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Differential Effects of NGF, FGF, EGF, cAMP, and Dexamethasone on Neurite Outgrowth and Sodium Channel Expression in PC12 Cells

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PC12 cells are a pheochromocytoma cell line that can be made to differentiate into sympatheticlike neurons by nerve growth factor (NGF). An essential component of the NGF-induced differentiation is the development of action potentials and sodium channels. Using whole-cell clamp we have confirmed that NGF produces a 5- to 6-fold increase in sodium channel density. The sodium channels induced by NGF are not different from those in cells not treated with NGF and are similar to those in other cell types. Basic fibroblast growth factor (FGF), another growth factor that causes PC12 cells to differentiate into sympathetic-like neurons, also produces a 5- to 6-fold increase in sodium current density with channels indistinguishable from those in PC12 cells treated and not treated with NGF. Basic FGF produces the same or somewhat larger increase in sodium channel density but much less neurite outgrowth. In contrast, epidermal growth factor does not produce neurite outgrowth but induces a small, reproducible increase in sodium channel density. Cyclic AMP produces spike-like processes but not neurites and results in a decrease in sodium current and sodium current density. Dexamethasone, a synthetic glucocorticoid, inhibits the increase in sodium current and sodium current density but does not antagonize the neurite outgrowth induced by NGF. Thus, although the increase in sodium channel expression induced by NGF and basic FGF parallels the changes in morphology that lead to neurite outgrowth, it clearly does not depend on them. The results show that different aspects of neuronal differentiation might be independently regulated by the microenvironment.

PC12 cells are a clonal cell line derived from a transplantable pheochromocytoma tumor in New England Hospital Deaconess rat (Greene and Tischler, 1976) with characteristics similar to precursors of adrenal gland cells. When grown in the presence of nerve growth factor (NGF), the cells differentiate into sympathetic-like neurons: The cells stop dividing, sprout neurites, and become electrically excitable (Greene and Tischler, 1976; Dichter et al., 1977; Rudy et al., 1982).

In the course of differentiation of PC12 cells by NGF, a number of early and late events are activated (Greene and Tischler, 1982; Greene, 1984). Early events are characterized by their rapid onset, usually occurring within minutes, whereas late events start to be observed between 24 and 48 hr. Many of the early events are transcriptionally independent, while the late events are frequently transcriptionally dependent. An example of an early event is the rapid change in the surface topography after the addition of NGF. These early morphological events (Connolly et al., 1979, 1984) are temporally associated with hydrolysis of phosphoinositides (Contreras and Guroff, 1987), activation of N-kinase (Rowland et al., 1987), changes in protein phosphorylation (Halegoua and Patrick, 1980; Yu et al., 1980; End et al., 1983; Landreth and Rieser, 1985; Cremins et al., 1986), and increased uptake of nutrients (McGuire and Greene, 1979). Interestingly, a number of early genes are induced by NGF. For example, Greenberg et al. (1985), Curran and Morgan (1985), Kruijer et al. (1985), and Milbrandt (1986) have observed a rapid increase in *c-fos*, a cellular oncogene and a transcription factor (Distel et al., 1987; Curran and Franza, 1988), peaking 15 min after the addition of NGF to PC12 cells. It is possible that *c-fos* acts as an intermediary for the late differentiation of PC12 cells by NGF.

Examples of late events induced by NGF are the outgrowth of neurites, the development of small vesicles that form in varicosities, and the development of electrical excitability. Late events also include the induction of several neuron-specific proteins such as GAP-43 (Basi et al., 1987; Karns et al., 1987), acetylcholinesterase (Lucas et al., 1980; Rieger et al., 1980), SCG-10 (Stein et al., 1988), Thy-1 (Richter-Landsberg et al., 1985; Dickson et al., 1986), NILE (Salton et al., 1983), MAP 1, and Tau (Drubin et al., 1985), 68K and other neurofilament proteins (Lee and Page, 1984; Dickson et al., 1986), VF8a (Levi et al., 1985), synapsin (Romano et al., 1987), and peripherin (Aletta et al., 1988), as well as the induction of a set of inducible clones (Leonard et al., 1987). Key to the development of electrical excitability is the transcriptionally dependent induction of TTX-sensitive sodium channels (Kirschenbaum et al., 1987; Mandel et al., 1988; C. Kentros, T. P. Snutch, M. Berger, et al., unpublished observations). The steady-state levels of sodium channel mRNA reach their peak within 2–3 hr after the addition of NGF but action potentials are not seen until after several days. Moreover, the increase in mRNA levels can be blocked by protein synthesis inhibitors (Kentros, Snutch, Berger, et al., unpublished observations).

Recently, acidic and basic fibroblast growth factor (FGF; see

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Gospodarowicz, 1986, for review) have been found to produce many of the same effects as NGF on PC12 cells (Togari et al., 1985; Wagner and D'Amore, 1986; Neufeld et al., 1987; Rydel and Greene, 1987; Schubert et al., 1987) and on chromaffin cells (Claude et al., 1988; Stemple et al., 1988). In PC12 cells, FGF has been found to induce neurite outgrowth, to produce phosphorylation of tyrosine hydroxylase, to induce MAP 1, Thy-1, and NILE, and to increase acetylcholinesterase activity (Rydel and Greene, 1987). This suggests that NGF and FGF acting at different receptors share some of the same signal-transduction pathways. In contrast, other factors such as epidermal growth factor (EGF) and cAMP lead to some of the early events such as protein phosphorylation and induction of c-*fos* but do not produce morphological differentiation (Connolly et al., 1984; Lee and Page, 1984; Leonard et al., 1987).

The effects of growth factors on PC12 cells are thought to be representative of the events that occur during neuronal differentiation, particularly for cells that derive from the neural crest. Adrenal chromaffin cells and sympathetic neurons are derived from the neural crest. The microenvironment of the neural crest determines whether the cell will become a sympathetic neuron or an adrenal chromaffin cell (Le Douarin, 1980, 1982). In the presence of NGF, the cell becomes a sympathetic neuron and in the presence of glucocorticoids, the cell becomes a chromaffin cell (Landis and Patterson, 1981). Adrenal chromaffin cells treated with NGF have been observed in culture to become sympathetic neurons as late as the neonatal stage (Doupe et al., 1985). The differentiation of neonatal adrenal chromaffin cells into sympathetic neurons by NGF can be antagonized by the synthetic glucocorticoid, dexamethasone (Unsicker et al., 1978; Tischler et al., 1982; Doupe et al., 1985). It follows, therefore, that NGF is activating a set of genes that gives the cell its neuronal phenotype and glucocorticoids act to inhibit these genes. However, in PC12 cells morphological differentiation by NGF is not antagonized by glucocorticoids (Greene and Tischler, 1976), while a number of mRNA transcripts induced by NGF are inhibited (Leonard et al., 1987; Federoff et al., 1988; Stein et al., 1988). This suggests that transcription of gene products involved in neurite outgrowth is no longer under the control of glucocorticoid-responsive elements in PC12 cells. Alternatively, steps subsequent to transcription, controlling steady-state transcript levels and amount of functional protein, are not responsive to glucocorticoids in these cells.

Since induction of sodium channels is a key feature of the late neuronal-like differentiation by NGF, in this paper we address the following questions: (1) Do other agents such as FGF, EGF, and cAMP that produce many of the early events produced by NGF, induce sodium channels either with electrophysiological properties identical to or different from those produced by NGF? (2) Is the increase in sodium channels by NGF as unaffected by dexamethasone as is neurite outgrowth? (3) To what degree does sodium channel induction correlate with morphological differentiation? These studies are important to understand the mechanisms that regulate sodium channel expression and suggest factors that may regulate neuronal excitability *in vivo*. The comparison of the effects of different agonists that influence PC12 cells may shed light on the control and coordination of different components of neuronal differentiation, e.g., neuronal excitability and morphological differentiation. Such investigations may lead to suggestions about specific intracellular messengers that regulate individual aspects of the neuronal phenotype.

Materials and Methods

Tissue culture. PC12 cells were cultured according to the method described by Greene et al. (1987). Briefly, PC12 cells, passage 29–45, were grown in RPMI with 10% horse serum heat inactivated at 56°C, 5% fetal calf serum, 25 U/ml penicillin, and 25 μg/ml streptomycin on rat tail collagen in 35-mm petri dishes (Falcon). The cells were fed every other day and passaged about once every week. These culture conditions were maintained when 300 μM 8-chlorophenylthio-cyclic adenosine monophosphate (8-CPT-cAMP, Boehringer Mannheim), 10 ng/ml EGF (generous gift of Frederick Maxfield, Dept. of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY), or 5 μM dexamethasone (Sigma, St. Louis, MO) was added. When cells were treated with 50 ng/ml NGF [2.5 S NGF was purified from mouse submaxillary gland according to the method of Mobley et al. (1976)] or 10 ng/ml bovine basic FGF (generous gift of Barbara Wold, Division of Biology, Caltech), the cells were grown in RPMI supplemented with 1% horse serum unless otherwise stated. We confirmed that NGF increased sodium current and sodium current density by the same amount in cells grown in either 10% horse serum and 5% calf serum or just 1% horse serum.

Electrophysiology. PC12 cells were patch-clamped in either the whole-cell or outside-out configuration, according to the method of Hamill et al. (1981) using an EPC-7 patch clamp (List Electronics, Greenville, NY) on a Nikon Diaphot inverted microscope (Garden City, NY) fitted with Hoffman interference contrast optics. The current signals from the patch clamp were filtered at 2200 Hz with a low-pass 8-pole Bessel filter (Frequency Devices, Haverhill, MA). The experimental protocol and data acquisition were run on an IBM XT or AT computer using PClamp software (Axon Instruments, Burlingame, CA). When recording, the cells were bathed in a saline solution containing (in mM) 140 NaCl, 2.8 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 Hepes-NaOH, pH 7.2. To block outward currents the pipette solution consisted of (in mM) 120 CsCl, 20 TEACl, 1 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, 10 Hepes-KOH, pH 7.2. The pipettes were pulled from VWR 100 lambda pipettes made of borosilicate glass and typically had resistances of 1–4 MΩ. Just prior to obtaining a gigaseal, the junction potential was zeroed. Membrane area was estimated from measurements of cell capacitance, assuming a specific capacitance of 1 $\mu F/cm^2$. The capacitance was measured from the integral of the capacity transient for a 10 mV depolarizing pulse from a holding potential of -90 mV.

Results

NGF and FGF produce neurite outgrowth and induce sodium channels

Figure 1 compares the effect of NGF and basic FGF on morphology with the amount of sodium current present in cells treated with the factors for 0, 2, and 10 d. By 2 d, cells treated with either NGF or basic FGF have begun to develop neurites and show an increase in sodium current. The magnitude of the sodium current as well as the density and length of neurites continues to increase as seen at 10 d of treatment with NGF or basic FGF. As shown in Figure 1, the density of neurites is much higher in NGF-treated cells than in basic FGF-treated cells. In addition, after 10 d of treatment, the neurites of cells treated with basic FGF are somewhat narrower and straighter than those in cells treated with NGF (see also Rydel and Greene, 1987). Although on average the sodium current in NGF-treated cells is somewhat greater than in cells treated with basic FGF (2194 $\pm$ 373 pA, mean $\pm$ SEM, $n = 12$, vs 1795 $\pm$ 206 pA, mean $\pm$ SEM, $n = 24$), the sodium current density is a little greater in cells treated with basic FGF than NGF (143 $\pm$ 13 $\mu A/cm^2$, mean $\pm$ SEM, $n = 24$, vs 119 $\pm$ 14 $\mu A/cm^2$, mean $\pm$ SEM, $n = 12$).

Effect of cAMP and EGF on ionic currents and morphology

Figure 2 compares the effects of 2 other agents, cAMP and EGF, on sodium currents and cell morphology. EGF causes an in-

A

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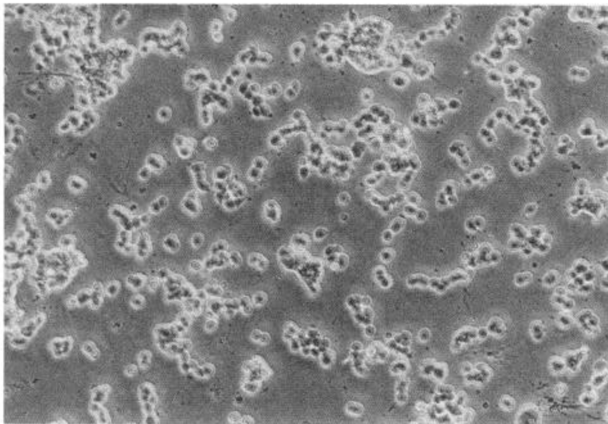

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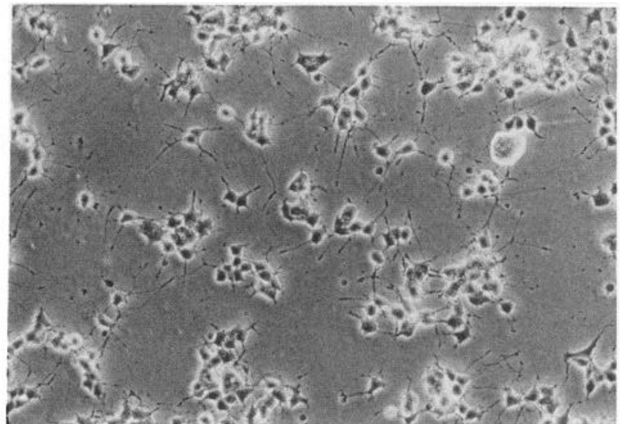

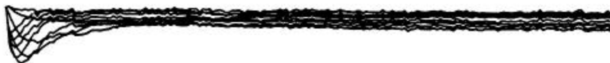

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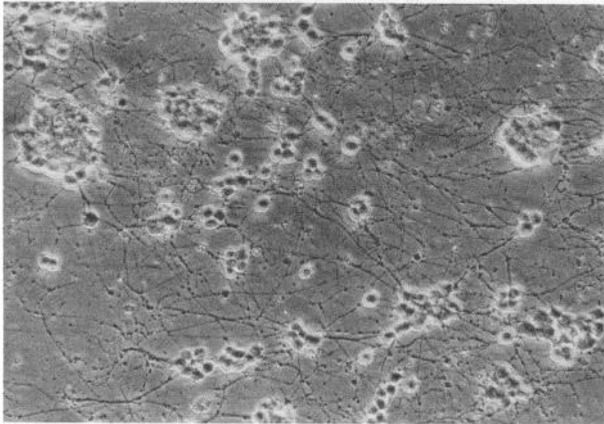

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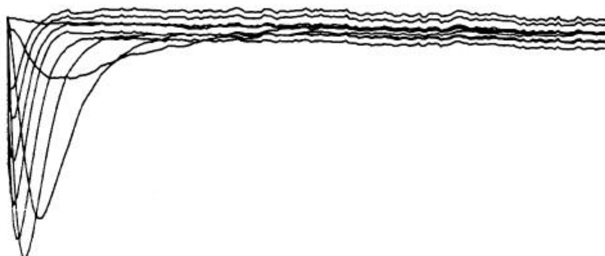

Figure 1. Effect of NGF (*A*) and basic FGF (*B*) on PC12 cell morphology and ionic currents. PC12 cell cultures were treated for 0, 2, or 10 d with NGF or basic FGF as described under Materials and Methods. Records are from sample cells under whole-cell patch clamp after blocking potassium currents as described under Materials and Methods. The cells were held at −90 mV, and depolarizing commands were given in 10 mV increments until +40 mV was reached.

B

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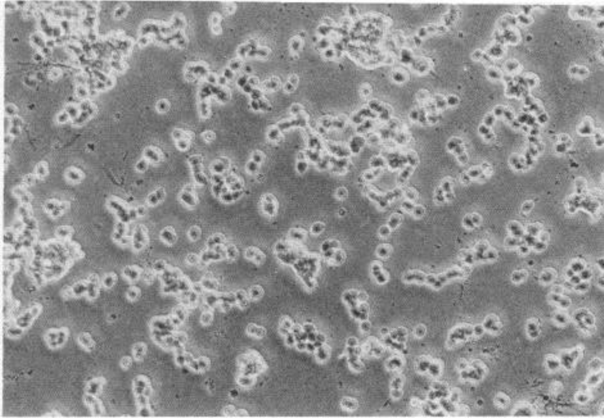

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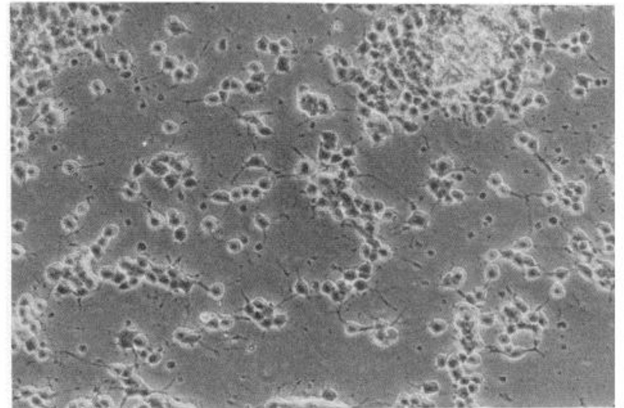

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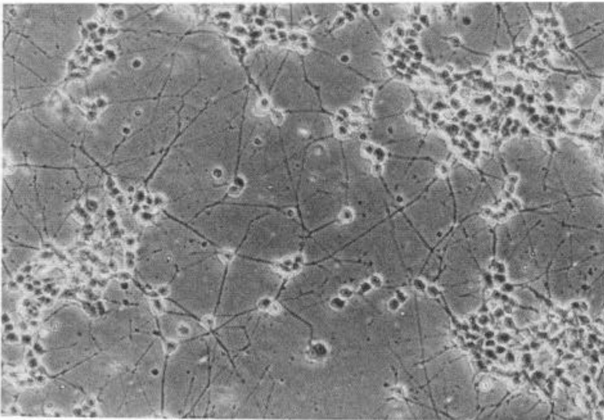

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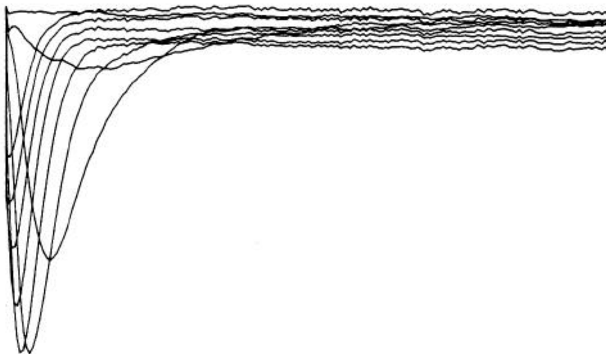

Figure 1. Continued.

A

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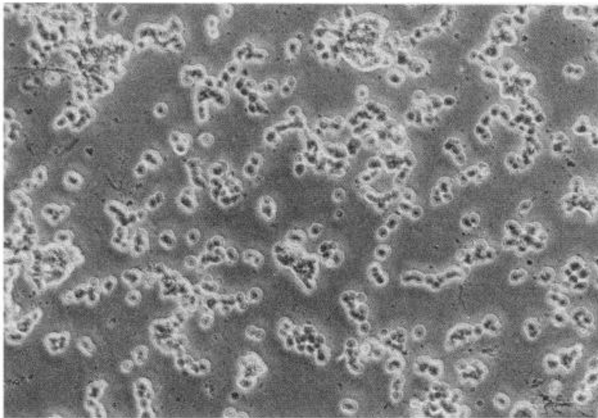

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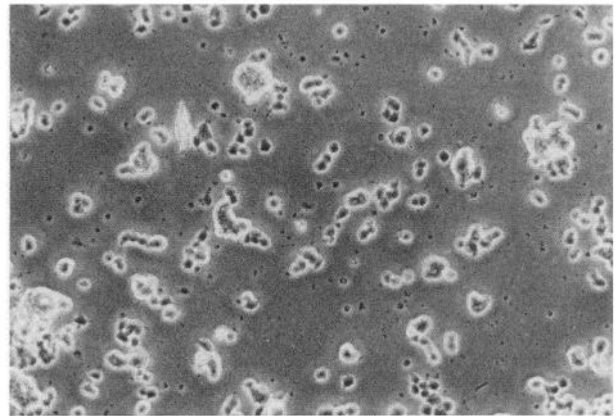

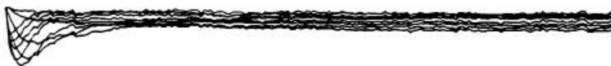

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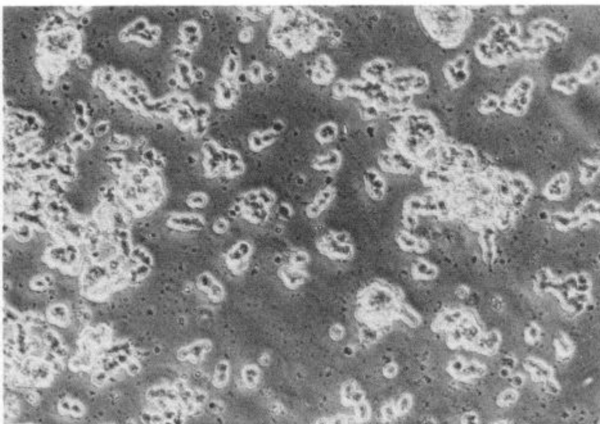

250 pA

1 ms

Figure 2. Effects of EGF (*A*) and cAMP (*B*) on PC12 cell morphology and ionic currents. PC12 cell cultures were treated for 0, 2, or 10 d with EGF or CPT-cAMP as described under Materials and Methods. Records are from sample cells under whole-cell patch clamp after blocking potassium currents as described under Materials and Methods. The cells were held at −90 mV, and depolarizing commands were given in 10 mV increments until +40 mV was reached.

crease in the rate of cell division without any significant change in morphology (Fig. 2*A*). However, there is a modest increase in both sodium current and sodium current density that appears to be time dependent. At 10 d, the sodium current and sodium current density for EGF-treated cells were the same as that produced by NGF and basic FGF at 2 d. On the other hand, cAMP produces only spike-like processes that become apparent at 2 d (Fig. 2*B*). As the spike-like processes appeared, the sodium

B

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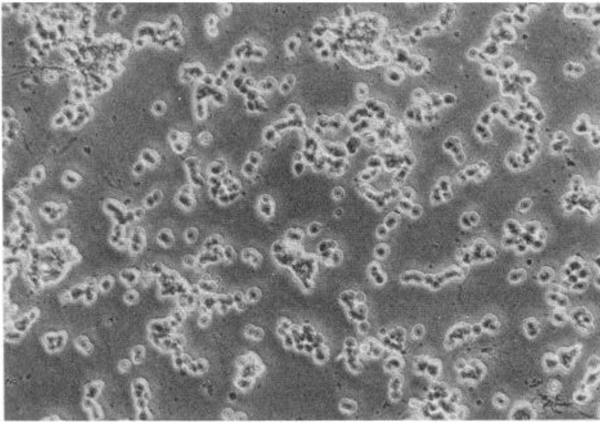

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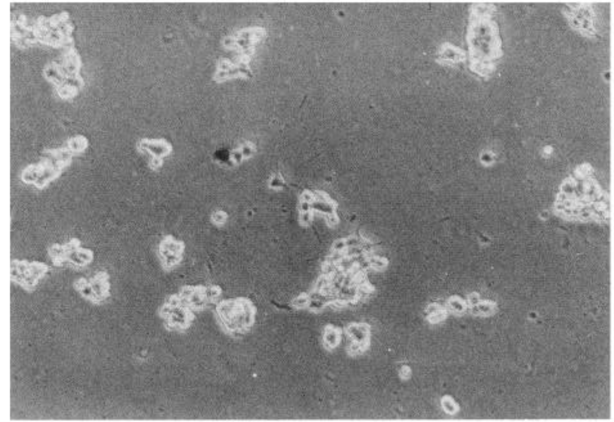

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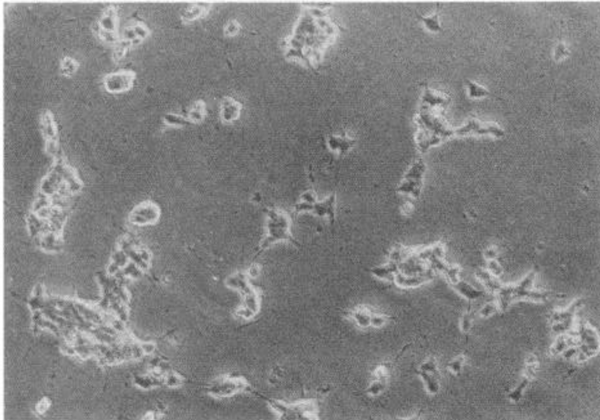

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Figure 2. Continued.

current began to disappear with an increase in calcium current. After a 10 d treatment, sodium currents are undetectable, but there is a significant increase in calcium current. The results of the effects of EGF, cAMP, NGF, and basic FGF on sodium current and sodium current density for several experiments are summarized in Figure 3. As shown in Figure 3, the small time-dependent increase in sodium current and sodium current density with EGF is reproducible.

Electrophysiological properties of sodium channels induced by NGF and FGF

Figures 4 and 5 illustrate the functional properties of sodium channels in untreated and NGF-treated PC12 cells in which potassium currents have been blocked. The amount of sodium current present in NGF untreated cells is quite variable. In 60% of the cells studied thus far (n = 78) sodium currents were not

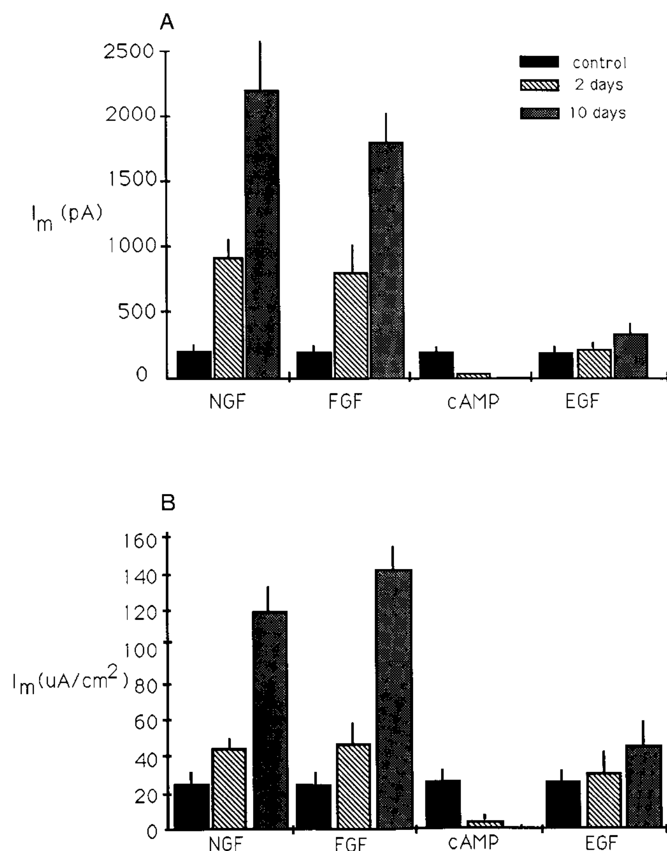


Figure 3. Mean effect of 50 ng/ml NGF (n = 24); 10 ng/ml basic FGF (n = 12); 300 μM CPT-cAMP (n = 11); and 10 ng/ml EGF (n = 13); on the magnitude of the peak sodium current (*A*) and peak sodium current density (*B*). Control cells, n = 23: *Error bars*: SEM.

detected. In the remaining cells, variable amounts of sodium current (20–150 pA of maximum peak sodium current) were observed (Fig. 4, *A–C*). In 3 of the cells studied we have also observed approximately 800 pA of maximum peak sodium current (not shown). In contrast, long-term treatment with NGF (10–14 d, n = 49) results in a more homogeneous cell population with respect to sodium current levels. Almost 100% of the cells exhibit more than 1000 pA of maximum peak sodium current. The amount of sodium current ranges between 900 to 2500 pA. A typical PC12 cell treated with NGF for 2 weeks is shown in Figure 4*D*. The properties of the sodium currents are very similar before and after NGF treatment. The voltage dependence of activation and inactivation (Fig. 5, *A–C*) and the time constants of inactivation (Fig. 5*D*) are all very similar before or after NGF treatment. In a separate set of experiments we compared the kinetics of the sodium current induced by NGF with the sodium current induced by basic FGF. The sodium currents induced by NGF and FGF have similar current–voltage characteristics and steady-state inactivation curves and are both completely blocked by 100 nM TTX (data not shown).

To further analyze the kinetic characteristics of the sodium channels induced by NGF and by FGF, single sodium channel records were obtained. During 25 msec depolarizations from a holding potential of −90 mV, inward channels are observed which activate rapidly and usually inactivate within the first 2 msec. When single-channel records are averaged, the current has kinetic characteristics similar to the macroscopic sodium current. The single sodium channel records show an average conductance of 11 pS, both for NGF- and basic FGF-treated cells, as well as for untreated cells (data not shown).

Dexamethasone suppresses sodium current induction but not neurite growth

We find (Figs. 1, 2) that the degree of neurite outgrowth does not always correlate with the amount of sodium channel induction. Whereas neurite outgrowth is significantly weaker with FGF treatment, sodium channel induction is similar or even greater with FGF than with NGF. Furthermore, EGF produces a small increase in sodium channels but no neurite outgrowth. The effects of dexamethasone on NGF-induced sodium currents and morphology described below also show a dissociation between the regulation of neurite growth and sodium channel expression.

Figure 6 shows that dexamethasone in the presence of NGF does not prevent neurite outgrowth; as shown in Figure 7, however, dexamethasone inhibits the NGF induced increase in sodium current and sodium current density. The small increase in sodium currents (Fig. 7*A*) in the presence of dexamethasone and NGF compared to control cells is due entirely to an increase in membrane area ($13.5 \pm 3.2 \times 10^{-6}$ vs $7.8 \pm 2.1 \times 10^{-6}$ cm^2) and therefore does not reflect an increase in sodium channel density (Fig. 7*B*). No significant effect on either sodium current or sodium current density was observed with 5 μM dexamethasone alone.

Discussion

We have previously shown that except for the appearance of a small population of TTX-resistant sodium channels in PC12 cells grown under certain conditions (Rudy et al., 1987), the sodium channels present in PC12 cells before and after long-term NGF treatment are indistinguishable. The electrophysiological studies shown here confirm and extend these observations. The amount of sodium current found here can account for the quantitative measurements of functional sodium channels or STX binding sites that have been reported in PC12 cell populations (Rudy et al., 1982, 1987). However, as shown here, these numbers reflect the averages of cells with diverse properties, particularly in PC12 cells not treated with NGF. The majority of cells not treated with NGF seem to lack functional sodium channels (Fig. 4). Therefore, in those undifferentiated cells where sodium current is lacking, the size of sodium channel induction by NGF is significantly greater than the average reported for cell populations.

Like NGF, FGF has been found to affect the survival and differentiation of neurons in the central and peripheral nervous systems (reviewed in Logan and Logan, 1986; Barde et al., 1989), and to induce neurite outgrowth in PC12 cells (Togari et al., 1985; Wagner and D'Amore, 1986; Rydel and Greene, 1987; Neufeld et al., 1987; Schubert et al., 1987). This is the first report showing that FGF can also induce sodium channels and thus that it may influence neuronal excitability. The sodium channels induced by FGF have electrophysiological characteristics indistinguishable from those induced by NGF. This suggests that FGF and NGF acting at different receptors (Togari et al., 1985) either share some signal-transduction pathways or have a transduction pathway that converges to activate the same late events, i.e., morphological differentiation and sodium channel induction.

In contrast to NGF and FGF, EGF and cAMP, agents that produce some of the early events of NGF and FGF, produced

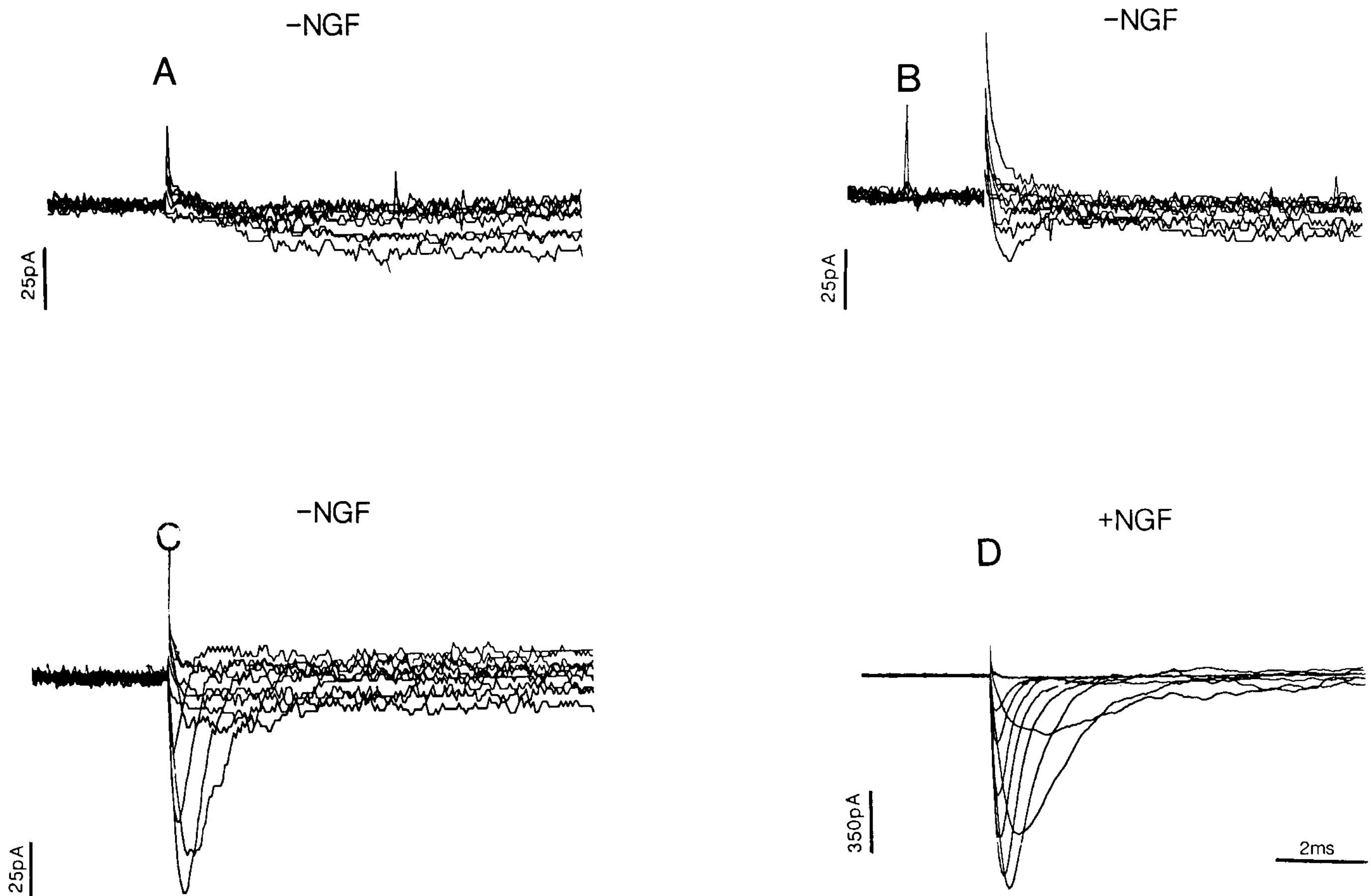


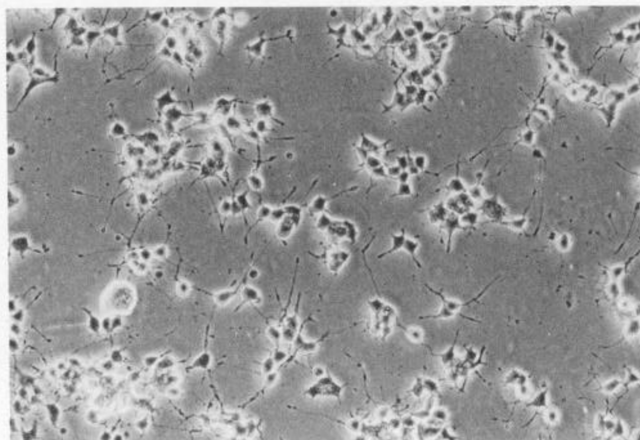
Figure 4. Sodium and calcium currents in PC12 cells. Records obtained in the whole-cell clamp configuration of the patch-clamp technique as described under Materials and Methods. *A–C,* Cells grown in the absence of NGF; *D,* cells grown in the presence of NGF for 14 d. The membrane potential was held at −80 mV, and depolarizing pulses were applied from −50 mV to +50 mV in 10 mV increments. Potassium currents were blocked as described under Materials and Methods.

little or no increase, respectively, of sodium current and sodium current density. Sodium channel mRNA induction by NGF in PC12 cells depends on protein synthesis (Kentros et al., 1990), suggesting that an intermediary protein is required to activate the sodium channel gene. The product of an early immediate gene such as c-*fos* induced by NGF and FGF (Greenberg et al., 1985; Curran and Morgan, 1985; Kruijer et al., 1985; Milbrandt, 1986) might be a good candidate. However, the results of the experiments with EGF and cAMP, agents known to also elevate c-*fos* (Greenberg et al., 1985), suggest that the elevation of c-*fos,* if necessary, is not sufficient by itself to activate sodium channel transcription.

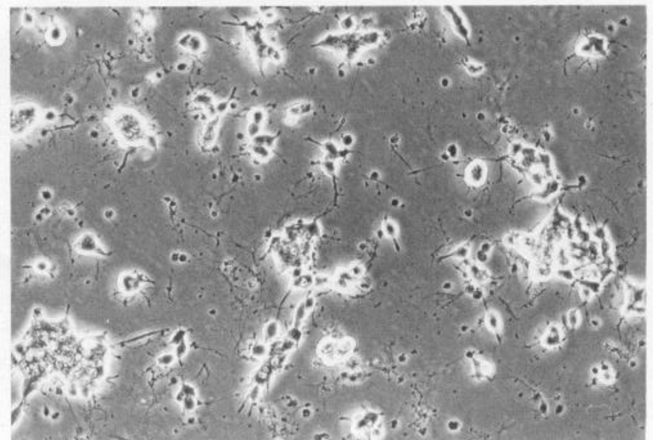
The time course of sodium channel induction by NGF and FGF parallels the time course of morphological differentiation induced by these agents. However, we find that FGF produces a similar or even greater increase in sodium channel density than NGF, despite its much weaker ability to induce neurite outgrowth. We also find that EGF produces a small increase in sodium channel density but no neurite outgrowth. Thus, the same density of sodium channels is found in cells treated with NGF for 2 d, with FGF for 2 d, or with EGF for 10 d. However, the resulting cells differ markedly in morphology, with many, few, and no neurites, respectively. Furthermore, we find that dexamethasone inhibits sodium channel induction but not neurite outgrowth. These results suggest that morphology and sodium channel expression are regulated independently and must involve, at least in part, distinct mechanisms. The sodium channel must belong to the class of transcripts reported by Leonard et al. (1987) and Stein et al. (1988) that are FGF and NGF inducible and dexamethasone suppressible. In contrast, some gene products associated with neurite outgrowth might belong to the class of transcripts described by Leonard et al. (1987) that are NGF inducible but not dexamethasone suppressible. Alternatively, neurite outgrowth may require posttranslational modifications of gene products that might not be responsive to glucocorticoids. The differences between the effects of NGF and FGF on neurite outgrowth may similarly result from a differential activation of some genes or different posttranslational mechanisms. Whatever the mechanism, our data show that not all components of neuronal differentiation need to respond equally to a particular agent and, therefore, that different elements of a neuronal phenotype can be independently regulated by the microenvironment. This emphasizes the complexity of neuronal differentiation, evident in the various phenotypes obtained even by just comparing, quantitatively, 2 components of the cell. It gives an indication of how the microenvironment, containing different mixtures of effectors, might regulate different phenotypes. This complexity can be understood in terms of the converging and diverging signal pathways of different growth factors and the presence of multiple regulating elements in genes. The ability of the local milieu to regulate cell morphology independently of sodium channel expression could have impor-

Figure 5. Macroscopic properties of sodium currents in PC12 cells. Data were derived from records of the difference current obtained before and after the application of 1 μM TTX. *A* and *B*, Current–voltage relation for the −NGF (*A*) and the +NGF (*B*) cells. *C*, Voltage dependence of the sodium conductance (*closed symbols*) and voltage dependence of steady-state inactivation (*open symbols*) for the −NGF (*squares*) and +NGF (*circles*) cells. The conductance was obtained assuming a constant reversal potential of +50 mV and is plotted as a relative conductance (i.e., the conductance at the indicated voltage divided by the maximum conductance obtained at large depolarizations). Steady-state inactivation was obtained from the ratio of the peak current obtained at +20 mV over that obtained at the same membrane potential in a pulse preceded by a 100 msec prepulse to the indicated voltage. *D*, Time constants of inactivation as a function of voltage obtained by fitting, to a single exponential, the decay of the sodium current during a depolarizing pulse to the indicated voltage.

2 DAYS NGF



2 DAYS NGF+DEX



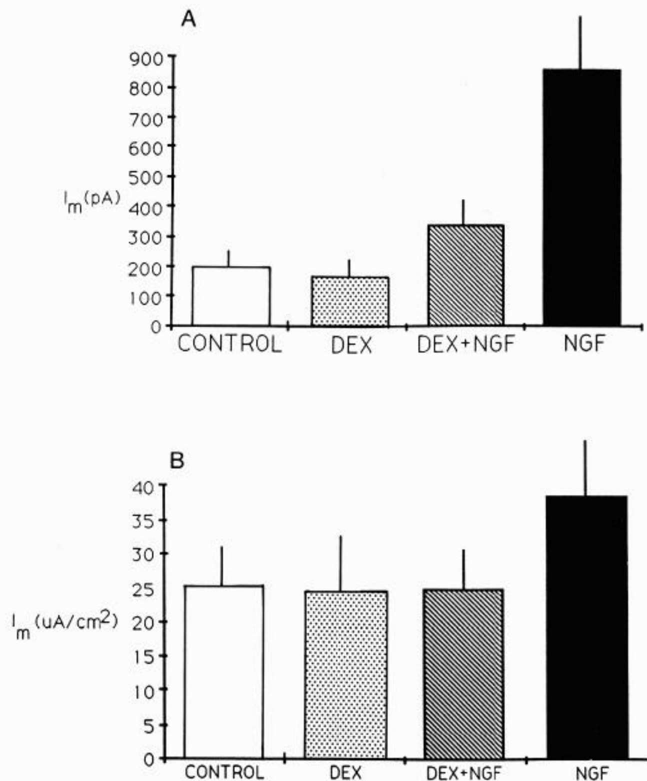


Figure 7. Dexamethasone (5 μM) inhibits peak sodium current (*A*) and peak sodium current density (*B*) of PC12 cells treated with 50 ng/ml NGF for 2 d. Average from 12 cells (control), 13 cells (dexamethasone), 12 cells (dexamethasone + NGF), 10 cells (NGF). *Error bars*: SEM.

tant consequences for active and passive electrical properties of neurons.

The signal-transduction pathways used by NGF, FGF, and EGF to induce sodium channel expression remain a mystery. cAMP has been suggested as a mediator of some of the effects of NGF (Schubert et al., 1978; Garrels and Schubert, 1979; Halegoua and Patrick, 1980; Cremins et al., 1986). Our data indicate that cAMP cannot act as a major second messenger for sodium channel induction by NGF, FGF, or EGF in PC12 cells since treatment of the cells with this agent leads to a decrease in sodium current. This is in contrast to the effects of cAMP on developing muscle cells (Offord and Catterall, 1989), where sodium channel message and protein are increased by cAMP. This suggests the existence of several sodium channels genes regulated by different factors. The inhibition of sodium current by cAMP is similar to the results observed by Greene et al. (1986) and Doherty et al. (1987), who found that pretreatment with forskolin or cholera toxin, both activators of adenylate cyclase, blocked several NGF-induced responses in PC12 cells. Moreover, these results indicate that any activation of adenylate cyclase by NGF or FGF is by itself insufficient to explain differentiation and may even lead to inhibition of some of the events initiated by NGF and FGF. Interestingly, we find that cAMP produces an increase in calcium current. It is well known that cAMP increases the opening probability of certain types of calcium channels (Reuter, 1983; Tsien, 1983). This is an immediate and short-lasting effect. Our data suggest that cAMP may also produce, in some cells, a long-term increase of calcium current density by regulating the numbers of expressed channels.

Note Added in Proof: Contrary to the observations described here, Kalman et al. (1990) found that cAMP produçes an increase in Na^+ currents in PC12 cells. They suggest that the NGF increase in Na^+ channel numbers involves the activation of cAMP-dependent protein kinase. We note, however, that the induction of Na^+ currents by either cAMP or by NGF reported by Kalman et al. is about an order of magnitude smaller than that observed by us after NGF or FGF treatment. Furthermore, while we observed a large increase in Na^+ current density after NGF or FGF treatment, their treated cells have current magnitudes similar to those of untreated cells that express Na^+ currents. It is possible that PC12 cells have 2 mechanisms to regulate Na^+ channel numbers. Other proteins are regulated by cAMP-dependent protein kinase in PC12 cells (see Discussion). However, the large induction of Na^+ channels described here and in previous publications (Rudy et al., 1982, 1987), like the NGF-induction of several other late genes in PC12 cells, appears not to be mediated by cAMP.

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Figure 6. Effect of dexamethasone on neurite outgrowth in cells treated with NGF for 2 d. Comparison of PC12 cell cultures treated with 50 ng/ml NGF for 2 d (*left*) with cells treated with NGF for 2 d in the presence of 5 μM dexamethasone (*right*).

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