

Protection by an ACTH₄₋₉ Analogue Against the Toxic Effects of Cisplatin and Taxol on Sensory Neurons and Glial Cells In Vitro

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Sensory neuropathy is a serious side effect of anti-tumour drugs such as cisplatin and taxol. There are indications that an analogue of the adrenocorticotrophic hormone 4–9 fragment (ACTH₄₋₉: Met(O₂)-Glu-His-Phe-D-Lys-Phe) can prevent these neurotoxic effects. We studied the potential protective effects of this analogue in cultures of chick dorsal root ganglia and rat Schwann cells treated with cisplatin or taxol to gain insight into the mode of action and characteristics of this neuroprotection. Neurite outgrowth of sensory neurons in vitro was dose-dependently inhibited by cisplatin and taxol; after 48 hr, 10 µg/ml cisplatin reduced outgrowth from 431 ± 17 µm to 220 ± 6 µm and 0.01 µg/ml taxol from 344 ± 3 µm to 200 ± 43 µm. Co-treatment of 10 µg/ml cisplatin with the ACTH₄₋₉ analogue (0.1 nM–1 nM) resulted in about 35% more outgrowth than cisplatin alone. In contrast, the analogue could not prevent taxol neurotoxicity. Migration of neurons and satellite cells from the DRG-body is completely inhibited by 10 µg/ml cisplatin. Taxol had no effect on the migration of these cells. In addition, cisplatin was more toxic to Schwann cells than taxol; 3–10 µg/ml cisplatin significantly reduced their laminin content, total protein, 2',3'-cyclic nucleotide 3'-phosphodiesterase activity, and cell division. The ACTH₄₋₉ analogue (0.01 nM–100 nM) had no effect on the migration of cells out of the DRGs and could not prevent the toxic effect on the Schwann cells. These data support our hypothesis that the neuroprotective effect of ACTH₄₋₉ analogue is brought about by a direct action on neurons, possibly by replacing a Schwann-/satellite-cell derived trophic factor.

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INTRODUCTION

Cisplatin and taxol are both potent anti-tumour drugs. Cisplatin is mainly used in the treatment of ovarian and testicular cancer (Gerhenson et al., 1981) and taxol is prescribed to patients with cisplatin refractory ovarian carcinoma (McGuire et al., 1989). Treatment with these drugs is dose-limited because of side-effects, of which the most serious is a sensory neuropathy (Mollman et al., 1988; Lipton et al., 1989). In rats treated with cisplatin or taxol the sensory nerve conduction velocity decreases significantly, but in the same animal the motor nerve conduction velocity is not affected (De Koning et al., 1988; Müller et al., 1990; Hamers et al., 1993b), indicating that treatment with either drug leads to a development of a pure sensory neuropathy in these animals. In vitro taxol and cisplatin inhibit neurite outgrowth from organotypic cultures of chick dorsal root ganglia (DRG) in a dose-dependent way (Mandys et al., 1994).

The oncolytic drug cisplatin (cis-diamminedichloroplatinum) causes morphological changes in DRGs. These changes, observed in animal and culture studies, include 1) an increase in lysosomes in the neurons of the spinal ganglia (Müller et al., 1990), 2) nucleolar segregation, 3) disorganization of ribosomes, 3) neuron shrinkage, and 4) hypertrophy of satellite cells (Tomiwa et al., 1986). In the DRG the main target for cisplatin seems to be the satellite cells, since cisplatin-DNA complexes have been localized in these cells but not in the neurons (Terheggen et al., 1989). In cultures of dorsal root ganglia the non-neuronal (i.e., glial) cells are more affected by cisplatin (Blisard et al., 1992) than neurons.

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Taxol is a complex taxane derivative, isolated from the plant *Taxus brevifolia*. This anti-tumour drug promotes tubulin polymerization in a cell-free system (Kumar, 1981). Taxol treatment of DRG-spinal cord explants results in the formation of an unusual amount of microtubuli and the formation of microtubule-endoplasmic reticulum arrays in DRG neurons (Masurovsky et al., 1981). In these cultures taxol treatment leads to a disorganization of organelle systems of the satellite and Schwann cells (Masurovsky et al., 1983). In the DRG-spinal cord organotypic cultures the oligodendroglia show more changes in morphology (i.e., increase in microtubuli) than the astroglia (Masurovsky et al., 1985). Furthermore, in cultures of dissociated DRG cells taxol inhibits growth cone motility (Letourneau and Ressler, 1984), induces the formation of growth cones varicosities (Sinclair et al., 1988), affects endocytic activity (Sinclair et al., 1988), and inhibits branching (Letourneau et al., 1986). Axonal transport is not affected by taxol (Horie et al., 1987). The effect of taxol on growth cone motility can be reversed by rinsing out the taxol (Sinclair et al., 1988), but there is also partial recovery when taxol remains in the culture medium (Mansfield and Gordon-Weeks, 1991).

Neurotrophic factors play an important role in the prevention or reduction of neurotoxic effects of cisplatin and taxol (Mollman et al., 1988). Peptides, derived from adrenocorticotrophic hormone (ACTH), have neurotrophic and neuroprotective potential (Bär et al., 1990; Gispén, 1990; Strand et al., 1991). An ACTH₄₋₉ analogue, Met(O₂)-Glu-His-Phe-D-Lys-Phe, has a beneficial effect on sciatic nerve regeneration (Van Der Zee et al., 1988), enhances neurite outgrowth from DRG cultures (Bär et al., 1992; Hol et al., 1994), and has neuroprotective actions in animal (De Koning et al., 1988; Gerritsen van der Hoop et al., 1988; Terheggen et al., 1989; Müller et al., 1990; Hamers et al., 1993a,b) and man (Gerritsen van der Hoop et al., 1990). Another neurotrophic factor, nerve growth factor (βNGF), involved in sensory and sympathetic ganglion development, has shown to have protective abilities in several neurotoxicity models (Peterson and Crain, 1982; Apfel et al., 1991).

In order to study the protective effects of the ACTH₄₋₉ analogue and to understand more about the mode of action of this peptide, we compared its effect in organotypic cultures of 12-day-old chick embryo DRGs that were exposed to taxol or cisplatin, two drugs with different mechanisms of action both resulting in inhibition of neurite outgrowth. Furthermore, to investigate the role of Schwann cells in neuroprotection/-toxicity we treated Schwann cells with cisplatin, taxol, and the ACTH₄₋₉ analogue. To complete our studies we compared the protective effects of the ACTH-analogue to βNGF in cultured DRGs that were damaged by cisplatin and taxol.

MATERIALS AND METHODS

Cultures

Dorsal root ganglia. Dorsal root ganglia of 12-day-old chick (developmental stage 37–38 according to Hamburger and Hamilton, 1951) were dissected and cultured in semi-solid medium as described before (Mandys et al., 1994). In short, DRGs were grown in petri dishes containing two layers of a mixture of agar and culture medium: the bottom layer contained 0.5% agar and the top layer 0.25% agar (DIFCO agar, Noble, Detroit, MI). The culture medium consisted of minimal essential medium (MEM, GIBCO, Paisley, Scotland) supplemented with 10% heat inactivated foetal calf serum (FCS, GIBCO), 100 U/ml benzylpenicilline (Centrachemie, Woerden, The Netherlands), 100 µg/ml streptomycin sulphate (Pharmachemie, Haarlem, The Netherlands), and 3 ng/ml βNGF (Boehringer, Mannheim, Germany). In the experiment on the effect of βNGF on outgrowth protection no basal amount of NGF was added (Fig. 3). To study migration of glial cells the DRGs were cultured under the same conditions (i.e., the same culture medium without agar) in poly-L-lysine (PLL, Sigma, St. Louis, MO) coated petri dishes. DRGs were cultured at 37°C in a humidified atmosphere under 6.5% CO₂ in air.

Schwann cells. Sciatic nerves from 2–5-day-old rat pups (Wistar) were dissected and collected in HEPES buffered Dulbecco's modification of Eagle's medium (DMEM; pH 7.4). The sciatic nerves were incubated for 45 min with 0.1% collagenase at 37°C, followed by 0.125% trypsin for 15 min. The trypsinization was stopped by adding 2 ml DMEM containing 10% FCS. The nerves were mechanically dissociated by passing 3 times through an 0.8 mm needle with a syringe. The cell suspension was filtered through a 50 µm filter to remove pieces of intact tissue. The cells were spun down (10 min, 500g) and plated in collagen coated 96-wells plates (laminin ELISA) or petri dishes (CNPase-assay). The culture medium consisted of DMEM supplemented with 10% FCS, 100 U/ml benzylpenicilline, 100 µg/ml streptomycin sulphate, and 6 g/L glucose. Schwann cells were cultured at 37°C in a humidified atmosphere under 6.5% CO₂ in air. For some experiments a Schwann cell line was used (passage 36), obtained from primary Schwann cells repeatedly stimulated with 3 µM forskolin (Van Breugel et al., in prep). These cells were cultured in poly-L-lysine (PLL) coated 96-wells plates under the same conditions as the primary Schwann cells.

Treatment

Dorsal root ganglia. Cisplatin (cis-diamminedichloroplatinum, Pharmachemie) and taxol (Bristol-Meyers Squibb, Princeton, NJ) were added to both layers of agar or to the culture medium at final concentrations of

10 $\mu\text{g/ml}$ (cisplatin) and 0.01 $\mu\text{g/ml}$ (taxol) (almost equipotent doses based on earlier experiments [Mandys et al., 1994]). Cisplatin (0.5 mg/ml) was dissolved in 0.9% NaCl. Taxol stock solution (6 g/L) was dissolved in Cremophor EL (Sigma)/dehydrated ethanol (50:50 v/v). Cisplatin and taxol were freshly diluted in MEM before each experiment. The ACTH₄₋₉ analogue was diluted in MEM and added to both layers of culture medium/agar in a final concentration of 0.1, 1, 10, or 100 nM.

Schwann cells. After 24 hr in culture the primary Schwann cells were treated with cisplatin (0.03–30 $\mu\text{g/ml}$) for 4 hr. The medium was replaced with culture medium with or without the ACTH₄₋₉ analogue (0.1–10 nM). After 1 week the protein amount was determined according to Bradford (1976), or the cells were fixed with methanol and the laminin content was measured with an ELISA as described before (Van der Neut et al., 1988). 2',3'-Cyclic nucleotide 3'-phosphodiesterase (CNPase) activity was measured after 12 days in vitro (Kurihara et al., 1981). The Schwann cell line was treated with 10 $\mu\text{g/ml}$ cisplatin or 0.01 $\mu\text{g/ml}$ taxol for 48 hr. One day after treatment the cells were pulsed with 10 μM 5-bromo-2'-deoxyuridine (BrdU) for 24 hr. Cells were fixed, protein amount was determined, and cell division was measured with a BrdU-ELISA.

Evaluation of DRG outgrowth. In each experiment 8 DRGs were studied per condition. The DRGs were observed after 24 and 48 hr in vitro under an inverted microscope and the outgrowth was quantified morphometrically (see Fig. 4D for a representation of the regular neurite outgrowth). In some experiments photographs or drawings of individual DRGs were evaluated with a digitizer (Graphtec, Japan). In later experiments the outgrowth was quantified with an image analysis system (TIM: 1 pixel = 171 μm^2). Both methods produced the same results. The mean neurite length (L) was calculated by 1) measuring the length of 5 separate neurites per DRG, measured from the edge of the ganglion to the end of the neurite, or 2) derived from the outgrowth surface (L = average radius of the total outgrowth – radius of the ganglion). During the experiment the investigator was not aware of the treatment of the DRGs.

Laminin ELISA. After fixation with methanol the Schwann cells were incubated with phosphate buffered saline (PBS) supplemented with 10% horse serum (PBS-H10; pH 7.4) for 30 min at 37°C to block nonspecific binding. The cells were rinsed 3 times with PBS and incubated with rabbit-anti-laminin 1:500 (EY Labs Inc., San Mateo, CA) in PBS-H10 for 60 min at 37°C. After rinsing with PBS the cells were incubated with peroxidase conjugated goat-anti-rabbit (Sigma) for 60 min at 37°C. The peroxidase was detected with 0.2% orthophenylene diamine and the absorbance was read at 490 nm (BioRad microplate reader 3550).

CNPase assay. Schwann cells were rinsed twice with 0.9% NaCl, harvested in 250 μl 0.01 M Tris/HCl pH 7.3, and homogenized with ultrasound (Branson sonifier B12, 70 W, 3 \times 15 sec). The samples were split in two for protein determination and CNPase activity. After 30 μl 0.66% cetavlon (N-hexadecyltrimethyl ammonium bromide) 0.067 M 2[N-morpholino]ethanesulfonic acid (MES, Sigma) was added to 20 μl sample they were left on ice for 10 min. The enzyme reaction was started with the addition of 150 μl of a MES (0.033 M) buffer containing 6.67 mM cAMP (Sigma) to the samples and incubated at 30°C for 20 min. The reaction was stopped with 300 μl 0.067 M Na₂CO₃, 0.33U alkaline phosphatase (30 min, 37°C). The colour reaction was started by adding 1 ml colouring reagent containing: 2% sodium dodecyl sulphate, 2% ascorbic acid, 4.8% H₂SO₄, 0.004 M (NH₄)₆Mo₇O₂₄·4H₂O (Merck, Darmstadt, Germany). After 45 min at 40°C the absorbance was read at 750 nm (Pye Unicam SP8-100 spectrophotometer) and the values were corrected for the amount of protein.

Characterisation of migrating cells. The DRGs were fixed with 4% paraformaldehyde for 15 min, rinsed 4 \times with Tris-buffered saline (TBS; 0.05M Tris, 0.9% NaCl, pH 7.4), and incubated overnight at room temperature with anti-neurofilament (RT97 1:50, Boehringer Mannheim), anti-Thy1.1 (1:250) or anti-S100 (1:2000) diluted in 0.05M Tris with 0.9% NaCl, 1% BSA, and 0.5% Triton X-100 (pH 7.4). Hereafter the DRGs were rinsed 4 \times with TBS and incubated with biotinylated goat-anti-rabbit or horse-anti-mouse antiserum (1:220; Vector) for 1 hr at room temperature, followed by incubation with streptavidin-conjugated FITC (Dakopatts: 1:100).

Statistics. Data were analysed with a Student's t-test or an analysis of variance followed by a supplementary t-test.

RESULTS

Dorsal Root Ganglia

Neurite outgrowth. Embryonal chick DRGs (mean diameter 562 \pm 7 μm) have a large outgrowth potential. After 48 hr in culture the neurites reached a mean length of 393 \pm 20 μm . Figure 1 shows the effect of 10 $\mu\text{g/ml}$ cisplatin (mean of 4 independent experiments) and 0.01 $\mu\text{g/ml}$ taxol (mean of 3 independent experiments) on the mean neurite length. After 48 hr in culture cisplatin reduced the outgrowth from 431 \pm 18 μm to 220 \pm 6 μm ($P < 0.001$) and taxol from 344 \pm 3 μm to 200 \pm 43 μm ($P < 0.05$).

Neurites of DRGs treated with cisplatin in combination with 10 nM of the ACTH₄₋₉ analogue were 35 \pm 3% longer ($P < 0.001$, mean of 4 independent experiments) compared to DRGs treated with cisplatin alone

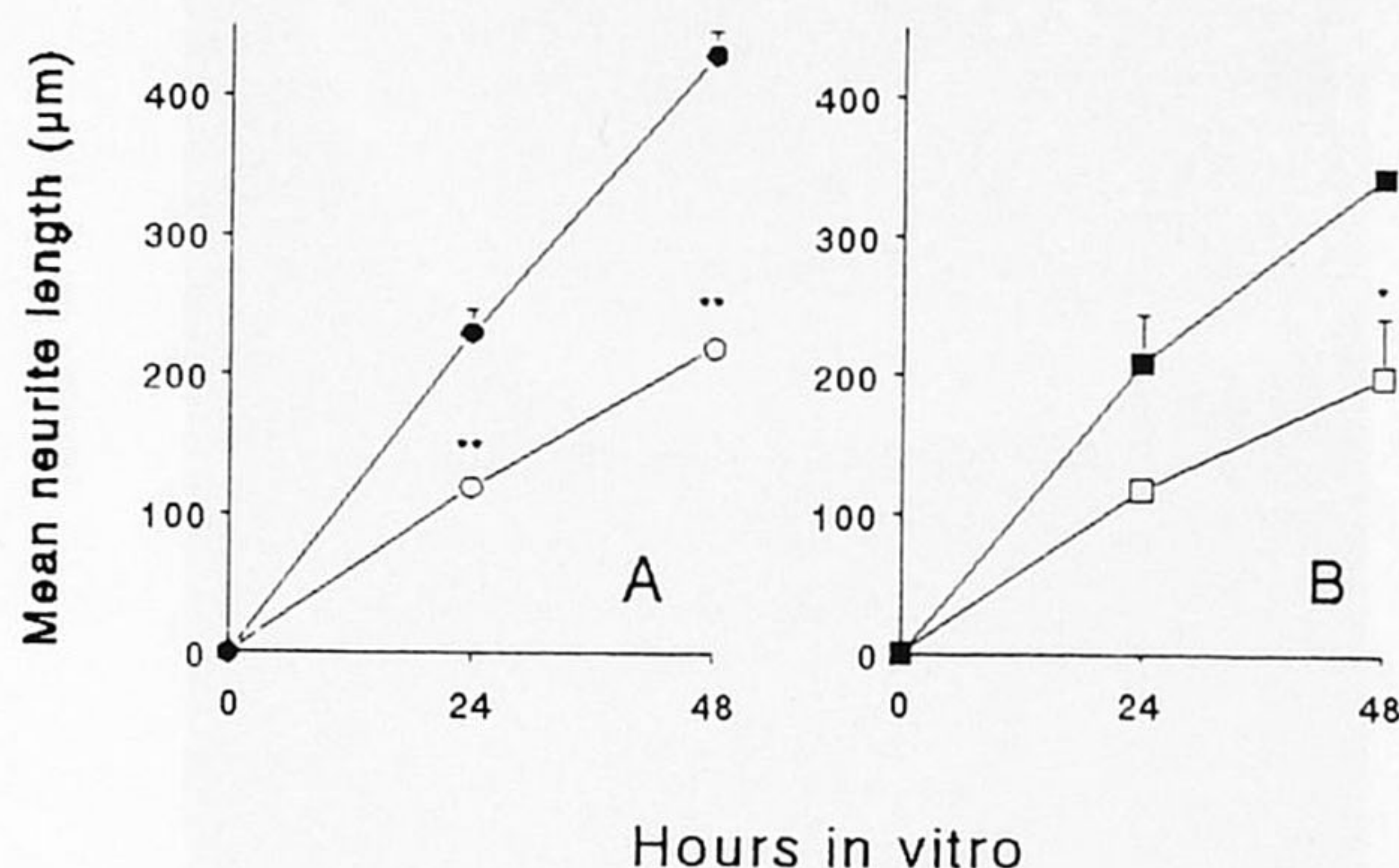


Fig. 1. Effect of 10 μg/ml cisplatin (A) and 0.01 μg/ml taxol (B) on the length of outgrowing neurites from chick ED 12 dorsal root ganglia after 24 hr and 48 hr in culture. ●, cisplatin vehicle (0.018% NaCl); ○, 10 μg/ml cisplatin; ■, taxol vehicle (0.0002% Cremophor EL/dehydrated ethanol [50:50 v/v]); and □, 0.01 μg/ml taxol. Values are represented as mean ± SEM of 4 independent experiments (cisplatin) or 3 independent experiments (taxol) (* $P < 0.05$, ** $P < 0.001$, Student's *t*-test, cisplatin or taxol versus vehicle).

(Fig. 2). All 3 concentrations of the ACTH₄₋₉ analogue tested were effective in preventing cisplatin induced inhibition of outgrowth. The taxol treated DRGs could not be protected by 1, 10, or 100 nM of the ACTH₄₋₉ analogue (Fig 2).

In our model βNGF prevented both cisplatin and taxol damage to DRGs (Fig. 3). The mean neurite length of the vehicle treated DRGs (no basal amount of βNGF added to the culture medium) was 90 ± 12 μm. Cisplatin (10 μg/ml) decreased the outgrowth to 58 ± 7 and taxol (0.01 μg/ml) decreased the outgrowth to 58 ± 6 μm ($n = 6-8$, $P < 0.001$, control versus cisplatin or taxol). βNGF (8 ng/ml) increased the outgrowth dose-dependently up to 177 ± 4 μm. βNGF prevention was only partial because under control condition 8 ng/ml βNGF would lead to an average neurite length of 412 ± 10 μm (data not shown).

Morphology and cell migration. Cisplatin and taxol both inhibited neurite outgrowth, but they differed strongly in their effect on glial cell migration. Migrating cells were characterized and were shown to be S-100 positive, demonstrating that these cells are satellite cells. None of the cells were positive for the fibroblast marker Thy 1.1. Few of the migrating cells were neurofilament (NF) positive, indicating that incidentally neurons migrate from the DRG body. Under control conditions DRG cultured on PLL-coated dishes form a network of neurites intermingled with satellite cells and a few NF-positive cells that have migrated from the DRG body (Fig. 4A). Cisplatin (10 μg/ml) completely prevents migration of these cells (Fig. 4B). The neurite network is

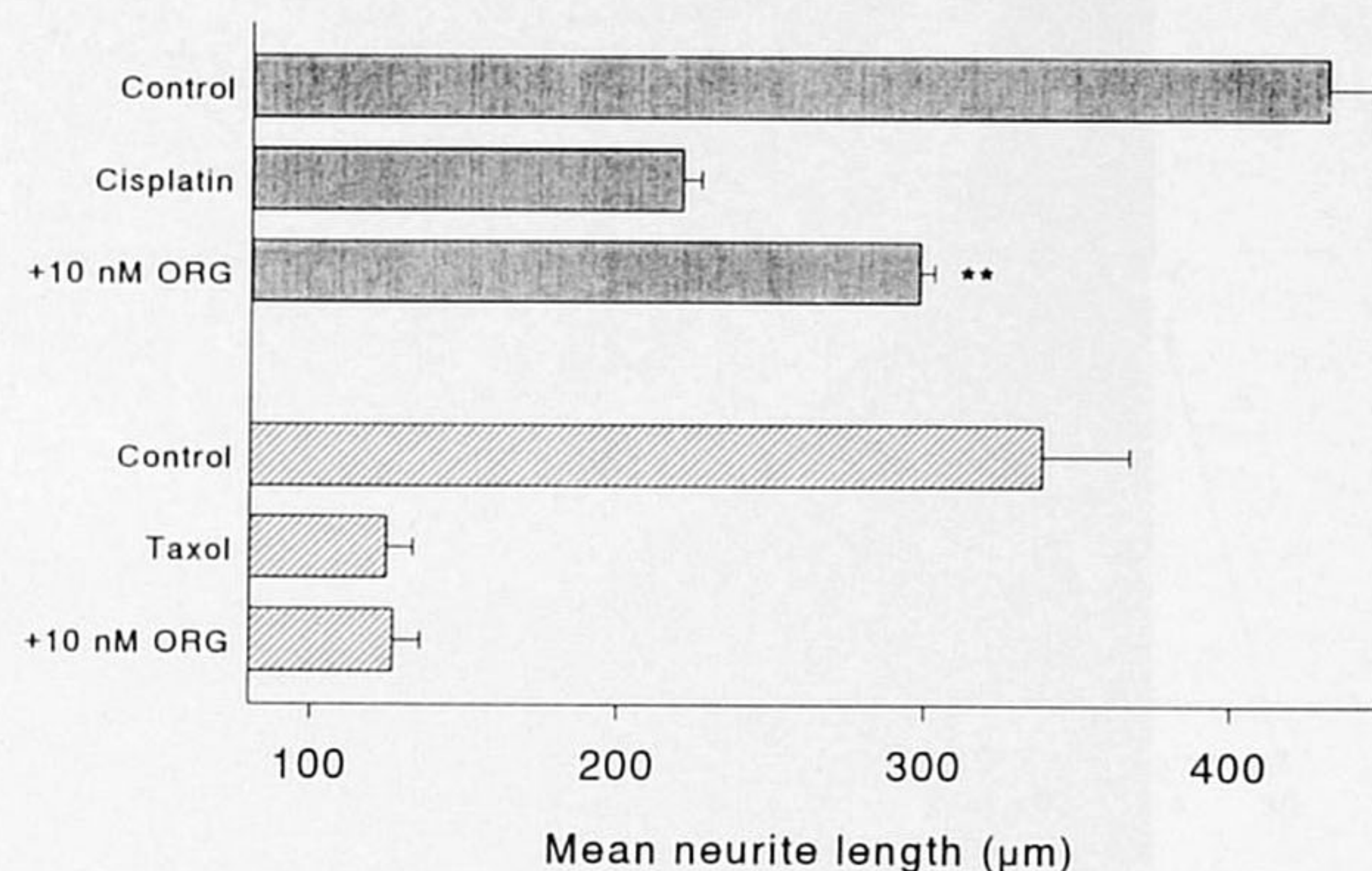


Fig. 2. Effect of the ACTH₄₋₉ analogue on the mean neurite length of chick ED12 DRG's treated with 10 μg/ml cisplatin or 0.01 μg/ml taxol. Values are expressed as mean ± SEM of 3 experiments (cisplatin) or of 1 representative experiment (taxol, $n = 8$) (** $P < 0.001$, Student's *t*-test cisplatin versus cisplatin-ACTH analogue).

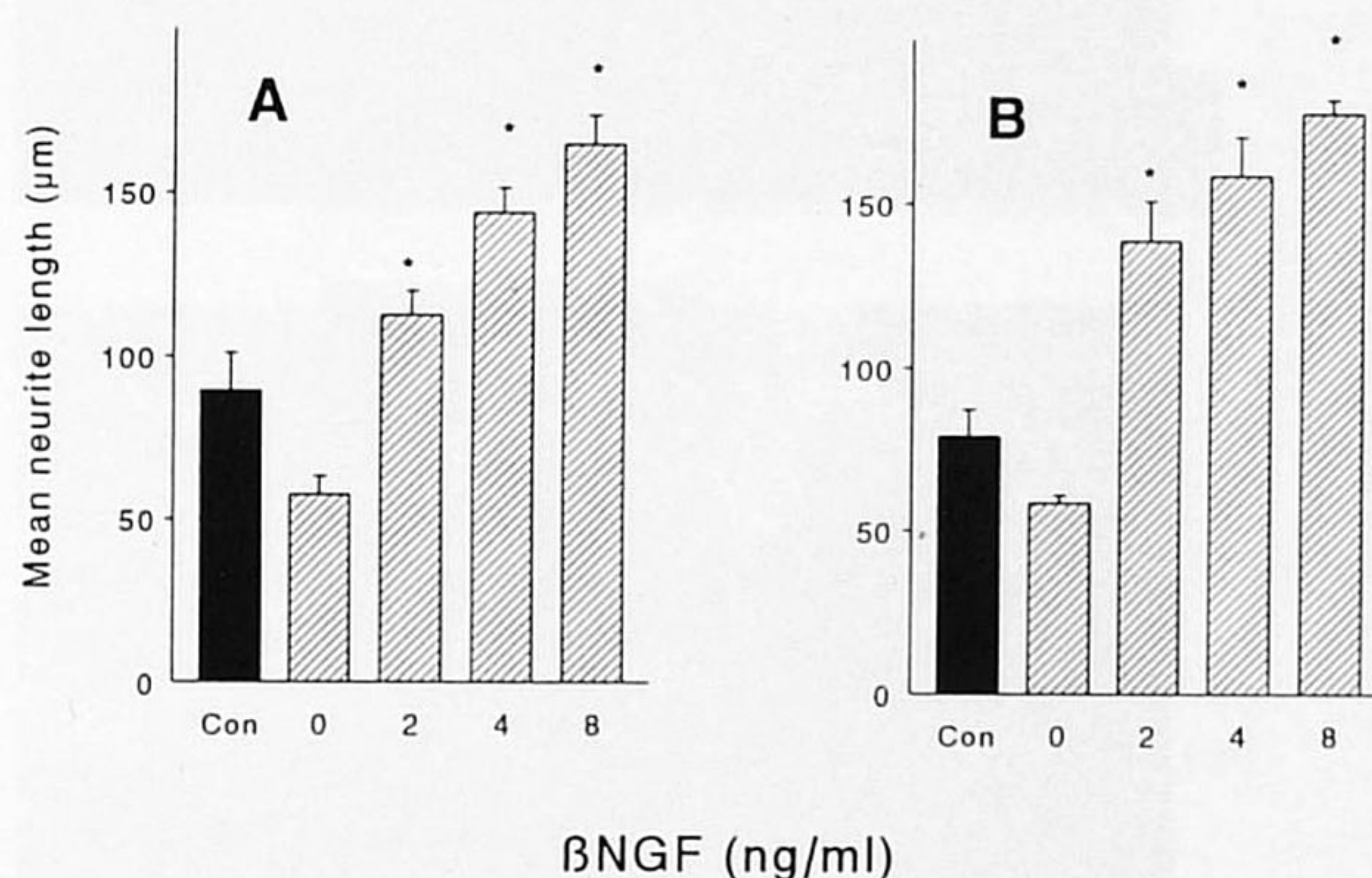


Fig. 3. Effect of βNGF on the mean neurite length of chick ED12 DRGs treated with (A) 0.01 μg/ml taxol and (B) 10 μg/ml cisplatin. In the control treatment (filled bar) no βNGF was added to the culture medium. Values are expressed as mean ± SEM of 1 experiment ($n = 6-8$) (ANOVA: A: $F = 39.57$, $df = 28$, $P < 0.001$; B: $F = 34.91$, $df = 31$, $P < 0.001$; * $P < 0.05$, supplementary *t*-test).

also changed by cisplatin treatment: it is less dense and the neurites are shorter and thicker. Taxol (0.01 μg/ml) has no effect on the number and type of migrating cells (Fig. 4C), but does affect the pattern of the neurite outgrowth. The neurites were shorter and their branching was reduced. Some neurites, however, were very thin and long, with almost no branches. These qualitative differences have been observed in 3 independent experiments.

Sciatic Nerve Schwann Cells

Cisplatin (0.03–30 μg/ml) reduced the amount of laminin and protein dose-dependently. Figure 5A shows

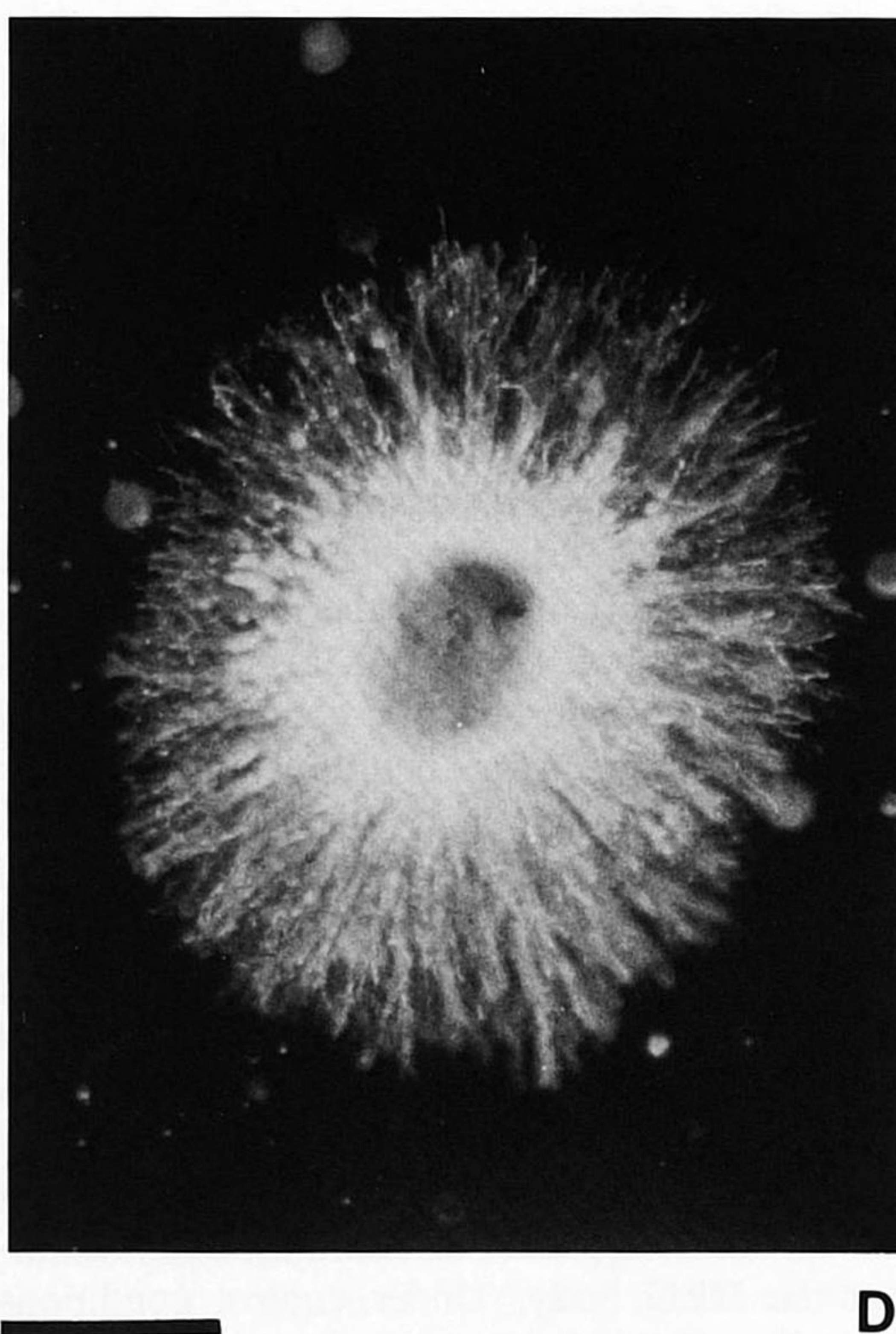
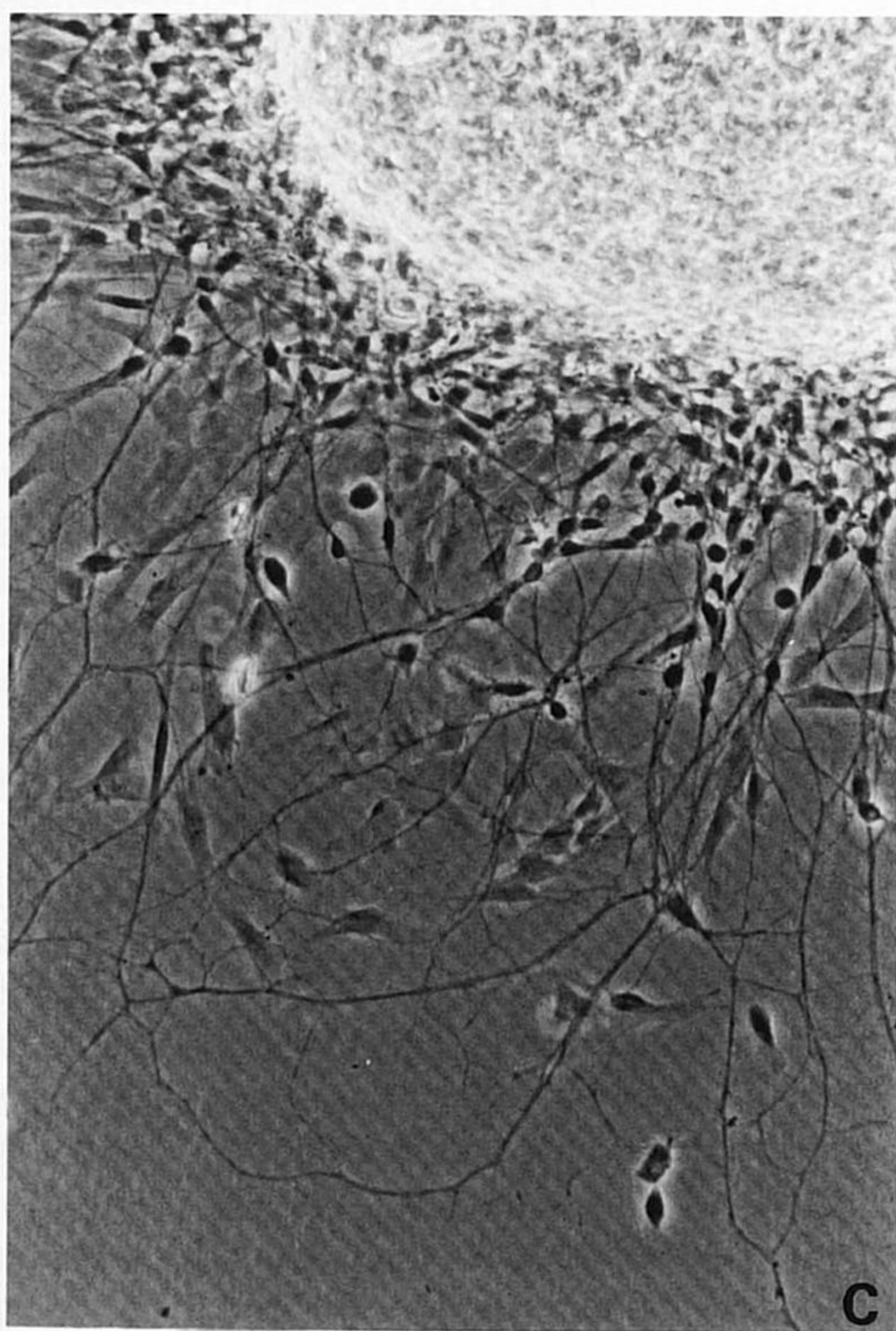
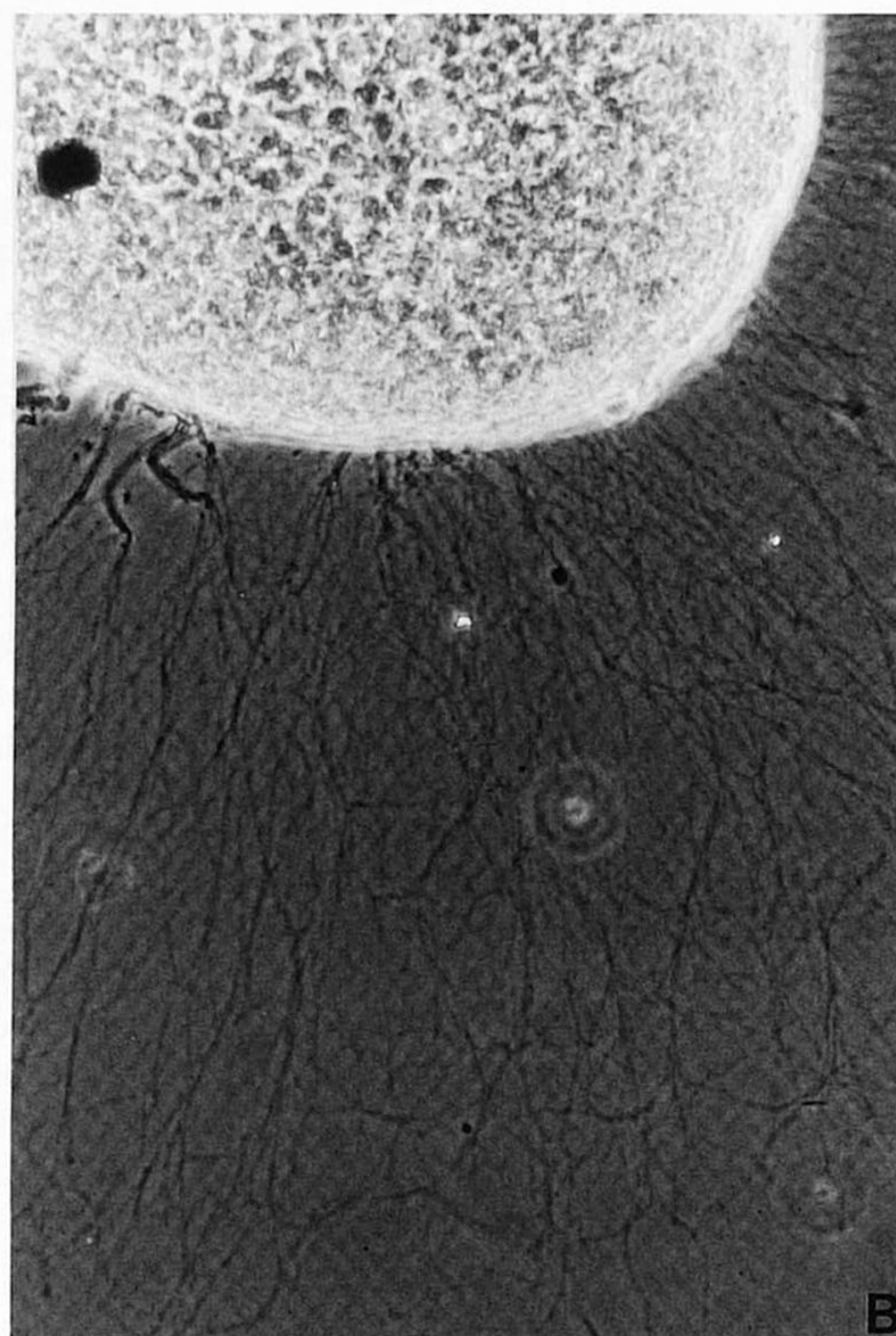
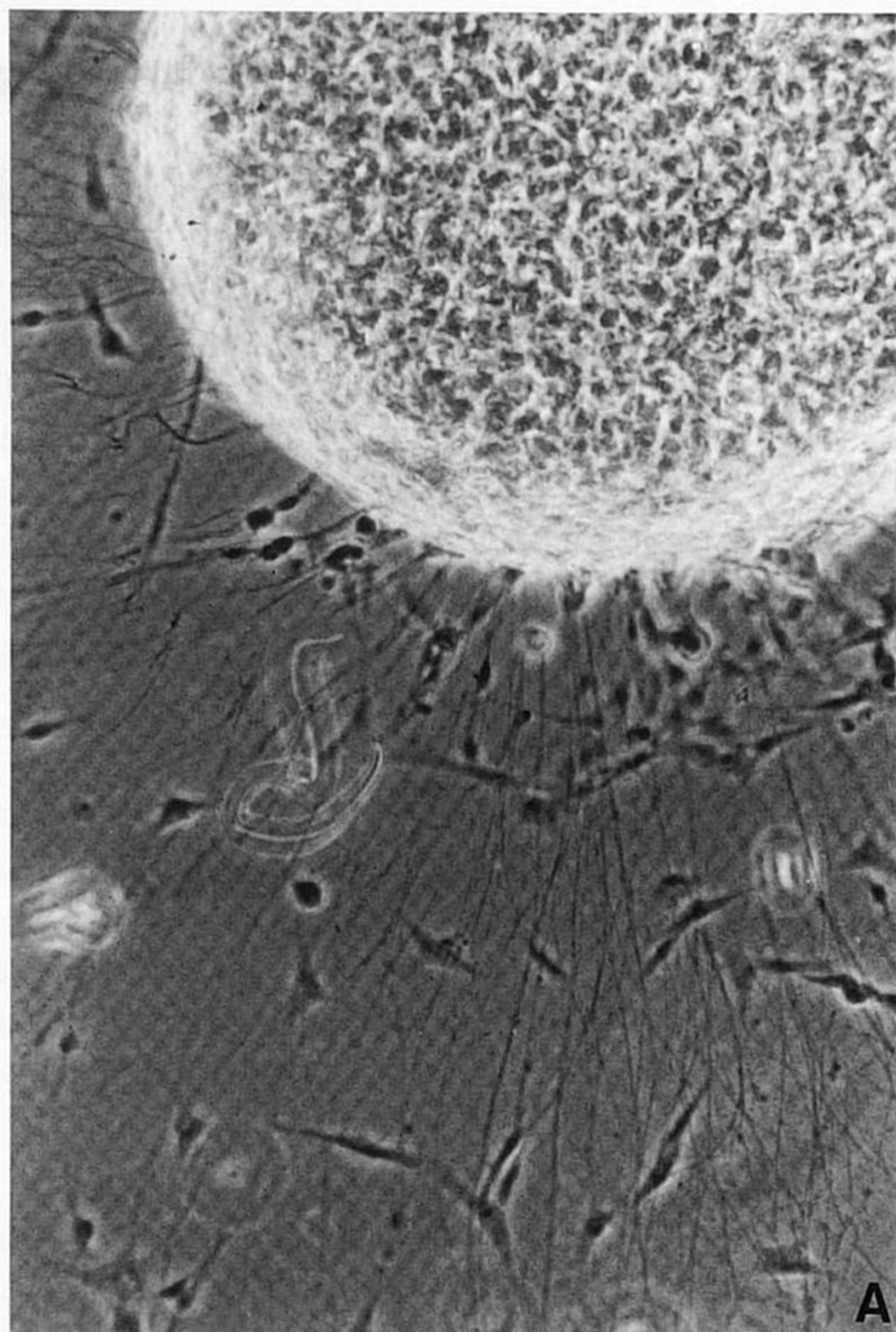


Fig. 4. Neurite outgrowth and cell migration from chick ED12 dorsal root ganglia 48 hr in vitro. **A:** Vehicle treated DRGs with migrated cells intermingled in neurite outgrowth. **B:** Effect of cisplatin treatment on the neurite outgrowth and cell migration. **C:** Effect of taxol on the neurite outgrowth. **D:** Representation of the regular outgrowth of a chick ED12 DRG cultured in agar (bar in A, B, and C is 160 μ M; bar in D is 550 μ M).

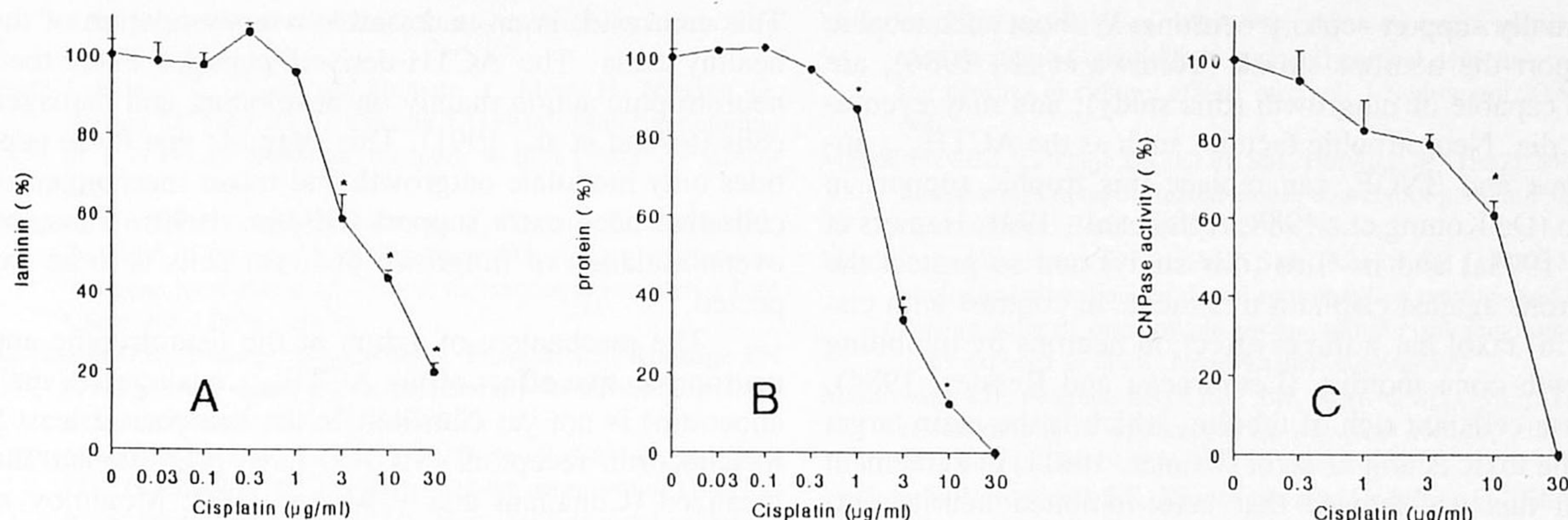


Fig. 5. Effect of cisplatin on (A) laminin content, (B) total protein, and (C) CNPase activity in cultured Schwann cells. Values are expressed as mean \pm standard error of 1 representative experiment. (ANOVA: A, $n = 3$ $F = 276.70$, $df = 16$, $P < 0.001$; B, $n = 2$ $F = 1065.45$, $df = 7$, $P < 0.001$; C, $n = 2$ $F = 9.90$, $df = 9$, $P = 0.014$; * $P < 0.05$, supplementary t-test.)

that after 1 week in culture a dose of 10 µg/ml cisplatin reduced the laminin content to $43 \pm 2\%$ of the control value ($n = 3$, $P < 0.05$). In the same experiment (Fig. 5B) 10 µg/ml cisplatin reduced the amount of protein to $12 \pm 0.4\%$ ($n = 2$, $P < 0.05$). After 12 days in culture cisplatin also reduced the CNPase activity dose-dependently (Fig. 5C): 10 µg/ml caused a 39% reduction of the enzyme activity ($n = 2$, $P < 0.05$). In almost all experiments 30 µg/ml cisplatin was lethal for the Schwann cells. Figure 6 shows that 10 nM of the ACTH₄₋₉ analogue could not prevent cisplatin induced reduction of the laminin content. Also, other concentrations of the analogue (0.001 nM up to 100 nM) were not effective in protecting the Schwann cells from cisplatin.

In addition, 10 µg/ml cisplatin affected the Schwann cell line. Cisplatin decreased the amount of protein with 53% ($n = 8$, $P < 0.001$) and the BrdU incorporation with 87% ($n = 8$, $P < 0.001$). When these cells were treated with 0.01 µg/ml taxol the amount of protein was decreased with 19% ($n = 8$, $P < 0.001$) and the BrdU incorporation with 35% ($n = 8$, $P < 0.001$, $n = 8$; results not shown).

DISCUSSION

Previous studies have shown that an ACTH₄₋₉ analogue, Met(O₂)-Glu-His-Phe-D-Lys-Phe, can protect against sensory neuropathy in cisplatin and taxol treated rats (De Koning et al., 1988; Hamers et al., 1993a,b). In order to examine the cell types involved and to learn more about the mechanism of action of the peptide, we studied the effects of this ACTH₄₋₉ analogue in cultured chick dorsal root ganglia treated with cisplatin and taxol. These ganglia, which contain only sensory neurons, are

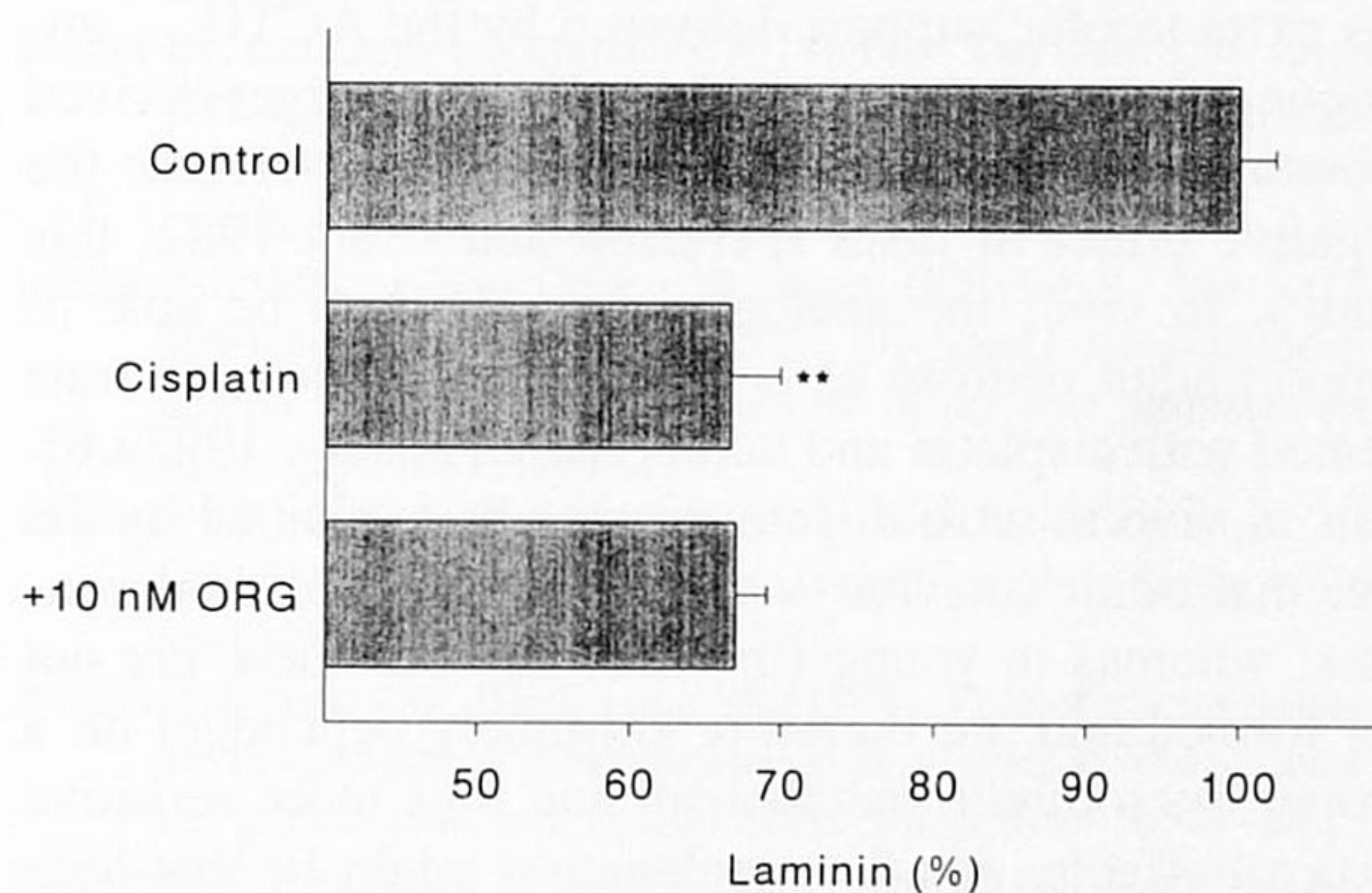


Fig. 6. Effect of the ACTH₄₋₉ analogue on the laminin content of Schwann cells treated with 10 µg/ml cisplatin. Values are expressed as mean \pm SEM of 1 representative experiment ($n = 4$). ** $P < 0.001$, Student's t-test cisplatin versus control.

primarily affected in cisplatin- and taxol-induced sensory neuropathy (Müller et al., 1990). Our results indicate that the ACTH₄₋₉ analogue can partially protect against the outgrowth inhibiting action of cisplatin. In the presence of this peptide the mean neurite length in DRG cultures treated with cisplatin was increased with 35%. In contrast the analogue had no positive effect on taxol neurotoxicity in the concentrations studied. The finding that the analogue could partially restore outgrowth after cisplatin but not after taxol treatment is probably due to the different mechanisms of action by which these oncolytics exert their neurotoxic action.

Cisplatin mainly affects satellite cells (Terheggen et al., 1989; Blisard et al., 1992; this study) and Schwann cells (Sodaar et al., 1989; this study) which

normally support sensory neurons. Without such trophic support the neurons shrink (Tomiwa et al., 1986), are less capable of outgrowth (this study), and may eventually die. Neurotrophic factors, such as the ACTH₄₋₉ analogue and β NGF, can replace this trophic support in vivo (De Koning et al., 1988; Apfel et al., 1991; Hamers et al., 1993a) and in vitro (this study) and so protect the neurons against cisplatin treatment. In contrast with cisplatin, taxol has a direct effect on neurons by inhibiting growth cone motility (Letourneau and Ressler, 1984). These cells are rich of tubulin, which is the main target of the toxic action of taxol (Kumar, 1981). In agreement with this we showed that taxol-inhibited neurite outgrowth did not diminish the migration of satellite cells out of the ganglia and only mildly reduced the BrdU incorporation in Schwann cells. Thus Schwann cells and satellite cells were less affected (this study) and could probably still produce their trophic support. However, since neuritogenesis is disturbed at the level of microtubules, this trophic support has not the required effect, nor has extra trophic support delivered by the ACTH₄₋₉ analogue. In contrast, a physiological target-derived growth factor of DRGs (β NGF) is able to overcome the negative effect of taxol (Peterson and Crain 1982; this study). In vivo, the analogue does seem to be able to support adult neurons as is evidenced by studies on rats treated with cisplatin and taxol (Hamers et al., 1993a,b). This in vivo/in vitro difference may be explained by the fact that adult (in vivo) neurons have established neurites, whereas in young (in vitro) neurons these are not yet formed and are therefore extremely dependent on a proper microtubule metabolism and thus more sensitive to taxol effects. Another explanation might be that both taxol (Masurovsky et al., 1981) and the ACTH₄₋₉ analogue (Müller et al., 1992) stimulate microtubuli formation. This means that if the prevention by the analogue is mainly due to an increase in microtubuli the peptide may not be able to exert a beneficial effect in taxol-treated DRGs, as taxol has already induced changes in the microtubuli.

It is important to determine what cells are involved in the prevention of the sensory neuropathy in vivo so that cell specific substances can be used to protect these cells and thus, sensory function in patients treated with cytostatics (Gispen, 1990). ACTH-derived peptides might play an important role in the prevention of cisplatin and taxol neurotoxicity in view of their relative safety. The ACTH₄₋₉ analogue has already been shown to be effective in a clinical trial of cisplatin-treated patients with ovarium cancer (Gerritsen van der Hoop et al., 1990). In contrast with the ACTH-derived peptides, neurotrophic factors, such as β NGF, are very potent growth promoting factors, which might stimulate outgrowth from damaged as well as undamaged target cells.

This can result in an undesirable overstimulation of the healthy cells. The ACTH-derived peptides exert their neurotrophic action mainly on developing and damaged cells (Strand et al., 1991). This suggests that these peptides only modulate outgrowth and repair mechanism of cells that need extra support (Gispen, 1990). Thus, no overstimulation of fullgrown or intact cells is to be expected.

The mechanism of action of the neurotrophic and neuroprotective effect of the ACTH₄₋₉ analogue (a melanocortin) is not yet clarified. In the last year at least 5 melanocortin receptors (MC1-5) have been cloned and localized (Chhajlani and Wikberg, 1992; Mountjoy et al., 1992; Gantz et al., 1993a,b). So far, none of them has been shown to have the ACTH₄₋₉ analogue as its agonist. Despite this we have some indications that the analogue acts via a subtype of the melanocortin receptor. The MC-receptors, belonging to the larger family of the 7-transmembrane spanning receptors, are adenylate cyclase coupled. In spinal cord neurons we found with α MSH and the ACTH₄₋₉ analogue (both melanocortins) an increase in cAMP formation within 15 min after treatment (Hol et al., 1991). This indicates that both peptides may act via a subtype of the melanocortin receptor. The presence of such receptors on DRG- and glial-cells remains to be demonstrated.

We conclude that the ACTH₄₋₉ analogue protects against cisplatin-induced outgrowth inhibition from chick dorsal root ganglia. Most probably the ACTH₄₋₉ analogue acts directly on the sensory neurons. The analogue was not able to prevent taxol neurotoxicity, cisplatin-induced Schwann cell death, cisplatin-induced satellite cell death, or inhibition of the migration of the satellite cells.

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