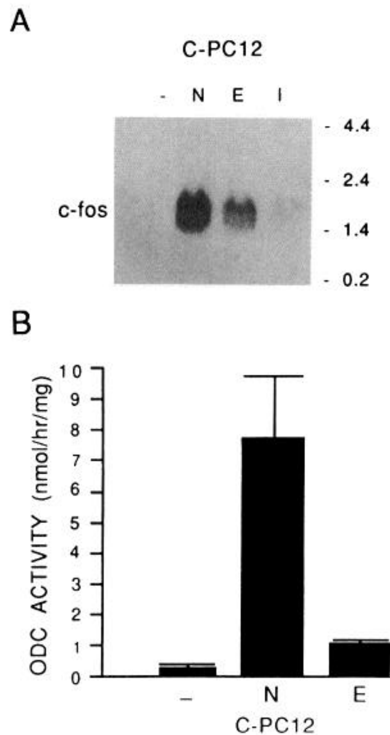

Figure 4. Responsiveness of wild-type PC12 cells to growth factor treatment. *A*, Northern blot analysis of c-fos mRNA expression in 20 μg samples of RNA from cells that were either untreated (−) or treated for 30 min with 100 ng/ml NGF (*N*), 100 ng/ml EGF (*E*), or 300 nM insulin (*I*). The position and molecular sizes of a commercially available RNA ladder (Bethesda Research Labs) are shown on the *right*. *B*, Ornithine decarboxylase (*ODC*) activity in cells that were either untreated (−) or treated for 5 hr with 100 ng/ml NGF or 100 ng/ml EGF. Error bars represent the SEM.

also determined in the 6-15 and 6-24 sublines overexpressing trkA. After treatment with NGF for 1 d, there was an increase in the proportion of cells with appreciable (>50 pA) Na currents (from 0/11 to 6/11 and 15/27 to 11/14, respectively). However, because of large increases in cell size, the Na current density remained essentially unchanged (Fig. 3*D*), consistent with the lack of change in Na current density in wild-type PC12 cells after 1 d treatment (data not shown). NGF treatment of the 6-15 and 6-24 cell lines for 7 d resulted in further increases in the proportion of cells (11/12 and 22/22, respectively) with appreciable Na currents, as well as significant ($p < 0.01$) increases in average Na current magnitude, cell size, and Na current density (Fig. 3*D*). Although the Na current densities in both sublines were comparable to those detected in wild-type PC12 cells, the Na current density in the NGF-treated 6-24 cells was lower than in NGF-treated 6-15 cells, presumably due to the extremely large increase in cell size that occurred in this PC12 subline (Fig. 3). Despite this variability, the overall results suggest that neither the rate nor the extent of induction of functional Na channels was appreciably influenced by overexpression of the trkA receptor.

Differential regulation of type II Na channel expression in PC12 cells by insulin, EGF, and NGF

Insulin, EGF, and NGF mediate their effects on PC12 cells through tyrosine kinase receptors, yet have clearly different biological effects, with insulin and EGF promoting growth and proliferation and NGF causing cessation of cell division and neuronal differentiation (for discussion, see Saltiel and Decker, 1991; Chao, 1992b). To determine whether the difference in the biological effects of these factors includes differences in the regulation of Na channel expression, we compared the effects of insulin, EGF, and NGF on Na channel expression in PC12 cells. The two different sublines of wild-type PC12 cells used in the analysis responded to all three factors, as indicated by increases in c-fos mRNA (Fig. 4*A*) and/or ornithine decarboxylase (ODC) activity (Fig. 4*B*). In addition, treatment of the wild-type PC12 cells with insulin elicited a rapid increase in the tyrosine phosphorylation of both the β-subunit of the insulin receptor and a key insulin receptor substrate (IRS-1) (Fanger, Kuhne, Lienhard, and Maue, unpublished observations). The results were consistent with previous studies (Huff et al., 1981), including those using PC12 cells expressing roughly equivalent numbers of functional receptors for these factors (Ohmichi et al., 1993).

To determine if the differential effects of insulin, EGF, and NGF included corresponding differences in Na channel mRNA expression, wild-type PC12 cells treated with these factors were analyzed using two different methods and two different probes for Na channel mRNA. In addition, because it has been postulated that the transient nature of the cellular responses to EGF receptor activation may be the basis for the differences in the actions of EGF and NGF (Heasley and Johnson, 1992; Qiu and Green, 1992), Na channel mRNA levels were analyzed in cells treated over a time course of 1–7 d. Northern blot analysis was carried out as in our previous studies (Ginty et al., 1992; Fanger et al., 1993), using a ^{32}P-labeled antisense probe that corresponds to a coding region highly conserved among Na channel genes (Mandel et al., 1988) and recognizes multiple types of Na channel mRNA (Cooperman et al., 1987; Sills et al., 1989). In response to NGF, there was a clearly detectable increase in Na channel mRNA by the earliest time assayed (24 hr), with a steady increase in Na channel mRNA throughout the time period investigated (Fig. 5*A*). In contrast, insulin did not cause an increase in Na channel mRNA (data not shown), while EGF caused only a slight increase in Na channel mRNA that gradually developed over the period of treatment (Fig. 5*A*). RNase protection assays were then used to analyze RNA from NGF-, insulin-, and EGF-treated cells, using previously developed procedures and a ^{32}P-labeled probe specific for type II Na channel mRNA (Fanger et al., 1993). Consistent with the results from the Northern blot hybridizations, NGF caused an increase in type II Na channel mRNA that was detectable at the earliest time assayed (24 hr) and gradually increased throughout the NGF treatment (Fig. 5*B*), while insulin failed to elicit an increase (data not shown), and EGF caused only a relatively small increase in type II Na channel mRNA over the time period investigated (Fig. 5*B*). The average results from several independent experiments are shown in Figure 6, where the Na channel mRNA signals in the RNase protection assays and Northern blot hybridizations were quantitated by densitometry, normalized to the signals representing cyclophilin mRNA, and then plotted relative to the level of Na channel mRNA in the untreated cells. As shown, the results obtained using both assays were virtually identical. In wild-type PC12 cells, NGF treatment caused a significant ($p < 0.05$) increase in Na channel mRNA by 1 d (2.1–2.3-fold), which gradually increased by 7 d (4.2–4.6-fold) and remained significantly different ($p < 0.01$) from levels found in untreated cells (Fig. 6). This contrasts to insulin treatment, which did not cause a detectable increase at any time,

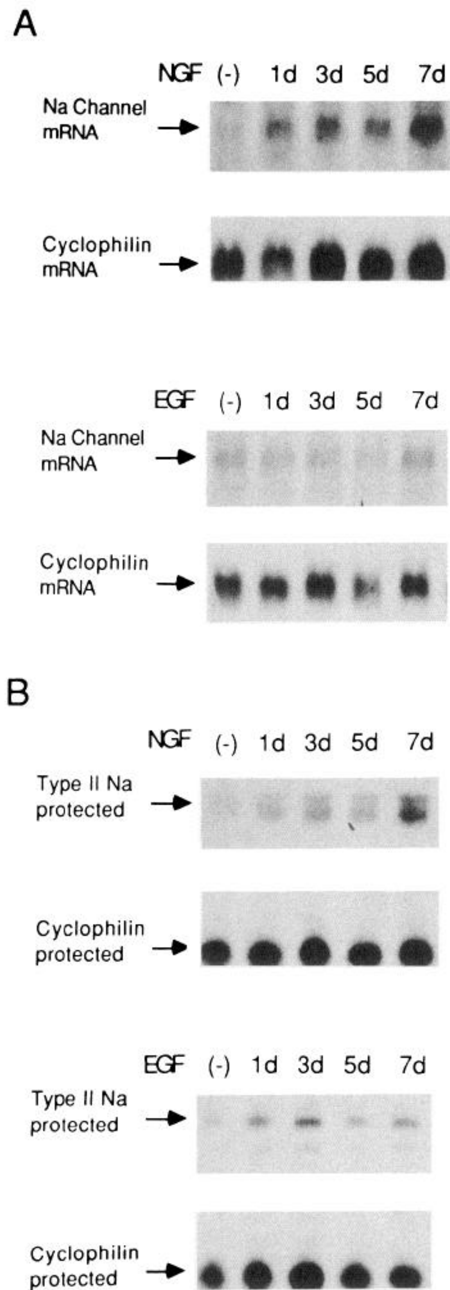

Figure 5. Time course of induction of Na channel mRNA in wild-type PC12 cells treated with NGF or EGF. Samples of RNA from cells that were either untreated (−) or treated with 100 ng/ml NGF or 100 ng/ml EGF for 1–7 d were analyzed using Northern blot hybridization (*A*, 40 μg samples) and RNase protection assays (*B*, 20 μg samples). In *B*, probes specific for type II Na channel mRNA and for cyclophilin mRNA were used, with the signals representing cyclophilin mRNA, which is constitutively expressed in PC12 cells and unaffected by growth factor treatment, used as an internal control for variations in RNA loading (see Materials and Methods). Only the signals representing the protected mRNA fragments are shown. In both *A* and *B* representative experiments are shown, with signals representing Na channel mRNA shown after 24 hr exposure to film and signals representing cyclophilin mRNA shown after 30 min exposure to film.

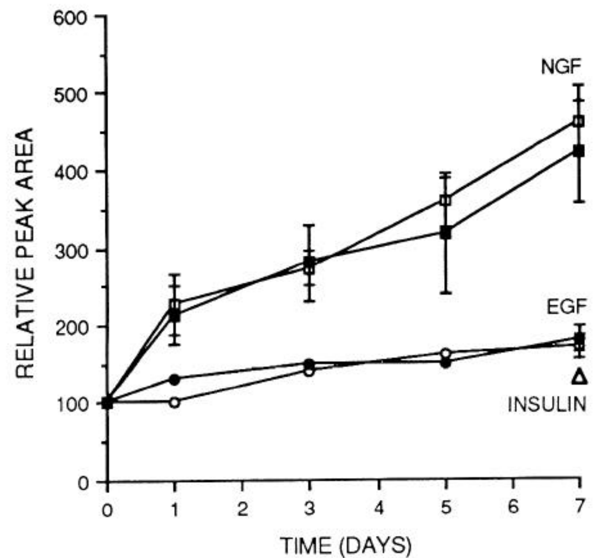

Figure 6. Relative time course of induction of Na channel mRNA in wild-type PC12 cells treated with NGF, EGF, or insulin. Na channel mRNA in cells treated with NGF (*squares*), EGF (*circles*), or insulin (*triangles*), was detected by Northern blot hybridization (*open symbols*) or by RNase protection assay using a type II Na channel specific probe (*solid symbols*). The signals were quantitated by densitometry, normalized to the signals representing the constitutively expressed cyclophilin mRNA to correct for variations in RNA loading, and then plotted relative to the level of Na channel mRNA in untreated cells. The average results from three separate experiments are shown, with error bars (representing the SEM) shown where the levels of expression were significantly greater than detected in untreated cells.

and treatment with EGF, which caused a gradual increase in Na channel mRNA that was significantly different ($p < 0.01$) from the levels in untreated cells only after treatment for 7 d, when an ~1.7-fold induction was detected (Fig. 6). Thus, both methods detected differential effects of these factors on Na channel mRNA expression and slight, yet detectable effects of EGF on Na channel mRNA levels. Furthermore, the close agreement between the changes in Na channel mRNA levels detected in the Northern blot experiments and the changes in type II Na channel mRNA levels detected in the RNase protection assays also indicated that the induction of type II Na channel mRNA could account for the increase in Na channel mRNA in PC12 cells during prolonged exposure to NGF and EGF.

To determine if the changes in Na channel mRNA in response to NGF and EGF were accompanied by corresponding changes in the expression of functional Na channels, whole-cell patch clamp recordings were used to measure the effects of EGF on Na current density in the wild-type PC12 cells used in the analysis of Na channel mRNA. In two different wild-type lines, there were relatively low levels of Na channel expression in the untreated cells, as indicated by the proportion of cells with appreciable (>50 pA) Na currents (9/20, 10/26), the average magnitude of the Na currents, and the Na current density (Fig. 7*A*). After treatment with 100 ng/ml NGF for 7 d, there was the expected increase in the proportion of cells with appreciable currents (21/21, 23/23), as well as significant ($p < 0.01$) increases in the average magnitude of the Na currents, cell size, and Na current density, the latter increasing ~5–6-fold in both sublines (Fig. 7*A*). In response to 100 ng/ml EGF for 7 d, there was an intermediate increase in the proportion of cells with appreciable Na currents (16/21, 14/21) and a 2.0–2.5-fold increase in the average magnitude of the Na currents. Coupled with only a slight increase in cell size (as indicated by cell membrane capacitance), this resulted in a 2.0–2.5-fold increase in Na current density in

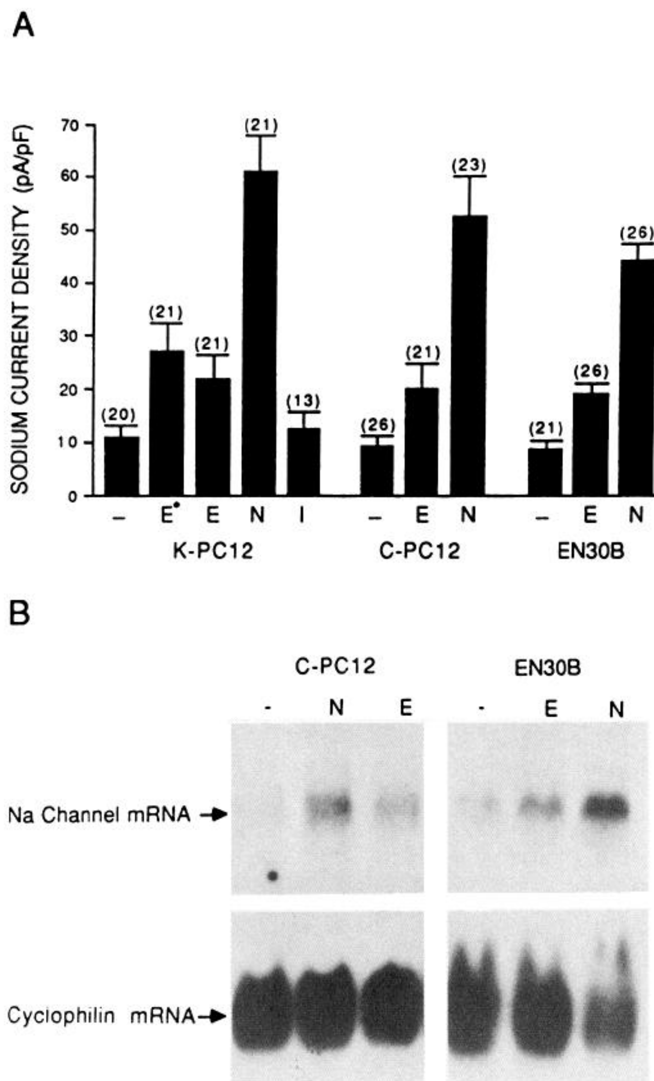

Figure 7. Na channel expression in wild-type PC12 cells and PC12 cells expressing an EGF receptor/p75 chimeric receptor. *A*, Average Na current density in wild-type PC12 subclones (*K-PC12, C-PC12*) and PC12 cells expressing an EGF receptor/p75 chimeric receptor (*EN30B*). Cells were either untreated (–) or treated for 7 d with 10 ng/ml EGF (*E**), 100 ng/ml EGF (*E*), 100 ng/ml NGF (*N*), or 300 nM insulin (*I*). The number of cells in each group is in parentheses above the bars. Error bars represent the SEM. Cell membrane capacitance (mean ± SEM) was 9.2 ± 0.5 pF for untreated, 9.3 ± 0.6 pF for EGF-treated (100 ng/ml), and 22.1 ± 1.2 pF for NGF-treated C-PC12 cells, 8.2 ± 0.8 pF for untreated, 9.2 ± 0.4 pF for EGF-treated (100 ng/ml), 9.1 ± 0.5 pF for EGF-treated (10 ng/ml), 18.7 ± 1.4 pF for NGF-treated, and 7.6 ± 0.6 pF for insulin-treated K-PC12 cells, 13.0 ± 0.8 pF for untreated, 23.2 ± 1.2 pF for EGF-treated, and 25.0 ± 1.3 pF for NGF-treated EN30B cells. *B*, Northern blot hybridization of total RNA (40 μg) isolated from wild-type PC12 cells or EN30B cells. Cells were untreated (–), or treated for 7 d with 100 ng/ml EGF (*E*) or 100 ng/ml NGF (*N*). Signals representing Na channel mRNA are shown after 24 hr exposure to film; signals representing cyclophilin mRNA are shown after 30 min exposure to film.

both sublines in response to EGF (Fig. 7*A*), clearly less than that induced by NGF and consistent with previous findings (Pollock et al., 1990). A similar increase in the proportion of cells with appreciable Na currents (16/21), Na current magnitude, and Na current density was also observed in cells treated with 10 ng/ml EGF (Fig. 7*A*). The results were not limited to wild-type PC12 cells or the presence of a particular type of serum in the culture medium, since in a stably transfected subline of PC12 cells maintained in medium containing neomycin and different serum components (see Materials and Methods), the proportion of EGF-treated cells with appreciable Na currents (14/23) was again between that found in untreated cells (10/25) and in NGF-treated cells (22/22), and there were ~2-fold increases in Na current magnitude and Na current density (see Fig. 10). The magnitude of these inductions was similar to the increases detected in the analysis of Na channel mRNA, and suggests that the modest increase in Na current density in PC12 cells that occurs in response to EGF can be accounted for by the relatively modest increase in type II Na channel mRNA expression.

In contrast to the effects of EGF and NGF, exposure to insulin for 7 d did not appear to increase Na channel expression in PC12 cells. In wild-type cells, insulin did not increase the proportion of cells with appreciable Na currents (6/13) when compared to untreated cells (9/20), nor did it cause increases in cell size, Na current magnitude, or Na current density (Fig. 7*A*). This was not unique to the culture medium or type of serum used, as insulin was also ineffective in increasing Na channel expression in a subline of PC12 cells maintained under slightly different culture conditions (see Fig. 10).

In addition to differences in the specific tyrosine kinase receptors with which NGF and EGF interact, NGF, but not EGF, can also interact with a 75 kDa transmembrane glycoprotein (p75) that is expressed in PC12 cells and many subpopulations of neurons (Johnson et al., 1986; Buck et al., 1987; Radeke et al., 1987). Although the role of p75 in NGF function is not clearly defined (for discussion, see Chao, 1992a; Meakin and Shooter, 1992), we made an initial investigation into the impact of this difference on Na channel regulation by analyzing a subline of PC12 cells (EN30B) expressing chimeric receptors consisting of the ligand binding domain of the EGF receptor and the transmembrane and cytoplasmic domains of the p75 receptor (Yan et al., 1991). In these cells, the response to EGF more closely resembles the response to NGF, including increases in neurite outgrowth and transin gene expression (Yan et al., 1991). In our experiments, the cells responded as expected, with neurite outgrowth observed in response to EGF as well as NGF (data not shown). In addition, when treated with NGF for 7 d, the proportion of EN30B cells with appreciable Na currents (>50 pA) increased (from 10/21 to 26/26) and there was a significant ($p < 0.01$) increase in the average magnitude of the Na currents and average Na current density (Fig. 7*A*). In contrast, when treated with EGF for 7 d the average Na current density in the EN30B cells was very similar to that in wild-type PC12 cells treated with EGF, and the density relative to untreated and NGF-treated EN30B cells was similar to the relative increases observed in wild-type PC12 cells (Fig. 7*A*). Furthermore, when Na channel mRNA expression was analyzed using Northern blot analysis, the increase in Na channel mRNA in EN30B cells treated with EGF (1.8-fold) was similar to that in wild-type PC12 cells treated with EGF (1.7-fold), rather than comparable to the NGF-mediated increases in EN30B (4.5-fold) and wild-type cells (4.1-fold) (Fig. 7*B*). Therefore, coexpression of the chimeric receptor did not elevate the level of Na channel expression induced by EGF to the level induced by NGF, and suggests that the difference in the ability of NGF and EGF to interact with p75 does not account for the difference in the ability of these two factors to induce Na channel expression.

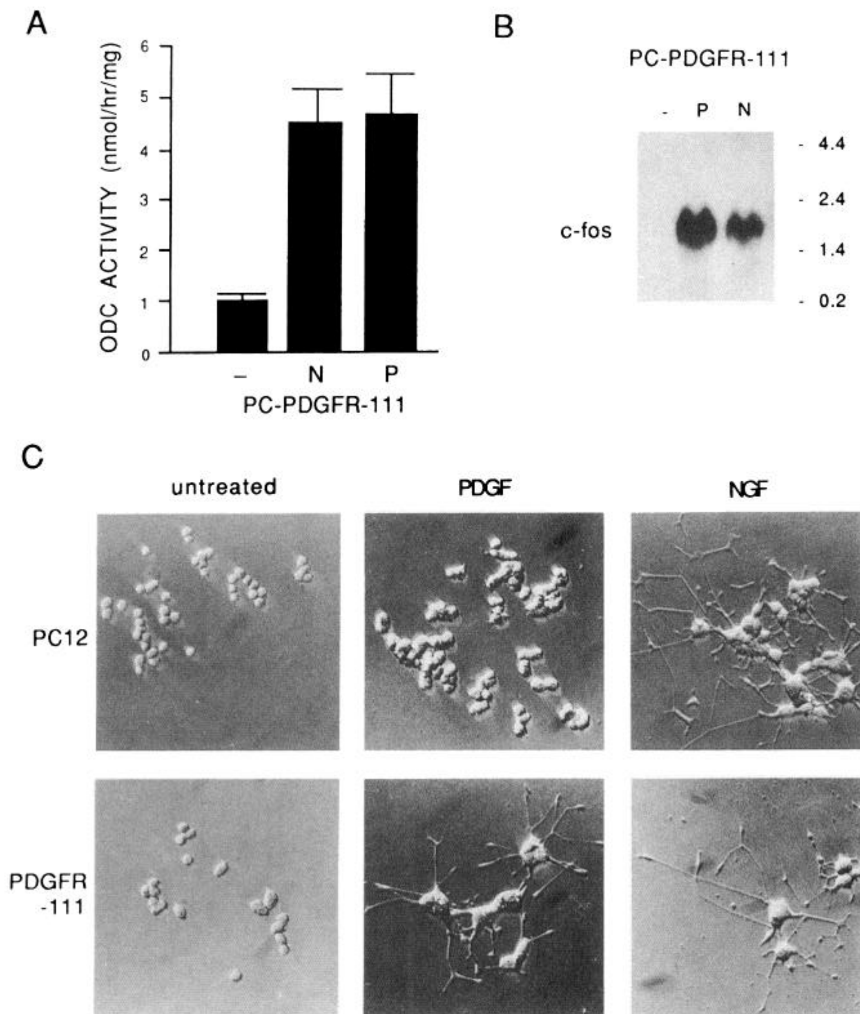

Figure 8. Responsiveness of PC12 cells stably expressing PDGF receptors to PDGF and NGF treatment. *A*, Ornithine decarboxylase (*ODC*) activity in PC-PDGFR-111 cells either untreated (−), treated with 100 ng/ml NGF (*N*), or treated with 30 ng/ml PDGF (*P*) for 5 hr. *B*, Northern blot analysis of c-fos mRNA expression in PC-PDGFR-111 cells either untreated (−) or treated for 30 min with 30 ng/ml PDGF (*P*) or 100 ng/ml NGF (*N*). The position and the molecular sizes (kb) of a commercially available RNA ladder (Bethesda Research Labs) are shown on the *right*. *C*, Photographs of PC-NEO-102 (*PC12*) and PC-PDGFR-111 cells either untreated or treated for 7 d with 30 ng/ml PDGF or 100 ng/ml NGF. The photographs show that while both sublines of PC12 cells extend neurites in response to NGF, only the PC-PDGFR-111 subline does so in response to PDGF. Cell bodies in the untreated PC12 cells are approximately 15 μm in diameter.

PDGF can regulate type II Na channel expression in PC12 cells expressing β-PDGF receptors

There is evidence to suggest that PDGF has a neurotrophic role during development of the nervous system (Smits et al., 1991). Although wild-type PC12 cells do not express PDGF receptors, if β-PDGF receptors are stably expressed in PC12 cells, the PDGF-mediated cessation of cell division, persistent MAP kinase activation, and neurite outgrowth are similar to that observed in the cells in response to NGF (Heasley and Johnson, 1992). In order to determine whether this apparent neuronal differentiation in response to PDGF includes an increase in Na channel expression, we analyzed two different PC12 sublines that stably express a cDNA encoding full length β-PDGF receptors (PC-PDGFR-102 and PC-PDGFR-111), as well as a subline of the parental PC12 cells stably transfected with an expression vector lacking PDGF receptor cDNA that simply confers neomycin resistance (PC-NEO-102). Analysis of this latter subline served as a control for the effects of transfection, the exposure to the antibiotic neomycin, and the effects of PDGF on wild-type cells. Indication that the PC-PDGFR-102 and PC-PDGFR-111 cells responded to our PDGF treatment included PDGF-mediated increases in ODC activity and c-fos mRNA that were comparable to that induced by NGF (Fig. 8*A*,*B*). In addition to these new findings, we also found that PDGF elicited neurite outgrowth in these cells comparable to that elicited by NGF (Fig. 8*C*), consistent with previous observations (Heasley and Johnson, 1992), and in contrast to the total lack of effect of PDGF on the PC-NEO-102 cells (Fig. 8*C*).

To determine if there was a change in type II Na channel gene expression in the PC-PDGFR-111 and PC-PDGFR-102 cells in response to PDGF, RNA samples from these cells were analyzed in RNase protection assays using a probe specific for type II Na channel mRNA (Fig. 9). In control experiments with PC-NEO-102 cells, exposure to NGF, but not PDGF, for 7 d increased the levels of type II Na channel mRNA (Fig. 9). In the PC-PDGFR-111 cells, exposure to PDGF for 7 d caused an increase in type II Na channel mRNA (Fig. 9) that was comparable to the response to NGF (5.4-fold vs 4.7-fold). Similar increases in type II Na channel mRNA were also detected when the PC-PDGFR-102 subline was treated with PDGF or NGF (data not shown).

To determine if the changes in Na channel gene expression in the PC-PDGFR-111 and PC-PDGFR-102 cells were accompanied by changes in functional Na channel expression, whole-cell patch-clamp recordings were used to compare the effects of NGF and PDGF on the expression of functional Na channels in the PC-PDGFR-102, PC-PDGFR-111, and PC-NEO-102

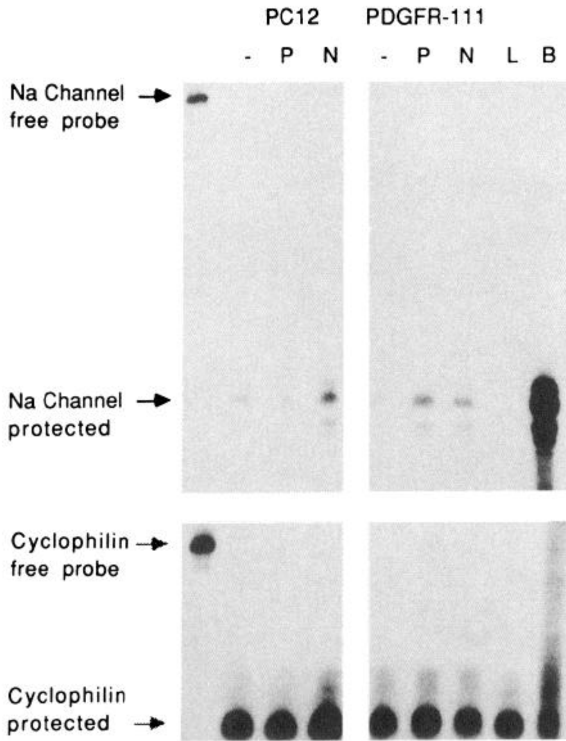

Figure 9. RNase protection analysis of the growth factor–mediated induction of type II Na channel mRNA in neomycin-resistant PC12 cells and PC12 cells stably expressing PDGF receptors. PC-NEO-102 cells (*PC12*) and the PC-PDGFR-111 subline of PC12 cells (*PDGFR-111*) were either untreated (−) or treated for 7 d with 30 ng/ml PDGF (*P*) or 100 ng/ml NGF (*N*). RNA samples from these cells, as well as RNA from rat liver (*L*) and rat brain (*B*), were analyzed using a 219 bp probe specific for type II Na channel mRNA, which protects a 128 bp fragment, and a 62 bp probe specific for cyclophilin mRNA, which protects a 37 bp fragment (see Fanger et al., 1993).

cells. As expected for the PC-NEO-102 control subline of cells, exposure to PDGF for 7 d had no effect on Na channel expression in these cells (Fig. 10*A,C*), while treatment with NGF for 7 d caused the proportion of cells with appreciable Na currents (>50 pA) to increase (from 2/10 to 9/10) and caused a significant ($p < 0.01$) increase in the magnitude of the Na currents and the average Na current density (Fig. 10*A,C*). In contrast, PDGF caused dramatic increases in Na channel expression in both the PC-PDGFR-111 and PC-PDGFR-102 cells. The proportion of PDGF-treated PC-PDGFR-111 and PC-PDGFR-102 cells with appreciable Na currents (23/25, 22/22) was similar to that observed in response to NGF treatment (22/22, 22/22), and the increases in the magnitude of the Na currents, average cell size, and average Na current density were not only significant ($p < 0.01$), but were also comparable to those elicited in the cells by NGF (Fig. 10*B,C*). The effects of PDGF were significantly ($p < 0.01$) greater than the effects of EGF or insulin on these cells, and the increased Na channel expression in PDGF-treated cells was evident when different types of serum were used in the culture medium (Fig. 10*C*). The five- to sixfold inductions in Na current density in response to PDGF were similar in magnitude to the PDGF-mediated induction of type II Na channel mRNA in these cells, and provided further indication that activation of PDGF receptors in these cells can induce Na channel expression.

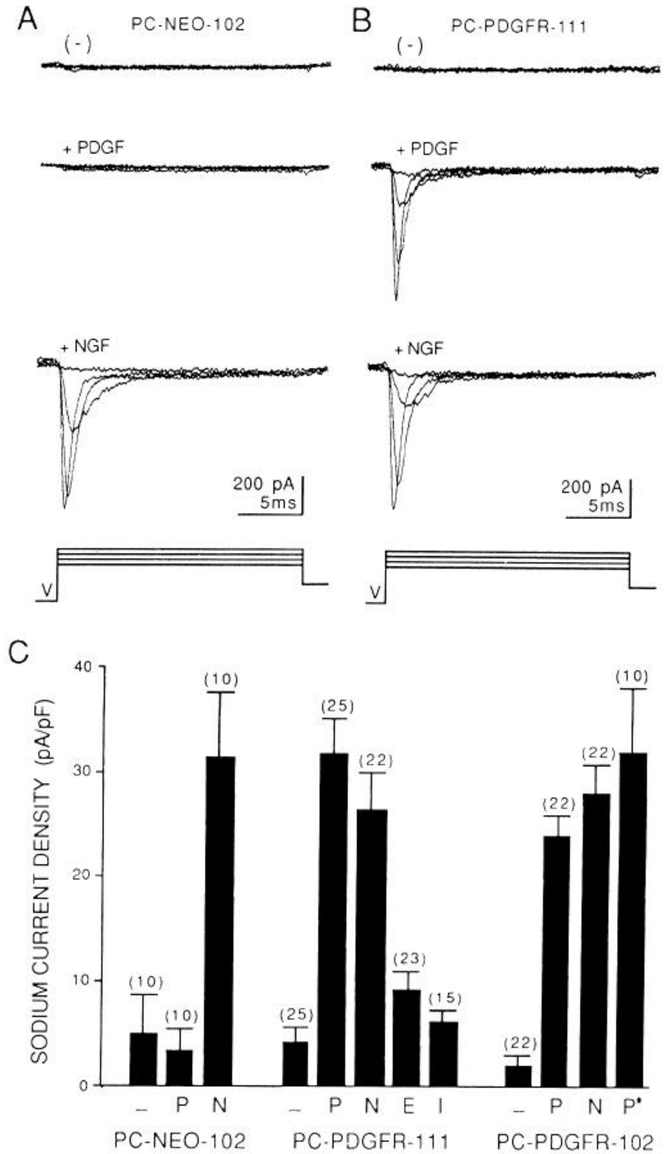

Figure 10. Na current density in PC12 cells stably expressing PDGF receptors. Representative recordings of voltage-dependent Na currents in PC12 cells stably expressing a gene conferring neomycin resistance (*A, PC-NEO-102*) and PC12 sublines stably expressing PDGF receptors (*B, PC-PDGFR-111*). Cells were either untreated (−) or treated for 7 d with 30 ng/ml PDGF or 100 ng/ml NGF. Superimposed current records illustrate the response to depolarizing pulses to −40, −30, −20, and −10 mV, from a prepulse potential of −120 mV and holding potential of −80 mV. *C*, Average Na current density in PC-NEO-102, PC-PDGFR-111, and PC-PDGFR-102 cells that were either untreated (−) or treated for 7 d with 30 ng/ml PDGF (*P*), 100 ng/ml NGF (*N*), 100 ng/ml EGF (*E*), 300 nM insulin (*I*), or 30 ng/ml PDGF in culture medium containing the serum routinely used to culture PC12 cells, rather than the plasma-derived, relatively PDGF-free serum (*P**). The number of cells in each group is in parentheses above the bars. Error bars represent the SEM. Cell membrane capacitance (mean ± SEM) was 5.8 ± 0.2 pF for untreated, 5.3 ± 0.2 pF for PDGF-treated, and 10.9 ± 0.9 pF for NGF-treated PC-NEO-102 cells, 9.1 ± 0.5 pF for untreated, 20.5 ± 1.8 pF for PDGF-treated, 18.4 ± 0.8 pF for NGF-treated, 10.0 ± 0.5 pF for EGF-treated, and 10.5 ± 0.5 pF for insulin-treated PC-PDGFR-111 cells, 8.4 ± 0.5 pF for untreated, 23.6 ± 2.8 pF for PDGF-treated, 18.2 ± 1.4 pF for NGF-treated, and 21.8 ± 2.8 pF for PDGF-treated (normal serum) PC-PDGFR-102 cells.

Discussion

There is considerable interest in the actions of growth factors and their receptors, from understanding the signal transduction mechanisms they utilize, to defining the cellular responses they govern and biological roles they play in nervous system development. However, in previous studies investigating the abilities of these receptors to elicit neuronal differentiation, the number of neuronal-specific responses examined has been small, and often limited to responses dependent on growth factor-induced *ras* activity. Therefore, to further delineate the capabilities of various receptor tyrosine kinases (RTKs) to induce neuronal differentiation, we have determined their ability to induce Na channel expression in PC12 cells. We demonstrate for the first time that activation of either trkB or trkC is capable of inducing type II Na channel mRNA expression in PC12 cells. The results further define the signaling capabilities of these receptors by showing that in addition to eliciting changes that are *ras*-dependent, such as neurite outgrowth, they can elicit neuronal-specific responses, like Na channel induction, that have been shown to be *ras*-independent (D'Arcangelo and Haleguoa, 1993; Fanger et al., 1993). We have also analyzed the effect of trkA overexpression on the NGF-mediated induction of Na channel expression. When compared to the various responses to NGF that were monitored in the initial characterization of the trkA-overexpressing cells (Hempstead et al., 1992), expression of type II Na channel mRNA appears to be most similar to that of peripherin mRNA, where the induction by NGF was not noticeably enhanced, yet the basal level of expression was slightly elevated. This is in sharp contrast to the augmented induction of transin mRNA. As previously suggested (Hempstead et al., 1992), the relative insensitivity of some late responses and not others may reside in differences in the regulatory mechanisms involved, consistent with the *ras*-dependent induction of transin and *ras*-independent regulation of Na channel expression (D'Arcangelo and Haleguoa, 1993; Fanger et al., 1993).

We also show for the first time that PDGF receptor activation in PC12 cells can induce type II Na channel mRNA and functional Na channel expression, as well as ODC activity and c-fos mRNA. Induction of Na channel expression is the first example of a neuronal-specific gene being induced by PDGF, and when combined with previous results (Heasley and Johnson, 1992) extends the list of responses that can be elicited in PC12 cells by PDGF receptor activation to include increases in ODC activity, c-fos mRNA, MAP kinase activity, neurite outgrowth, and Na channel expression. Thus, as previously suggested (Sasahara et al., 1991; Smits et al., 1991; Eccleston et al., 1993), PDGF may have a more expanded role in the nervous system than previously imagined, with effects not only on glial cells (for review, see Richardson et al., 1990), but on neuronal components of the nervous system as well.

The disparate biological effects of NGF, EGF, and insulin on PC12 cells have led to considerable analysis of the biochemical changes they elicit in an effort to identify mechanisms important to neuronal differentiation (for discussion, see Saltiel and Decker, 1991; Chao, 1992b). Here we demonstrate for the first time that while insulin is unable to induce Na channel expression in PC12 cells, EGF elicits a modest increase in Na channel mRNA that, like the response to NGF, appears to be due largely to an increase in the level of type II Na channel mRNA. This contrasts to the much larger increase in type II Na channel mRNA caused by NGF, which occurs with a time course similar to the NGF-mediated increases in peripherin and neurofilament-L mRNAs (Lindenbaum et al., 1988; Hempstead et al., 1992). This pattern of responses resembles the regulation of GAP-43 expression in PC12 cells, where insulin is ineffective and there is a muted response to EGF in comparison to NGF (Costello et al., 1990). Given the responses were compared in the same neuronal cellular environment, it suggests that mechanisms related to the receptors themselves, rather than cell-specific differences in cellular components, play an important role in determining the response.

Among the features that could account for the differential regulation of Na channel expression by various growth factors are differences in their receptors. For example, evidence suggests that in addition to the trk RTKs, p75 plays a biological role in the response to neurotrophins (Lee et al., 1992; Davies et al., 1993), though its exact role and participation in signal transduction are controversial (for discussion, see Chao, 1992a). However, with regard to Na channel expression in PC12 cells, there are several indications that ligand interactions with p75 probably do not play a role in the growth factor-mediated specificity. First, growth factors that interact with p75, such as NGF and BDNF, as well as those that do not, including bFGF and PDGF, can induce Na channel expression (Pollock et al.,1990; Ginty et al., 1992; present study). Second, as we show here, expression of an EGF receptor/p75 receptor chimera in PC12 cells does not increase the extent to which EGF induces Na channel expression. Finally, a mutant form of NGF that predominantly associates with trkA and not p75 appears sufficient for Na channel induction in PC12 cells (G. Fanger, P. Barker, E. Shooter, and R. Maue, unpublished observations). Upon examination of the RTKs, it is also clear that sequence similarity alone is not indicative of the ability to regulate Na channel expression. For example, based on sequence, the insulin receptor is more homologous to the trk RTKs than the PDGF receptor is (for discussion, see Saltiel and Decker, 1991), yet in PC12 cells PDGF receptor activation can induce many of the same changes as trk receptor activation, while activation of the insulin receptor is ineffective. As a result, although perhaps not surprising, it is of interest to discover that trkB and trkC can induce Na expression in PC12 cells similar to trkA. Identifying receptors with similar capabilities allows common structural features to be identified and analyzed as to their effect on Na channel induction. For example, the large carboxy-terminal tails of the EGF and insulin receptors, which have been implicated in negative regulation of receptor autophosphorylation (for discussion, see Saltiel and Decker, 1991), may suppress sustained signaling necessary for Na channel expression, and could serve as a focus for further analysis.

Differences in growth factor receptors may result in overlapping, yet distinct subsets of signaling mechanisms utilized by individual growth factors (for discussion, see Schlessinger and Ullrich, 1992). Many signaling pathways and intermediates have been implicated in mediating the actions of growth factors, including phospholipid turnover, cAMP, arachidonic acid, and a variety of protein kinases (for review, see Haleguoa et al., 1991). In many cases, signaling components are either coupled to activated receptors through direct interaction, as in the case of phospholipase Cγ and phosphotidylinositol-3-kinase, or indirectly through adapter or linking proteins, as in the case of the *ras*-associated cascade of protein kinases (for reviews, see Schlessinger and Ullrich, 1992; McCormick, 1993). These rapid changes trigger gene expression, through modification and ac-

tivation of preexisting transcription factors (Fu and Zhang, 1993; Silvennoinen et al., 1993) and activation of genes encoding proteins such as c-*fos*, c-*jun*, and *jun*-B (Bartel et al., 1989), which in turn serve as transcription factors (Gizang-Ginsberg and Ziff, 1990; for review, see Haleguoa et al., 1991). Among these changes, receptors that can induce Na channel expression in PC12 cells, including those for NGF, bFGF, and PDGF, cause persistent activation of MAP kinase, whereas receptors for insulin and EGF do not (Heasley and Johnson, 1992; Ohmichi et al., 1993). Furthermore, receptors that can induce Na channel expression can also cause rapid tyrosine phosphorylation of a nuclear protein (SNT), while EGF receptor activation is ineffective (Rabin et al., 1993). For Na channel regulation, the significance of specific signaling components such as these remains to be determined.

In the present study we have shown there are both intrinsic similarities and differences in the ability of growth factors and their RTKs to regulate neuronal Na channel expression. The results suggest that for a number of growth factors there may be important, yet currently unappreciated, roles in regulating Na channel expression during nervous system development and regeneration. With identification of an increasing number of growth factors and receptors within the nervous system (Berkemeier et al., 1991; Ip et al., 1992; Tsoulfas et al., 1993; Valenzuela et al., 1993; for review, see Chao, 1992a), determining the ability of these newly identified molecules to influence Na channel expression will be important for dissecting their cellular modes of action. Furthermore, analysis and comparison of RTKs, including those with alterations in their cytoplasmic domains because of alternative splicing (Klein et al., 1990; Middlemas et al., 1991; Tsoulfas et al., 1993; Valenzuela et al., 1993) or experimentally induced mutations (Kazlauskas and Cooper, 1989; Decker, 1993; Stephens et al., 1994), should allow selective elimination or activation of specific signaling pathways and identification of mechanisms important for Na channel expression and neuronal differentiation.

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