Neuronal Growth Factor Regulation of Two Different Sodium Channel Types Through Distinct Signal Transduction Pathways

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Abstract. Neuronal growth factors regulate the expression of voltage-activated sodium current in differentiating sympathetic neurons and PC12 cells. We show that, in PC12 cells, the NGF- and FGF-induced sodium current results from increased expression of two distinct sodium channel types. Sodium current results from the rapid induction of a novel sodium channel transcript, also found in peripheral neurons, and from the long term induction of brain type II/IIA mRNA. Expression of the type II/IIA sodium channel requires activation of the cyclic AMP-dependent protein kinase (A-kinase), whereas induction of the peripheral neuron type sodium channel occurs through an A-kinase-independent signal transduction pathway. These findings suggest that the two sodium channel types act in concert to ensure the generation of action potentials during neuronal differentiation.

The presence of voltage-activated sodium channels is one distinguishing feature of the neuronal phenotype. However, sodium channels in the vertebrate peripheral and central nervous systems exhibit a wide spectrum of structural diversity. This diversity arises by expression from a multiplicity of sodium channel genes, as well as by alternative splicing of mRNAs (Noda et al., 1986; Suzuki et al., 1988; Sarao et al., 1991; Yarowsky et al., 1991; Schaller et al., 1992; Gautron et al., 1992; Trimmer et al., 1990; Rogart et al., 1989; Kallen et al., 1990). Different tissues express different sodium channel types (Beckh et al., 1989; Gordon et al., 1987; Westenbroek et al., 1989; reviewed in Mandel, 1992). In addition, electrophysiological studies have revealed the co-existence of distinct sodium channel types in single neurons. Individual neurons which have been studied include cranial sensory neurons (Bossu and Feltz, 1984) and pyramidal neurons (French and Gage, 1985), vertebrate sympathetic neurons (Jones, 1987; Campbell, 1992), and invertebrate neurons (Gilly and Brismar, 1989). The regulatory pathways involved in the neuronal expression of individual sodium channel types have not been identified in any of these in vivo studies.

The clonal rat pheochromocytoma cell line, PC12 (Greene and Tischler, 1982), affords the opportunity to understand how the expression of sodium channels is regulated. Previous studies showed that treatment of PC12 cells with NGF or fibroblast growth factor (FGF) increased the density of sodium channels (O'Lague and Huttner, 1980; Rudy et al., 1987; Mandel et al., 1988; Pollock et al., 1990). The increase in sodium channel density by NGF was paralleled by increases in type II/IIA sodium channel mRNA, suggesting that the newly acquired ability to generate action potentials was due to increased expression of this channel type (Mandel et al., 1988). Because the NGF-induced increase in sodium current has been suggested to be mediated by the cyclic AMP-dependent protein kinase (A-kinase[1]; Kalman et al., 1990), we have hypothesized that the increase in expression of type II/IIA mRNA is also mediated by A-kinase. In opposition to this simple hypothesis, two electrophysiological studies have reported that treatment of PC12 cells with cyclic AMP analogs does not increase sodium channel expression (Pollock et al., 1990; Ifune and Steinbach, 1990), and a third study has suggested that the growth factor-induced sodium current requires post-translational effects of cyclic AMP (Ginty et al., 1992).

Here, through combined whole cell voltage-clamp and molecular hybridization studies, we have readdressed the question of whether neuronal growth factor induction of sodium channel genes occurs through a cyclic AMP-dependent pathway. Our findings confirm a cyclic AMP requirement for brain type II/IIA sodium channel expression, but also reveal a more complex mechanism underlying sodium channel gene regulation in PC12 cells. A second type of sodium channel is expressed in PC12 cells in response to growth factor treatment, and may account for a novel sodium channel type in peripheral neurons as first suggested on the basis of antibody studies (Wollner and Catterall, 1985). The discov-

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1. *Abbreviations used in this paper*: A-kinase, cyclic AMP-dependent protein kinase; dbcAMP, N^6,2'-O-dibutyryladenosine 3',5'-cyclic monophosphate.

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ery of this additional sodium channel type reveals a cyclic AMP-independent pathway through which growth factor treatment leads to the acquisition of excitable membrane properties.

Materials and Methods

Cell Culture

PC12 and PC12 subclones were grown on tissue culture dishes as previously described (Mandel et al., 1988). When required, NGF, purified from mouse submaxillary glands according to Mobley et al. (1976) and recombinant bovine basic FGF (kindly supplied by A. Baird, Whittier Institute) were added to the culture medium at final concentrations of 100 ng/ml and 10 ng/ml. N^6,2'-0-dibutyryladenosine 3':5'-cyclic monophosphate (dbcAMP) (Sigma Chemical Co., St. Louis, MO) was dissolved in DME (Gibco Laboratories, Grand Island, NY) at 40 mM and diluted into the culture medium to a final concentration of 1 mM.

The PC12 subclone A126-1B2, which is deficient in A-kinase activity (Van Buskirk et al., 1985), was provided by J. A. Wagner (Cornell Medical School, NY). The PKI-4 PC12 subclone, which expresses the A-kinase inhibitor protein (PKI), was generated by cotransfecting PC12 cells with two plasmids: pRSV-PKI (Day et al., 1989), and pSV_2-NEO (Southern and Berg, 1982). For the stable transfections, $\sim 4 \times 10^6$ PC12 cells were electroporated in 0.4 ml DME containing 18 μg pRSV-PKI and 2 μg pSV_2-NEO, using a Gene Pulser (Bio-Rad Laboratories, Richmond, CA). 2 d after transfection, the cells were replated in medium containing 800 μg/ml geneticin (Gibco Laboratories). After 2 to 3 wk, geneticin-resistant clones were isolated and screened for the ability to produce neurites in response to treatment with NGF but not dbcAMP. A geneticin-resistant subclone, PKI-4, which fulfilled this criterion was used in the present study.

Northern Blot Analysis

Total cellular RNA was isolated from PC12 or PC12 subclones according to the method of Cathala et al. (1983). PC12 RNA and RNA purified from rat tissues (Chirgwin et al., 1979) was electrophoresed as previously described (Cooperman et al., 1987), and then electrophoretically transferred to nylon membranes (Duralon-UV; Stratagene Corp., La Jolla, CA). The blots were cross-linked using a Stratalinker UV crosslinker (Stratagene Corp.) and hybridized to ^{32}P-UTP-labeled antisense RNA probes using the following linearized templates: pRB211 (Cooperman et al., 1987), pNach2 (Cooperman et al., 1987), p1B15 (cyclophilin; Danielson et al., 1988), and pSP-PKI, containing 183 bp of the synthetic PKI gene (Day et al., 1989). RNA probes were synthesized with SP6 polymerase according to the manufacturers instructions (Promega Corp., Madison, WI). The blots were washed twice in 0.1 × SSC, 0.1% NaDodSO4 for 20 min at 68°C and then subjected to autoradiography with preflashed XAR-5 film (Eastman Kodak Co., Rochester, NY). Levels of sodium channel mRNA were determined by densitometric analysis of the autoradiograms using a Joyce Loeble densitometer and normalized to the similarly quantitated levels of cyclophilin mRNA.

Electrophysiology

Whole cell voltage-clamp was used to measure sodium current from PC12 cells. The studies were performed by holding the cell membrane potential at −80 mV and stepping to various potentials for 10 ms durations, at 5 s intervals. The outward potassium current was largely suppressed by substituting CsCl for KCl in the patch pipette. The external solution contained (in mM) 140 NaCl, 1 KCl, 1 $CaCl_2$, and 10 Hepes (pH 7.2). The pipette solution contained (in mM) 140 CsCl, 1 EGTA, and 10 Hepes (pH 7.2). Data was digitized at 50 μs intervals via an ITC-16 A/D converter and acquired on a ATARI Mega-4 computer. Current measurements and analysis were performed on individual stored current traces using Instrutech software (Acquire and Review).

Whole cell recordings were performed on cells within 1 h of placing the cells in the external recording solution. This was a time before any obvious signs of deterioration, such as the loss of adhesion and granulation which are seen after several hours in recording solution. Sodium currents were very stable under whole cell voltage clamp recording, provided that no changes in cell access resistance were observed. To minimize the tendency of membrane to seal over the electrode tip, thereby increasing access resistance, a slightly negative pressure was maintained at the back of the electrode. Even with these precautions, it is likely that we underestimated the actual current, because of the presence of neurites. However, the error was minimized by recording from cells 2 d after either NGF or dbcAMP treatment, when neurites were reasonably short in length (see Results). Additionally, after treatment of the cells with either NGF or dbcAMP, we consistently chose cells, based on their sodium current kinetics and voltage-dependence of activation, which could be reasonably well voltage-clamped. A two tailed T-test was performed to determine the significance of differences in currents between control and NGF- or dbcAMP-treated cells and reported in the legend to Fig. 4.

Results

PC12 Cells Express Transcripts Encoding Two Different Sodium Channel Alpha Subunits

To characterize the sodium channel mRNAs present in undifferentiated PC12 cells, Northern blots were first probed with a cRNA probe, pRB211 (Cooperman et al., 1987), which encodes a highly conserved region of the alpha subunit and cross-hybridizes with all previously identified sodium channel mRNAs. Using this probe we detected three sodium channel transcripts of approximately 9.5, 10 and 11 kb in undifferentiated PC12 cells (Fig. 1 *A*, first lane). The transcripts were present in very low amounts relative to levels of sodium channel transcripts in brain (Fig. 1 *A*).

To determine which of the three sodium channel transcripts was derived from the type II gene, the mRNA was hybridized with a probe containing 5' untranslated sequences specific for this gene (Cooperman et al., 1987). Only the two smaller transcripts (9.5 and 10 kb) were detected with the type II gene-specific probe (Fig. 1 *B*), consistent with previous studies using rat brain mRNA and presumably reflecting the differential use of two polyadenylation sites (Beckh et al., 1989). The same two transcripts were also identified when a probe specific for sequences in the 3' untranslated region of the type II gene was used (data not shown). Recently, it was shown that the type II gene is alternatively spliced in the coding region to yield two different transcripts, type II and type IIA (Sarao et al., 1991; Yarowski et al., 1991). Because our cRNA probes do not discriminate between these two alternatively spliced variants, we have followed the convention of referring to the transcripts and encoded proteins as type II/IIA.

The third and largest transcript (~11 kb) identified in PC12 mRNA did not hybridize with probes specific for the type II/IIA mRNAs, and likely represents a new sodium channel family member. In addition to PC12 cells, the transcript is present in mRNA obtained from DRG and from superior cervical ganglia (SCG), but is not detected in Northern blots using brain mRNA (Fig. 2), suggesting that the large mRNA is expressed preferentially in the peripheral nervous system. We have tentatively designated the novel 11-kb transcript type as Peripheral Nerve type 1 (PN1). The additional sodium channel-related transcript detected in DRG and SCG RNA (Fig. 2) is presently unidentified.

The Type II/IIA and PN1 Transcripts Are Induced with Different Time Courses by Neuronal Growth Factors

Sodium current in PC12 cells is increased substantially by treatment with either NGF or FGF. The time course of so-

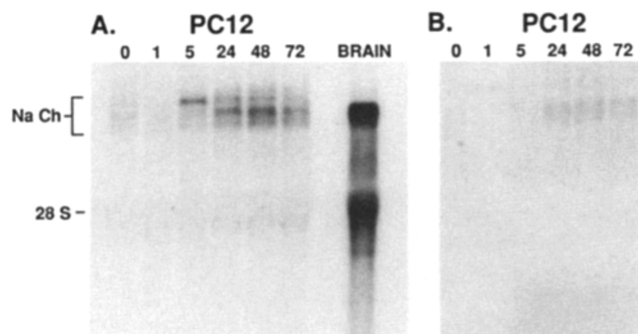

Figure 1. Two types of sodium channel mRNA are induced by NGF with distinct time courses. PC12 cells were incubated in the presence of 100 ng/ml NGF for the indicated hours. Total RNA was isolated from PC12 cells or from rat brain and subjected to Northern blot analysis as described in Materials and Methods. Duplicate blots were hybridized with antisense RNA probes generated from the generic sodium channel template pRB211 (*A*) or from the type II/IIA gene-specific template pNach2 (*B*). The positions of sodium channel transcripts (*Na Ch*) and the larger ribosomal RNA subunit (*28S*) are indicated.

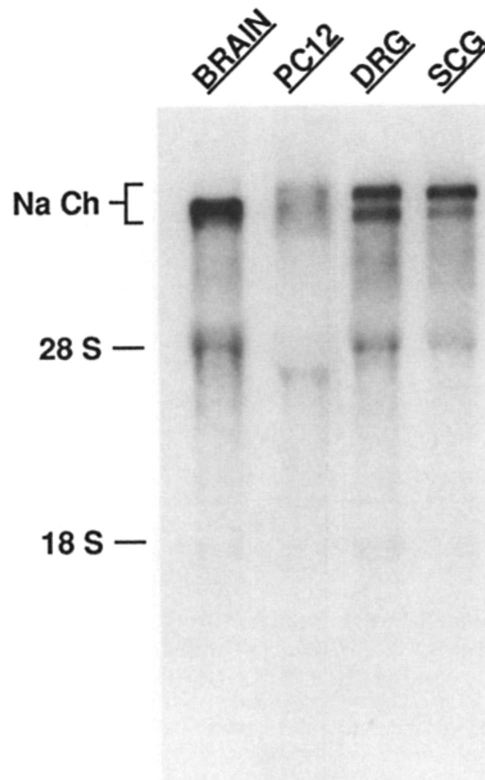

Figure 2. A PC12 sodium channel mRNA comigrates with a transcript specifically expressed in the peripheral nervous system. Total RNA was isolated from rat brain, dorsal root ganglia (*DRG*), superior cervical ganglia (*SCG*) or from PC12 cells treated with NGF for 1 d; ~2 μg of RNA isolated from the indicated tissues and 10 μg of RNA isolated from PC12 cells were subjected to Northern blot analysis as described in Materials and Methods. The blot was hybridized with an antisense RNA probe generated from the generic sodium channel template pRB211. The positions of sodium channel mRNA (*Na Ch*) and the ribosomal RNA subunits (*28S* and *18S*) are indicated.

dium channel mRNA induction by NGF or FGF was examined by Northern blot analysis using the conserved antisense transcript of pRB211. As shown in Fig. 1 *A*, although the steady state levels of all three sodium channel transcripts increased after treatment with NGF, the time courses of induction were quite distinct. The two type II/IIA-specific transcripts exhibited a similar time course of induction. Levels of type II/IIA mRNA increased fourfold within 24 h, and further increased to a maximum of sixfold after 2 d. In contrast, PN1 transcripts exhibited a much faster and more transient induction in response to NGF. This mRNA was induced maximally (fivefold) within 5 h of NGF treatment, and declined to a lower steady state level of induction (threefold) over the next several days. The kinetics of induction of both PN1 and type II/IIA mRNAs in response to FGF were similar to those seen in response to NGF (Fig. 3 *A*).

A-kinase Mediates Growth Factor Induction of Type II/IIA, but not PN1, mRNA

The differential kinetics of induction in response to neuronal growth factor treatment suggested that the two sodium channel mRNA types were regulated through different signal transduction pathways. Because at least one electrophysiological study has indicated that A-kinase activity is required for NGF induction of functional sodium channels (Kalman et al., 1990), we examined whether this kinase was also involved in increasing levels of sodium channel mRNA. As shown in Fig. 3 *A*, treatment of PC12 cells with the cyclic AMP analog dbcAMP resulted in a threefold increase in steady state levels of type II/IIA sodium channel mRNA. The time course of the response, as well as the maximal level of induction, were similar to that observed after treatment with NGF or FGF (threefold for each in Fig. 3 *A*).

To further examine the involvement of the A-kinase pathway in sodium channel regulation, we utilized PC12 cell lines in which A-kinase activity was dramatically reduced. First, type II/IIA mRNA levels were measured in a PC12 subline, A126-1B2, which is deficient in the type II cyclic AMP-dependent protein kinase (Van Buskirk et al., 1985). The basal level of type II/IIA mRNA in these cells was too small to be quantitatively analyzed (Fig. 3 *B*). Treatment of A126-1B2 cells with saturating levels of dbcAMP (1 mM) resulted in the appearance of some type II/IIA mRNA (Fig. 3 *B*), which likely reflects the low level of A-kinase activity in these cells (Van Buskirk et al., 1985). However, there was no detectable induction of type II/IIA transcripts in A126-1B2 cells after treatment with NGF or FGF (Fig. 3 *B*). In a complementary approach, stably-transfected PC12 cell lines were established which expressed a transfected A-kinase inhibitor peptide (PKI) cDNA (Day et al., 1989). Northern blot analysis of one of the transfected cell lines (PKI-4) showed a high level of PKI mRNA (data not shown). Moreover, functional PKI peptide was produced in this cell line because transient transfection of a chimeric reporter gene containing a cyclic AMP-inducible element (CRE; Montminy et al., 1986) did not lead to increased reporter activity (data not shown; see also Montminy et al., 1986). The expression of functional inhibitor protein in the PKI-4 cells

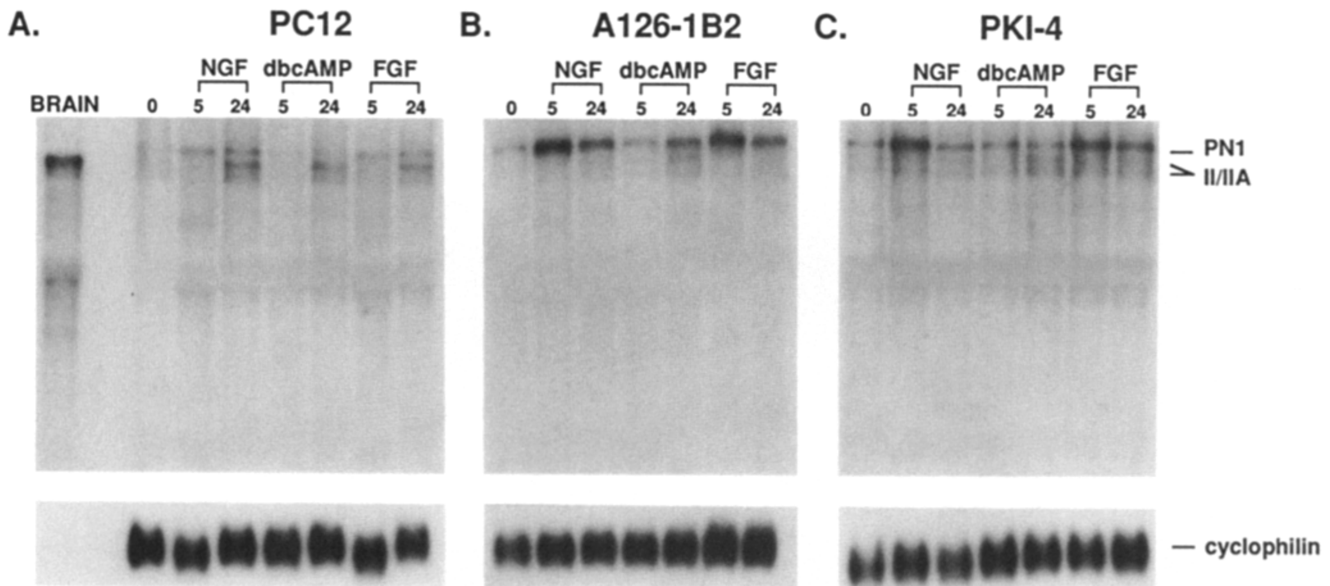

Figure 3. A-kinase mediates the induction of the type II/IIA, but not PN1, sodium channel mRNA by neuronal growth factors. PC12 cells (*A*), A-kinase deficient cells, A126-1B2 (*B*) or PKI-4 cells expressing the A-kinase inhibitor peptide (*C*) were incubated for the indicated hours in the presence of 100 ng/ml NGF, 1 mM dbcAMP or 10 ng/ml FGF. Total RNA was isolated from these clonal cells (10 μg/lane) or from rat brain (2 μg/lane) and subjected to Northern blot analysis as described in Materials and Methods. Blots were hybridized with an antisense probe generated from the generic sodium channel template pRB211. To insure that equal amounts of RNA were loaded in each lane, blots were subsequently hybridized with an antisense probe directed against the internal control cyclophilin mRNA. The positions of the PN1 sodium channel mRNA and the two transcripts generated from the type II gene (*II/IIA*) are indicated. The increase in PN1 mRNA levels in A126-1B2 cells treated with dbcAMP (*B*) was not reproducible in other experiments.

severely blunted the increase in type II/IIA mRNA levels compared to control PC12 cells. Treatment of PKI-4 cells with dbcAMP resulted in only a twofold increase in type II/IIA mRNA levels (Fig. 3 *C*). Neither NGF nor FGF treatment of these cells resulted in any increase in type II/IIA transcript levels (Fig. 3 *C*).

In contrast to the induction of type II/IIA mRNA, induction of PN1 mRNA by neuronal growth factors was not mediated through A-kinase activation. Activation of A-kinase by dbcAMP in PC12 cells did not result in any increase in PN1 mRNA levels (Fig. 3 *A*). Furthermore, normal increases in the level of PN1 mRNA were obtained in A126-1B2 cells treated with NGF or FGF (Fig. 3 *B*), an induction of eightfold at 5 h of NGF treatment decreasing to a fourfold induction at 24 h. Expression of the A-kinase inhibitor peptide in the PKI-4 cell line also did not prevent induction of PN1 mRNA by either NGF or FGF (Fig. 3 *C*).

Induction of Both PN1 and Type II/IIA mRNAs Correlates with Induction of Sodium Current in PC12 Cells

NGF treatment of PC12 cells results in an increase in sodium current when measured between 3 and 7 d after exposure to the growth factor (Dichter et al., 1977; O'Lague and Huttner, 1980; Rudy et al., 1987; Mandel et al., 1988; Kalman et al., 1990; Ginty et al., 1992). We found that a fivefold increase in sodium current above the mean baseline level was detected as early as 24 h after NGF treatment. The average current continued to increase over the next 4 d, but large variations in the amount of sodium current were observed at all time points (see Fig. 4 *A*). When expressed as percentage of cells which showed sodium currents (>10 pA), increases of between 80–90% were observed within 2 d of NGF treatment (Fig. 4 *A*). The current amplitudes at the 3–5 d time points are likely to be underestimates of the actual current amplitude because of the inability to record the current contributed by neurites. Consequently, in all remaining experiments, we restricted our recordings to cultures treated with NGF for 2 d, when neurites were less numerous and shorter in length (Greene and Tischler, 1976).

The correlation between sodium current and the levels of PN1 and type II/IIA mRNAs suggested that these channel types both contribute to the NGF-induced current. To further test this idea, recordings were made from cells under conditions in which either PN1 or type II/IIA mRNAs were selectively expressed. The A-kinase-deficient (A126-1B2) or A-kinase-inhibited (PKI-4) cells selectively expressed high basal levels of PN1 mRNA relative to PC12 cells (compare in Fig. 3, *A–C*). Cells from both cell lines also showed a three- to fivefold higher level of basal current compared to PC12 cells (Fig. 4 *B*, see also Ginty et al., 1992). After 48 h of NGF treatment, a further twofold increase in sodium current was observed in both cell types (Fig. 4 *B*), which is similar to the increase in PN1 mRNA levels observed in the same cell types after 24 h of NGF treatment (see Fig. 3, *B C*). As expected, treatment of A-kinase deficient cells with dbcAMP did not result in any significant induction of sodium current compared to NGF treatment; mean currents were 238 ± 434 pA (n = 24) for control, 262 ± 392 pA (n = 18) for dbcAMP-treated, and 928 ± 1120 pA (n = 10) for NGF-treated cells.

To selectively induce type II/IIA mRNA, PC12 cells were

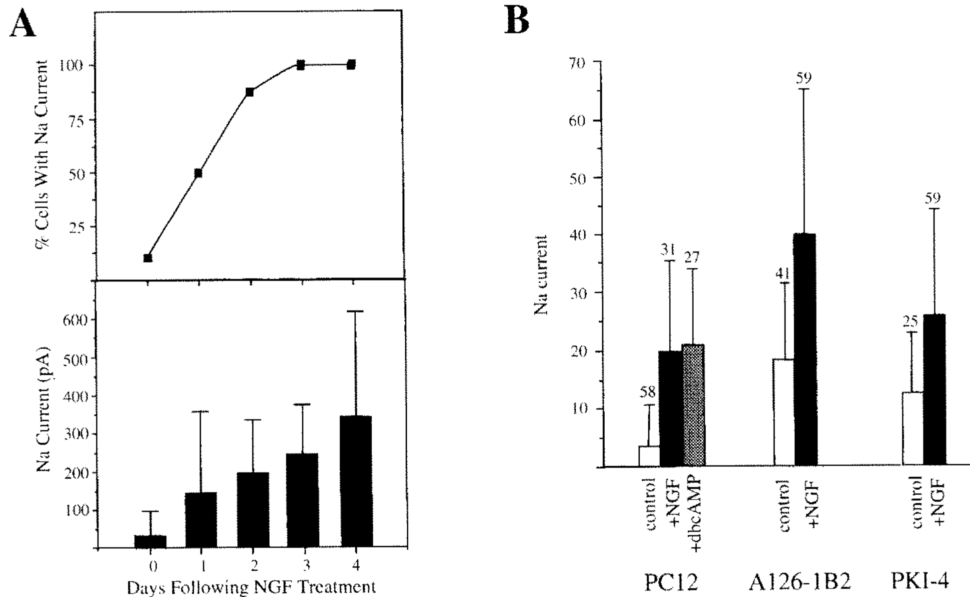

Figure 4. Sodium current induction in response to NGF or bdcAMP. (*A*) The time course of sodium current induction after treatment with NGF. PC12 cells were considered to be positive for sodium current if a TTX sensitive current >10 pA was recorded. (*B*) Comparisons of sodium current density in PC12 cells, A-kinase deficient cells (*A126-1B2*) and cells expressing the A-kinase inhibitor peptide (*PKI-4*). For each cell line, nontreated cells are indicated by unfilled columns. Currents were normalized to cell capacitance in order to correct for differences in cell size. The capacitance was estimated from the transient compensations provided on the EPC-7 patch clamp amplifier (list electronic). The numbers on top of the columns indicate *n* values, and the error bars represent the standard deviations of the mean. For each PC12 cell line, differences in current before and after treatment with NGF or dbcAMP are significant ($P < 0.01$).

treated with dbcAMP (see Fig. 3 *A*). After 2 d, measurements of sodium current in these cells indicated a five- to sixfold induction, which was comparable to the induction following NGF treatment (Fig. 4 *B*). This increase in current also compared favorably to the three- to fourfold induction of type II/IIA mRNA in PC12 cells treated with either NGF or dbcAMP for 24 h (Fig. 3 *A*). Thus, as observed for PN1, the increase in type II/IIA mRNA levels can account for the increase in sodium current.

Evidence was sought for differences in either the kinetics of macroscopic sodium current decay or the voltage-dependence of current activation for the two sodium channel types. Comparisons of the current-voltage relations for NGF-treated A126-1B2 cells (selectively expressing PN1 mRNA) and dbcAMP-treated PC12 cells (selectively expressing type II/IIA mRNA) revealed no clear differences (Fig. 5). Both channels activated between −50 and −40 mV with peak currents measured between −25 and −10 mV. Comparisons of current half-decay in NGF-treated A126-1B2 cells and the dbcAMP-treated PC12 cells also revealed no significant differences over the range of potentials between −50 mV and +20 mV.

Discussion

Our findings support the hypothesis that increased expression of type II/IIA sodium channels contributes to the voltage-activated sodium current in NGF-treated PC12 cells. Results from three different experimental approaches further indicate that NGF and FGF induction of type II gene expression is mediated through activation of A-kinase. First, stimulation of A-kinase activity, by treatment of PC12 cells with dbcAMP, leads to an increase in type II/IIA mRNA levels. The increase in type II/IIA transcript levels by dbcAMP is comparable, in both magnitude and time course, to the increase in transcript levels seen after treatment with NGF or FGF. Second, in a PC12 subclone deficient in A-kinase, levels of type II/IIA mRNA do not increase after treatment with NGF or FGF. Third, type II/IIA mRNA levels are not induced in a NGF- or FGF-treated PC12 subclone in which A-kinase activity is inhibited by expression of a transfected cDNA encoding the A-kinase inhibitor peptide, PKI.

The type II sodium channel gene is the first example of an ion channel gene regulated by NGF or FGF through an identified signal transduction pathway. The involvement of

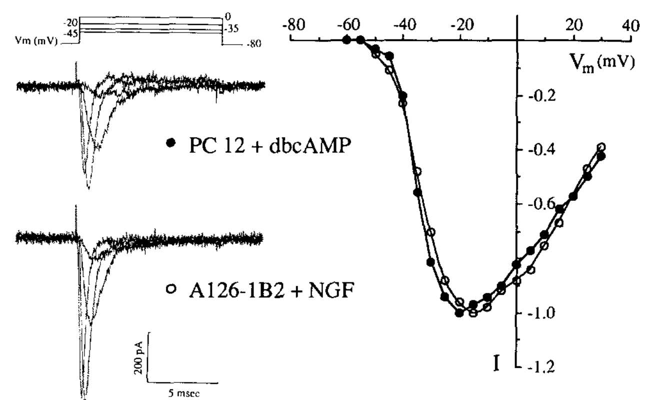

Figure 5. Comparisons of current kinetics for sodium channel types. Representative current traces and current-voltage relations are shown for cells expressing principally type II/IIA sodium channels (*filled circles*) and cells expressing principally PN1 sodium channels (*open circles*). The currents recorded for the two cells were normalized in order to compare the current-voltage relationships.

A-kinase activity in the NGF signal transduction pathways is consistent with biochemical studies showing A-kinase-dependent phosphorylation of tyrosine hydroxylase by NGF (Cremins et al., 1986) at a site directly phosphorylated by A-kinase (McTigue et al., 1985; see also Haycock, 1990). Although a previous study had reported that NGF-induced increases in sodium current involve the cyclic AMP-dependent pathway (Kalman et al., 1990), the selectivity of this pathway for the type II/IIA sodium channel was not recognized. It is likely that the regulation of type II/IIA sodium channel mRNAs by neuronal growth factors and dbcAMP is, at least in part, at the transcriptional level. No classic cyclic AMP-responsive element (CRE) is present in the cloned type II 5′ flanking region (Maue et al., 1990). However, PC12 cells transiently transfected with type II-reporter fusion genes show a two- to threefold increase in reporter activity after treatment with either NGF or dbcAMP (S. K. Kraner and G. Mandel, unpublished data).

A post-translational role for A-kinase in the NGF-induction of sodium channels has recently been suggested by Ginty et al. (1992). This suggestion was based on the finding that in A-kinase-deficient PC12 cells grown in suspension culture, sodium currents were not increased by NGF, despite an increase in sodium channel transcript levels. There could be at least two reasons for the difference in results between Ginty et al. (1992) and our present study. First, the Northern blot analysis in the study of Ginty et al. (1992) did not resolve sodium channel mRNA species of different sizes. A selective block of type II/IIA mRNA in A-kinase-deficient PC12 cells may have been obscured by the induction of the second sodium channel mRNA which we have described. Second, in PC12 cells cultured in suspension, NGF treatment results in lower overall sodium channel mRNA levels than in adherent cultures (Ginty et al., 1992). The low level of induction seen in Ginty et al. (1992), could have further compromised the ability to detect the partial increase in sodium current after treatment of A-kinase-deficient cells with growth factor. The inability of cAMP to elevate sodium current in PC12 cells reported in some other studies (Pollock et al., 1990; Ifune and Steinbach, 1990) is still unexplained, but could be due to the use of different derivatives of cAMP.

The identification of a second type of sodium channel in PC12 cells, termed PN1, was based on the ability of a probe containing conserved sodium channel sequences to hybridize to this mRNA species in Northern blot analysis. Unlike type II/IIA transcripts, PN1 transcripts are not detected by RNA probes specific for the type II gene (Cooperman et al., 1987; Beckh et al., 1989), and the brain type I and type III transcripts are distinctly smaller sizes on RNA gels (Beckh et al., 1989; Noda et al., 1986). It is likely that PN1 mRNA encodes a predominant sodium channel alpha subunit present in the peripheral nervous system. Evidence for this assertion is that the 11 kb PN1 transcript is prevalent in peripheral ganglia such as dorsal root ganglia and superior cervical ganglia, but is not seen in brain, and that sodium current is induced in NGF-treated PC12 subclones when PN1 mRNA is also preferentially induced. Confirmation of the identity of PN1 sodium channels awaits cloning of its cDNA and generation of specific antibodies to unique epitopes in the primary sequence.

In contrast to the findings with the type II sodium channel gene, NGF- and FGF-induction of PN1 mRNA levels did not require activation of A-kinase. Levels of PN1 mRNA were not increased by treatment of PC12 cells with dbcAMP. Additionally, the increase in levels of PN1 mRNA in response to growth factors was not prevented in a PC12 transfectant line expressing the A-kinase inhibitor peptide or in the PC12 subclone deficient in A-kinase activity. The second messenger pathway by which growth factors induce PN1 sodium channel mRNA has not yet been identified, but is independent of the activities of Src, Ras and Raf (D'Arcangelo and Halegoua, 1993) which mediate the actions of NGF on several other genes (D'Arcangelo and Halegoua, 1993; Guerrero et al., 1988; Szeberenyi et al., 1990; Thomas et al., 1991; Wood et al., 1993).

Electrophysiological studies have revealed the co-existence of sodium channels with different functional properties in individual sensory neurons (Campbell, 1992). However, in these studies, it was not possible to distinguish the current contribution of individual sodium channel types with similar functional properties. The ability to differentially express distinct sodium channel mRNAs in PC12 cells has revealed the coexistence of structurally distinct sodium channels with similar functional properties. Two explanations to account for functional redundancy can be envisaged. As discussed in Westenbroek et al. (1989), it is possible that primary sequence differences in type II/IIA and PN1 sodium channels are important for their targeting to different regions in a neuron. Alternatively, the advantage of expressing two sodium channel types with similar functional properties may be related to the fact that they are induced through distinct signal transduction pathways. The differential regulation provides a means of ensuring action potential generation at specific times during development and in a cell type-specific manner. Elucidation of the signal transduction pathway which induces PN1 sodium channel gene expression should shed further light on the biological significance of the multiplicity of neuronal sodium channels.

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