Effects of the Tumor Promoter 12-O-Tetradecanoylphorbol-13-acetate on Neurite Outgrowth from Chick Embryo Sensory Ganglia

L. Hsu, D. Natyzak, and Jeffrey D. Laskin

Department of Anatomy, School of Osteopathic Medicine [L. H., D. N.], and Department of Environmental and Community Medicine, Rutgers Medical School [J. D. L.], University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854

ABSTRACT

The addition of the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) to defined growth medium stimulated an intense neurite outgrowth from chick sensory ganglia explants. The development of radial neurites was concentration dependent. At low concentrations of TPA (1.6 to 16 nM), neurites were long but moderate in numbers. At higher concentrations (160 to 480 nM), TPA produced dense, short neurites that formed thick fascicles. This reduced outgrowth in length was not related to cytotoxicity and was found to be reversible when the tumor-promoting agent was removed. Neurite outgrowth was also accompanied by an increase in proliferation of nonneuronal cells. Blocking the proliferation of nonneuronal components by the mitotic inhibitor 1β-d-arabinofuranosylcytosine did not inhibit neurite outgrowth, suggesting that the TPA-induced neurite differentiation was independent of nonneuronal cells. When TPA was tested in the presence of nerve growth factor, the induction of neurite differentiation was not affected. Other active tumor promoters including phorbol-12,13-didecanoate and mezerein also elicited neurite outgrowth, while nonpromoting agents such as phorbol and 4α-phorbol-12,13-didecanoate were ineffective.

INTRODUCTION

The phorbol ester, TPA, is a low molecular weight plant diterpene that is the most potent tumor-promoting agent in the 2-stage mouse skin carcinogenesis assay (3). TPA is neither carcinogenic nor mutagenic alone but will greatly accelerate tumor development in animals pretreated with a subthreshold-initiating dose of a carcinogen. In a number of cell culture systems, TPA has been shown to modulate development by inhibiting or inducing differentiation (for a review, see Ref. 32). Stereospecific high-affinity binding sites for phorbol esters have been localized on a variety of cell surfaces (8), but are notably concentrated in brain tissue (9). In chick brain, binding activity of the tumor-promoting analogue of TPA, [3H]PDBU increased during development (21). Recently, autoradiographic localization of [3H]PDBU in the rat fetus indicated that phorbol ester receptors may play a role in regulating axonal elongation and nerve bundle formation in addition to promoting neurite development. The extent of nonneuronal cell outgrowth and neurite length was markedly decreased in cultures treated with high concentrations of TPA. The distinct morphological changes induced by TPA treatment suggest that, within the peripheral nervous system, the phorbol ester may play a role in regulating axonal elongation and nerve bundle formation in addition to promoting neurite development. In light of recent reports localizing receptors for TPA-like agents in the developing brain, our study emphasizes the importance of examining the role of phorbol ester-like compounds in nervous tissue.

MATERIALS AND METHODS

Materials. TPA, flavin mononucleotide, ara-C, and progesterone were purchased from Sigma Chemical Co., St. Louis, MO. DMSO was from Fisher Chemical Co., Springfield, N.J. Phorbol, PDD, and 4α-PDD were purchased from Consolidated Midland Corporation, Brewster, N.Y. Mezerein was generously donated by Dr. Debra Laskin, College of Pharmacy, Rutgers University. Ham's F-12 medium with glutamine was purchased from Flow Laboratories (Rockville, MD). NGF (7S), insulin, transferrin, and selenium were purchased from Collaborative Research, Waltham, MA. Chemical reagents for electron microscopy were from Electron

Received January 10, 1984; accepted July 13, 1984.
Microscopy Sciences, Ft. Washington, PA.

Composition of Growth Media. TPA (Sigma) was initially dissolved in DMSO (1 mg/ml) and diluted in culture medium just before use. The stock solution was maintained at -20°. TPA was added to the cultures at concentrations from 0.16 to 480 nm. At the highest concentration of TPA used, the amount of DMSO added to the growth medium was less than 0.002%. This amount of solvent had no effect on ganglionic development. Other tumor promoters were similarly prepared. Phorbol esters were added to either Ham's F-12 medium with glutamine or the same medium supplemented with hormonal and essential growth factors including insulin (5 μg/ml), transferrin (5 μg/ml), selenium (0.19 ng/ml), and progesterone (0.28 ng/ml). This supplemented growth medium provided a defined, serumless standard we refer to as complete medium.

In one series of experiments, NGF (75) was added at the concentration of 10 ng/ml in complete medium to study the combined effects of TPA and NGF. In another experiment, the mitotic inhibitor ara-C (8.9 μM) was added to the complete medium together with TPA (27).

Tissue Culture. Dorsal root ganglia from lumbar and sacral regions of 9-day-old White Leghorn chick embryos (Shamrock Poultry Farm, New Brunswick, NJ) were excised and collected in growth medium. Individual ganglia were explanted into single units of 96 multwell dishes (Costar, Cambridge, MA). Each well was previously coated with a mixture of 3 parts of diluted rat tail collagen and 1 part of 0.05% Flavin mononucleotide (19). Ganglia were fed with growth medium (50 μl/well) and maintained at 37° in a humidified atmosphere of 1% CO2 in air.

Assay of Ganglionic Development. Evaluations of the effects of TPA on ganglionic development were based on: (a) neurite length; (b) the density of neurite outgrowth; and (c) the area of explant outgrowth including both neuronal and nonneuronal components. Ganglionic cultures were examined for explant outgrowth using a phase microscope after 1 and 2 days in culture. For measurements of length and density, ganglia were fixed in 10% buffered formalin and processed for silver staining (13). All cultures were scored blind, and measurements from at least 20 ganglia for each test or control conditions were obtained. Each experimental series was repeated at least twice.

Neurite lengths were measured in silver-stained preparations as described by Spinelli and Ishii (30). To measure neurite density, each ganglion was divided into 4 quadrants, and each quadrant was visually estimated and ranked. Sparse growth was scored as 1, moderate growth as 2, and heavy growth as 3. The area of explant outgrowth which included both neurites and nonneuronal cells was determined by subtracting the area of the ganglionic cell mass from the area of the whole explant according to the equation for 2 ellipses as described by Coughlin et al. (7):

\[
\text{Outgrowth} = \pi(D_x d_x/4 - D_y d_y/4)
\]

where \(D_x\) and \(d_x\) are the major and minor diameters, respectively, of the entire explant, and \(D_y\) and \(d_y\) are the major and minor diameters, respectively, of the ganglionic cell mass.

The mean areas of outgrowth from explants, neurite length, and neurite density in test and control groups were subjected to either one or 2-way analysis of variance. The differences observed between groups were further subjected to Duncan's multiple-range test and t tests.

Electron Microscopy. Ganglia maintained on collagen-coated coverslips were fixed in 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1 w cacodylate buffer at pH 7.2. After postfixation in 2% osmium tetroxide and gradual dehydration in alcohols and propylene oxide, cultures were infiltrated and embedded with Epon-Araldite. Prior to sectioning, glass coverslips were removed. Thin sections of about 100 nm were stained with lead citrate (25) and uranyl acetate and were examined with a Phillips 300 electron microscope.

RESULTS

Effects of TPA in Ham's F-12 Medium. When chick sensory ganglia were maintained in unsupplemented growth medium (Ham's F-12) which lacked trophic agents such as NGF, insulin, or serum proteins, neurite differentiation was minimal. The addition of low concentrations of TPA (1.6 to 16 nm) to these cultures did not significantly improve explant development. After 2 days in culture, only an occasional outgrowth of nonneuronal cells and neuritic fibers was apparent (Chart 1; Table 1). Such poor development was similar to that of ganglia cultures maintained in Eagle's medium alone or with very low levels of NGF (17). When the concentration of TPA was increased to 80 or 160 nm, neuritic differentiation was enhanced. In these cultures, the outgrowth of fine neurites was accompanied by nonneuronal cells spreading from the periphery of the explant. With further increases in the concentration of TPA (160 to 480 nm), thicker outgrowths of neurites were observed (Fig. 1). At the highest concentration of TPA examined (480 nm), a decrease in neurite length was noted when compared to ganglia treated with lower dosages of the tumor promoter (Chart 1). From these results, we conclude that the addition of TPA to culture medium improved survival of the explants and promoted neurite development in a dose-dependent manner.

Effects of TPA in Complete Medium. In order to examine more fully the neurite-promoting effects of TPA, we modified the

---

**Chart 1. Effects of increasing concentrations of TPA on the length of neurites from sensory ganglia. Points represent the mean length of neurites measured from more than 30 to 40 ganglia after 2 days of growth in culture. Ganglia were grown in Ham's F-12 medium with (C) and without (A) hormonal and growth factor supplements. Bars, S.E.**

<table>
<thead>
<tr>
<th>TPA concentration (nm)</th>
<th>Mean neurite density in Ham's F-12</th>
<th>Mean neurite density in complete medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.95 ± 0.06</td>
<td>0.95 ± 0.06</td>
</tr>
<tr>
<td>1.6</td>
<td>1.04 ± 0.06</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>16</td>
<td>1.85 ± 0.07</td>
<td>1.85 ± 0.07</td>
</tr>
<tr>
<td>80</td>
<td>2.00 ± 0.06</td>
<td>2.00 ± 0.06</td>
</tr>
<tr>
<td>160</td>
<td>2.57 ± 0.08</td>
<td>2.57 ± 0.08</td>
</tr>
<tr>
<td>480</td>
<td>2.30 ± 0.09</td>
<td>2.30 ± 0.09</td>
</tr>
</tbody>
</table>

---

\(a\) Density was based on visual estimation of silver-stained fibers, ranking sparse growth as 1, moderate as 2, and dense as 3. Density significantly different for 0, 80, and 160 nm (p < 0.0001%).

\(b\) Density significantly different for 0, 16, 160, and 480 nm (p < 0.0001%). Thirty to 40 ganglia were tested for each concentration of TPA.

\(c\) Mean ± S.E.
control medium (Ham's F-12) by the addition of hormones and growth factors known to improve survival of neuronal cultures without additional serum proteins (2, 28). In this complete medium, short, fine neurites extended from the explant over a background of nonneuronal cells by the second day in culture (Fig. 2). This level of development in control sensory ganglia was not unlike that of ganglionic explants maintained in defined (N,) medium without NGF which also contains the same formula of growth supplements (28).

When TPA was incorporated into the complete medium, neurite development was enhanced affecting both the length and density of neurite outgrowth. Furthermore, the area of explant outgrowth including nonneuronal cells was also affected by TPA. These effects were dose dependent with distinct differences between cultures in low concentrations when compared to those maintained at high concentrations of TPA. In complete medium, the neurite-promoting effects of TPA were evident at concentrations as low as 16 nM. In these cultures, density of neurite outgrowth was significantly enhanced when compared to the sparse development seen in controls maintained in complete medium without TPA (Table 1; Figs. 2 and 3). Enhancement of neurite differentiation by the addition of TPA was also reflected in the lengths of neurites. Neurites from explants treated with 16 nM TPA were significantly longer than those of control ganglia (Chart 1; Figs. 2 and 3). Processes from TPA-treated ganglia were slimmer, consisting of single or a few neurites joined together. The thickness of most of these processes was usually less than 10 µm in diameter. At the ultrastructural level, they were identified as axonal processes typically containing bundles of neurotubules, neurofilaments, and vesicular profiles (33) (not shown). TPA also affected the extent of nonneuronal cell proliferation from the core of the explant as assayed by the area of neurotubules, neurofilaments, and vesicular profiles (33) (not shown). TPA also affected the extent of nonneuronal cell proliferation from the core of the explant as assayed by the area of neurotubules, neurofilaments, and vesicular profiles (33) (not shown). TPA also affected the extent of nonneuronal cell proliferation from the core of the explant as assayed by the area of neurotubules, neurofilaments, and vesicular profiles (33) (not shown). TPA also affected the extent of nonneuronal cell proliferation from the core of the explant as assayed by the area of neurotubules, neurofilaments, and vesicular profiles (33) (not shown).

In appearance, the NGF + TPA (160 nM) cultures resembled those treated with TPA (160 nM) alone with shortened outgrowth periods as low as 16 nM. In these cultures, density of neurite outgrowth in area produced by treatment with low concentrations supplemented medium (160 nM) was limited to 0.3 to 0.5 sq mm. These results indicate that the effects of TPA which regulate the extent of elongation are dependent on the continuous presence of the tumor promoter in the growth medium.

We also found that the ability of plant diterpenes to promote neurite differentiation in ganglia explants was correlated with their tumor-promoting activity (Table 2). PDD and mezerein, both active tumor promoters (12), elicited neurite outgrowth. Phorbol and 4aPDD, which are inactive as tumor promoters (12), did not cause neurite outgrowth in the concentration range tested.

Effects of TPA in Combination with NGF. We next examined the effects of TPA on neuritic outgrowth in the presence of NGF, a well-characterized peptide known to induce neurite development in peripheral ganglia (18). We found that the addition of NGF (10 ng/ml) in complete medium elicited a halo of fine neurites within 20 hr of explantation (Fig. 6). When TPA was added at low concentrations (16 nM) to the growth medium, together with NGF (10 ng/ml), neurite length was unaffected. However, we observed a significant increase in neurite density when compared to ganglia maintained in NGF alone, or in low concentrations of TPA (Table 3). In addition, the combined effects of NGF and TPA frequently produced thicker neurite outgrowths when compared with the effects of NGF or 16 nM TPA alone (Fig. 7). When NGF was combined with higher concentrations of TPA (160 nM), a marked reduction in neurite length was observed when compared to cultures treated with NGF alone or with NGF and 16 nM (Table 3; Fig. 9). Neurite density in cultures treated with both NGF and 160 nM TPA showed a small increase (2.43 ± 0.06 to 2.63 ± 0.06) from cultures treated with NGF alone (p < 0.002%). In appearance, the NGF + TPA (160 nM) cultures resembled those treated with TPA (160 nM) alone with shortened outgrowth of thick neurite fascicles.

Effects of the Mitotic Inhibitor ara-C. The enhanced outgrowth in area produced by treatment with low concentrations included both the neuritic fibers and the spread of nonneuronal cells, when compared to that of control ganglia (Chart 2).
Effects of TPA and other tumor promoters on neurite outgrowth

Expiants of 9-day-old chick embryonic sensory ganglia were treated with TPA and other tumor promoters at the concentrations indicated. Density of neurite outgrowth was visually estimated and ranked.

<table>
<thead>
<tr>
<th>Culture groups</th>
<th>Mean neurite density $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control $^a$</td>
<td>0.99 ± 0.03</td>
</tr>
<tr>
<td>TPA 15 nm</td>
<td>1.83 ± 0.18</td>
</tr>
<tr>
<td>TPA 160 nm</td>
<td>2.40 ± 0.32</td>
</tr>
<tr>
<td>Phorbol 26 nm</td>
<td>1.00 ± 0.05</td>
</tr>
<tr>
<td>Phorbol 260 nm</td>
<td>1.10 ± 0.04</td>
</tr>
<tr>
<td>4$\alpha$PDD</td>
<td>0.85 ± 0.10</td>
</tr>
<tr>
<td>15 nm</td>
<td>1.38 ± 0.12</td>
</tr>
<tr>
<td>150 nm</td>
<td>1.76 ± 0.20</td>
</tr>
<tr>
<td>Mezerein 15 nm</td>
<td>1.73 ± 0.07</td>
</tr>
<tr>
<td>150 nm</td>
<td>2.20 ± 0.23</td>
</tr>
</tbody>
</table>

$^a$ All derivatives tested in complete medium, TPA, and mezerein were significantly different than control cultures ($p < 0.01$%) as was PDD ($p < 0.05$%). Phorbol and 4$\alpha$PDD were significantly not different from control. Eighteen to 24 ganglia were treated with each tumor promoter.

$^b$ Density was based on visual estimation of silver-stained fibers, ranking sparse as 1, moderate as 2, and dense as 3.

| Mean ± S.E. |

Effects of TPA and ara-C on neurite outgrowth

Expiants of 9-day-old embryonic chick sensory ganglia were treated with TPA in complete medium with or without the addition of the mitotic inhibitor ara-C (8.9 $\mu$m). Area of outgrowth including neurite and nonneuronal cells and neurite density were assayed after 2 days in culture.

<table>
<thead>
<tr>
<th>Culture groups</th>
<th>Mean neurite density $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control $^c$</td>
<td>2.70 ± 0.48</td>
</tr>
<tr>
<td>Control + ara-C $^c$</td>
<td>2.59 ± 0.54</td>
</tr>
<tr>
<td>16 nm TPA</td>
<td>3.30 ± 0.63</td>
</tr>
<tr>
<td>16 nm TPA + ara-C $^c$</td>
<td>3.57 ± 0.53</td>
</tr>
<tr>
<td>160 nm TPA $^d$</td>
<td>1.67 ± 0.63</td>
</tr>
<tr>
<td>160 nm TPA + ara-C $^d$</td>
<td>1.97 ± 0.61</td>
</tr>
</tbody>
</table>

$^a$ Based on area obtained by subtracting explant cell mass from whole explant

$^b$ Density was based on visual estimation of silver-stained fibers, ranking sparse outgrowth as 1, moderate outgrowth as 2, and heavy outgrowth as 3.

$^c$ Mean area of outgrowth of control, TPA (16 nm), TPA (160 nm), significantly different ($p < 0.01$%); density of neurites for these groups is significantly different ($p < 0.05$%).

$^d$ Mean ± S.E.

4). It should be noted that despite this increase, cultures treated with high doses of TPA (160 nm) plus ara-C remained shorter in their outgrowth when compared to cultures treated with lower doses (16 nm TPA or 16 nm TPA plus ara-C).

The addition of ara-C affected the density of neurite outgrowth in both control and TPA-treated cultures. In the presence of ara-C, increased sprouting of fine neurites was observed in all cultures (Table 4). In control cultures and in ganglia treated with 16 nm TPA, the enhanced neurite formation remained as emergence of delicate and fine processes. In cultures treated with high concentrations of TPA (160 nm), plus ara-C, neurite processes were clearly seen without the intervening nonneuronal cellular background. Neurites fanned out from the periphery of the explant frequently fusing in the distal portions of the outgrowths (Fig. 8). From these results, it is apparent that blocking proliferation of nonneuronal cells did not inhibit neurite differentiation induced by TPA treatment. However, the addition of the mitotic inhibitor to the growth medium affected both the extent and density of outgrowth either directly or by elimination of the background cells.

DISCUSSION

Our results indicate that the addition of TPA to the growth medium of sensory ganglia explants with and without hormone and growth factor supplements promoted neurite differentiation. Depending on the concentrations of TPA used, distinct morphological changes in the development of the explants were evident. When TPA was incorporated into medium without hormones and growth factors, it promoted survival of ganglionic explants and induced neurite differentiation. In complete medium supplemented with hormones and growth factors, low concentrations of TPA (1.6 to 16 nm) stimulated the outgrowth of nonneuronal cells which was accompanied by the development of long, fine neurites. At higher concentrations (160 to 480 nm), the overall extent of neurite and glial outgrowth was reduced by TPA treatment, but the density of fibers was enhanced producing

4610 CANCER RESEARCH VOL. 44
TPA Effects on Neurite Outgrowth

explants with short, thick fascicles. Other active tumor promoters including PDD and mezerein also enhanced neurite differentiation, while the inactive compounds phorbol and 4α-PDD had no effect. At the present time, we cannot offer any explanation for the mechanism by which tumor promoters affect neuronal survival and neurite outgrowth. In unsupplemented medium, the dose of TPA needed to produce an effect was 10-fold greater than that used in supplemented medium. In this case, we cannot rule out the possibility that when TPA was incorporated in the complete medium, synergistic interactions of the tumor promoter with insulin, transferrin, progesterone, and/or selenium contributed to the overall enhanced neurite differentiation. The presence of insulin in complete medium is likely to be partly responsible for neurite outgrowth. When incorporated at relatively high, nonphysiological concentrations (29) or with fetal calf serum (1), this hormone has been shown to be effective in promoting neurite development in cultures of dissociated neurons. However, in ganglia explant cultures, the addition of 0.4 unit of insulin improved explant survival but did not produce significant outgrowth of neurites (17). In our complete medium and in a similarly defined (N₃) medium (28), sensory ganglia explants developed a sparse outgrowth of neurites on collagen and polyornithine-coated substrates. It should be noted, however, that our control cultures of ganglia maintained in complete medium were consistently less differentiated than TPA-treated cultures in terms of neurite length and density (Figs. 2, 3, and 5).

The biphasic dose-response and morphological features of these TPA-treated ganglia were strikingly similar to those observed in ganglia maintained in medium supplemented with NGF. At low concentrations of NGF (1 to 10 ng/ml), long neurites were elicited forming a radial halo around the explant (17, 18, 26). With higher concentrations of NGF (20 to 200 ng/ml), dense halos with exceedingly short fibers were formed (18, 26). It was suggested that treatment of NGF at high concentrations caused an increased side-to-side adhesion of neurites as a result of the presence of cell adhesion molecules (26). Increased adhesion of neurites formed thicker fascicles which were then prevented from further elongation because of the increased tension created along their axis causing the retraction of growth cones (4, 26). We also observed a reduction in neurite length and a thickening of neurite fascicles in cultures treated with higher concentrations of TPA. The initiation of neurite development was not prevented, since our results indicated that neurite density was enhanced. It is possible that TPA was affecting axonal elongation by alterations in cell surface adhesivity. It has been reported that TPA can stabilize adhesion of PC12 cells (6) and reduce the detachment of neurites from the underlying substrate (10). TPA can also alter the surface adhesivity properties of dissociated cerebellar neurons (16). It is also possible that at high concentrations of TPA, other effects unrelated to adhesivity may directly alter axonal elongation producing short neurites.

In the analysis of the effects of TPA on neuronal tissue, one must take into consideration the concentrations of the tumor promoter used. In the present study, application of low concentrations of TPA (16 nm) enhanced neurite length, density, and area of outgrowth of nonneuronal cells, while high concentrations of TPA (180 to 480 nm) reduced neurite length and area of outgrowth but enhanced neurite density. Dose-dependent effects of TPA producing either an inhibitory or stimulatory effects on several other cell types have also been reported. In human neuroblastoma cells, low concentrations of TPA (16 nm) enhanced differentiation of noradrenaline and neuron-specific enolase (22, 23). At concentrations below or above 16 nm, differentiation of these biochemical markers was less than optimal. In mouse neuroblastoma cells, higher concentrations of TPA (160 nm) were found to inhibit spontaneous or induced differentiation of neurite processes (15). Furthermore, pigmented cells from the neural crest were irreversibly prevented from differentiation with concentrations of TPA above 200 nm (11). Additional studies are needed to clarify the basis for such dose-related effects of TPA which can be either inhibitory or stimulatory.

Our morphological evidence based on silver-stained preparations and electron-microscopic observations firmly supports the hypothesis that TPA enhances neurite differentiation in ganglionic explants. Previous studies indicate, however, that TPA was inhibitory to neurite outgrowth in organized neuronal explants (14). When TPA was added to ganglionic cultures maintained in growth medium containing NGF, a reduced outgrowth response was observed after 2 days in culture when compared to the addition of NGF alone (14, 30). In the present study, we found that the addition of TPA with NGF produced cultures with dense outgrowths of neurites from sensory ganglia. The addition of TPA at low concentrations to growth medium together with NGF did not affect neurite length but significantly increased neurite density. These results suggest that TPA and NGF stimulate neurite outgrowth by different mechanisms. In other studies, TPA did not block the effects of NGF on adhesivity (6), nor did it alter NGF-receptor binding activities in PC12 cells (6). Furthermore, the effects of TPA were additive with NGF in terms of increasing the phosphorylation of specific NGF-sensitive slow-migrating proteins (10). These results and our present findings differ from that of Ishii and Spinelli (14, 30), who demonstrated reduced neurite differentiation in ganglia explants treated with NGF and TPA.

Addition of TPA to sensory ganglia also stimulated proliferation of nonneuronal cells which formed part of the outgrowth area. Removal of these cells by a mitotic inhibitor did not adversely affect the initiation or maintenance of TPA-induced neurite outgrowth. However, some regulation of axonal sprouting and elongation by nonneuronal cells during ganglionic development was evident, since treatment with ara-C also produced an increase in both the density and extent of neurite outgrowth. Other in vivo and in vitro studies have reported enhanced survival and increased sprouting in neuronal tissue treated with ara-C or other mitotic inhibitors (5, 24, 27).

Our results indicate that the tumor promoter TPA may serve as a useful agent for studying neuritogenesis. In addition to its ability to enhance neurite differentiation, its effects on organized explants suggest an involvement in cell-cell interaction and nerve bundle formation. Such regulatory effects on axonal elongation and fasciculation may in turn be significant in understanding guidance mechanisms and substrate interactions during the development of the nervous system.

REFERENCES


Fig. 6. Sensory ganglia treated with NGF (10 ng/ml) in complete medium for 2 days. A halo of fine neurites radiates from the explant. Silver stain, × 163.

Fig. 7. Sensory ganglia treated with NGF (10 ng/ml) and TPA (160 nw) in complete medium for 2 days. Neurites are dense and long. Silver stain, × 157.

Fig. 8. Neuritic processes from ganglionic explant treated with 160 nw TPA and ara-C in complete medium after 2-day frequent fusing at distal portions. Nonneuronal cells are limited in number. Silver stain, × 407.

Fig. 9. Sensory ganglia treated with NGF (10 ng/ml) and TPA (160 nw) in complete medium for 2 days. Neurite length and area of outgrowth are reduced, and dense neurites form fascicles. Silver stain, × 145.

4614 CANCER RESEARCH VOL. 44
Effects of the Tumor Promoter 12-O-Tetradecanoylphorbol-13-acetate on Neurite Outgrowth from Chick Embryo Sensory Ganglia

L. Hsu, D. Natyzak and Jeffrey D. Laskin