Journal of Neuroscience Research 44:142–148 (1996)

Upregulation of B50/GAP-43 Protein mRNA in Rat Dorsal Root Ganglia During Cisplatin Intoxication

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Expression of the growth-associated protein B50 (GAP-43) mRNA in dorsal root ganglia (DRG) of rats was studied by in situ hybridization. In response to treatment with the neurotoxic agent cisplatin, B50 mRNA expression was significantly enhanced following a cumulative cisplatin dose of 14 mg/kg. In the untreated age-matched control animals, only half of the ganglion cells exhibited expression of B50 mRNA (mean hybridization signal, 10 times background), whereas at a cumulative cisplatin dose of 14 mg cisplatin every neuron exhibited well above background expression (mean hybridization signal, 34 times background). Cotreatment with a neuroprotective $ACTH_{4-9}$ analog known to prevent cisplatin neuropathy in rats did not affect the overall expression of B50 mRNA. However, in the subpopulation of large sensory neurons, B50 mRNA content was significantly higher in the group cotreated with the $ACTH_{4-9}$ analog as compared with the saline-cotreated group after 14 mg/kg of cisplatin. We conclude that in analogy with the well-known upregulation of B50 mRNA following mechanical nerve lesions, treatment with the neurotoxic drug cisplatin also leads to an increase in B50 mRNA expression. This observation lends strength to the hypothesis that in neuropathies an imbalance between regenerative and degenerative mechanisms exists. The ability of the larger sensory neurons to retain an increased B50 mRNA expression better after cotreatment with the peptide than without may be related to stimulation of regenerative processes by this $ACTH_{4-9}$ analog. © 1996 Wiley-Liss, Inc.

Key words: B50/GAP-43, in situ hybridization, cisplatin neurotoxicity, ORG 2766

INTRODUCTION

The B50 (GAP-43) protein is a neural calmodulin-binding phosphoprotein that was originally described by Zwiers et al. (1976) and that is prominently expressed in the developing and regenerating nervous system (Skene, 1989). Expression of B50 protein is intimately associated with the formation of nerve fibers both during development and following lesioning of peripheral nerves. The protein is, however, also found in certain populations of mature neurons, including hippocampal neurons and olfactory bulb mitral cells (Oestreicher et al., 1986; Neve et al., 1987; Verhaagen et al., 1989), dorsal root ganglia (DRG) (Verge et al., 1990), and Schwann cells in degenerating peripheral nerve stumps (Curtis et al., 1992; Plantinga et al., 1993). In DRG neurons, B50 mRNA expression has been shown to be upregulated after mechanical nerve damage (Basi et al., 1987; Van der Zee et al., 1989; Verge et al., 1990; Chong et al., 1994). The high expression of B50 during neuronal growth and regeneration (Skene and Willard, 1981; Verhaagen et al., 1986; Skene, 1989), and the observation that higher-than-normal expression levels of B50 mRNA in PC12 cells lead to accelerated initial neurite outgrowth rates and increased sensitivity to NGF (Yankner et al., 1990), suggest a central role for this protein in the processes of nerve outgrowth and regeneration.

During the last decade it was shown that ACTH and several of its fragments enhance functional and histological recovery of a peripheral nerve crush (Strand et al., 1989; Bär et al., 1990). Moreover, the degradation resistant $ACTH_{4-9}$ analog ORG 2766 was shown to protect from neuropathies such as that induced by cisplatin in rats (De Koning et al., 1988; Hamers et al., 1991b, 1993; Gerritsen van der Hoop et al., 1988, 1994). In humans, a cisplatin induces a sensory neuropathy with preferential disease of the thick myelinated fibers responsible for

Received May 5, 1995; revised June 20, 1995; accepted October 27, 1995.

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propriocepsis and vibration sense (Gerritsen van der Hoop et al., 1990; Hamers et al., 1991b), while the small unmyelinated fibers responsible for pain and temperature sense remain relatively spared (Thompson et al., 1984; Elderson et al., 1989). In the rat model, a significant decrease in sensory nerve conduction velocity (dependent on thick fiber function) is observed after cumulative cisplatin doses of 12 mg/kg and above, whereas motor nerve conduction velocity is not affected (De Koning et al., 1988); moreover, a shift in diameter distribution towards smaller axonal diameters in the sural nerve has been observed (Gerritsen van der Hoop et al., 1994). The neuropathy in the rat model is readily reversible; sensory nerve conduction velocity returns to control values within 10 weeks after discontinuation of 20 mg cisplatin per kg treatment (Hamers et al., 1993), indicating that regenerative mechanisms are easily mobilized. It is not known how cisplatin exerts its neurotoxic effect: the facts, however, that the DRG, in contrast to the spinal cord, are highly exposed to cisplatin, and that sensory neurons but not motor neurons are particularly affected by cisplatin treatment (Thompson et al., 1984), point towards a primary action of cisplatin at the level of the cell body.

Upregulation of B50 expression occurs early during neuronal regeneration after mechanical lesions of the peripheral nervous system (Van der Zee et al., 1989; Verge et al., 1990; Woolf et al., 1990), and the amount of B50 protein is correlated with the number of sprouts (Van der Zee et al., 1989). In a model for acrylamide-induced neuropathy, B50 protein content in peripheral nerve axons was shown to increase after onset of this neuropathy (Bisby and Redshaw, 1987), indicating that B50 upregulation can also occur in toxic nerve damage. The latter observation indicates that during development of a neuropathy, both degenerative and regenerative processes can occur together. It has been hypothesized that under normal conditions regenerative and degenerative factors balance each other, and that neuronal damage develops only if degeneration wins out over regeneration. This might also explain the fast recovery from cisplatin-induced neuropathy observed in the rat model (Hamers et al., 1993): removal of the noxious stimulus leads to an immediate regenerative response. According to this hypothesis, application of neurotrophic substances would push the balance between degeneration and regeneration towards the latter (Gispen, 1990; Apfel et al., 1991).

Early application of αMSH after a peripheral nerve crush enhances the sprouting response (Verhaagen et al., 1987). ORG 2766, like αMSH, enhances this initial sprouting response after mechanical nerve damage, and the peptide protects against several toxic neuropathies (Bär et al., 1990). We wanted to know whether an increase in B50 expression is involved in the beneficial effects of ORG 2766 in models for neurological damage. This question has been hard to answer in models of mechanical nerve damage because of the fast and dramatic increase in B50 expression induced by the lesion itself. We speculated, however, that early effects of ORG 2766 on B50 expression might be more clearly visible in a model such as cisplatin-induced neuropathy, in which neuronal damage develops more slowly.

We have previously studied B50 mRNA expression levels in individual olfactory bulb cells by semiquantitative analysis of in situ hybridization signal (Verhaagen et al., 1993). In the present study we used the same method to quantify the amount of B50 mRNA in DRG cells of rats treated with cumulative doses of cisplatin. We tested the hypotheses 1) that degenerative and regenerative phenomena occur together after neurotoxic damage by cisplatin (is B50 mRNA expression enhanced in dorsal root ganglion cells during cisplatin treatment?), and 2) that ORG 2766 pushes the balance towards regeneration, as evidenced by a further increase in B50 mRNA expression after ORG 2766 treatment.

MATERIALS AND METHODS

In a pilot experiment, a dose-dependent increase in B50 mRNA expression was observed in response to cumulative doses of cisplatin of 10, 14, and 18 mg/kg. Based on these data we investigated B50 mRNA expression during early (1, 2, and 4 mg/kg) and advanced (14 mg/kg) stages of cisplatin intoxication.

Animals and Experimental Outline

Sixty young adult male Wistar rats (250 g) of an inbred strain (originally obtained from TNO, Zeist, The netherlands) were injected biweekly with cisplatin (Bristol-Meyers Squibb, Weesp, The Netherlands, 1 mg/kg i.p.). ORG 2766 (H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH, Organon BV, Oss, The Netherlands, 75 μg/kg s.c.), dissolved in saline, was injected every 48 hr starting immediately after the first cisplatin injection. Saline served as placebo. Age control animals (saline/saline) were included as well. Otherwise, animals were treated with cisplatin/saline or cisplatin/ORG 2766 for 4 days (1 mg cisplatin/kg), 1 week (2 mg/kg), 2 weeks (4 mg/kg), and 7 weeks (14 mg/kg). Four–7 animals per time point and per treatment were used. Four days after the last cisplatin injection, the rats were killed by decapitation, dorsal columns were immediately excised, and the dorsal roots of L4–L6 were collected. The DRG were carefully dissected from the roots, presented on RNAase-free agarose gel, embedded in Tissue-Tek (Miles Inc., Elkhart, IN), snap-frozen in dry ice-cooled isopentane, and stored at −80°C until further processing for in situ hybridization (ISH).

In Situ Hybridization

Six-μm cryostat sections were mounted at poly-L-lysine-coated microscope slides (five sections per slide) and fixed in 2% paraformaldehyde in phosphate buffer, pH 7.4, for 25 min, rinsed in phosphate-buffered saline (PBS), and kept in 70% ethanol overnight. The next day, in situ hybridization with antisense ^{35}S-labelled B50 mRNA probe (3.5×10^5 cpm/slide) was performed. The preparation of the probe and the procedure used for in situ hybridization have been described in detail previously (Verhaagen et al., 1990a). The sections were coated with Kodak NTB-2 emulsion and developed after an exposure time of 4 days. Cells were visualized by counterstaining with hematoxylin.

Quantification of B50 mRNA Levels in DRG Cells

The autoradiographic signal over all individual ganglion cells in which a nucleus was clearly visible in a given section was quantified by measuring the relative fraction of the cytoplasmic surface area of individual ganglion cells covered with silver grains. Between 70–150 cells per DRG were processed. We used an Olympus BH-2 microscope and a video camera linked to a personal computer equipped with the TIM image analysis system (DIFA Measuring Systems, Breda, The Netherlands). Individual ganglion cells were visualized using a 100× oil-immersion objective under bright-field illumination (field, 40 × 58 μm, height × width). Since the cell surface area covered by silver grains was measured, rather than the actual number of grains, a correction was made for grain overlap. The computed expression level was linearly related to the grain density and the concentration of bound radioligand (Richardson et al., 1989). Background expression level in each ganglion was assessed by measuring five randomly chosen fields without ganglion cells. Expression levels in individual ganglion cells were subsequently normalized with reference to background labelling (Verge et al., 1990).

Statistics

Methods used for statistical evaluation are indicated together with the results. Differences between groups were considered statistically significant if $P < 0.05$.

RESULTS

Selectivity of the Probe

We demonstrated that the procedure for ISH reveals the specific cellular distribution of B50 mRNA by comparing labelling by the antisense B50 mRNA probe with labelling by an antisense olfactory marker protein (OMP) mRNA probe in both DRG and olfactory epithelium. The expression patterns observed with the two probes were consistent with the previously reported expression patterns of B50 and OMP in these tissues (Verge et al., 1990; Verhaagen et al., 1990b) (data not shown).

Correlation Between Cell Size, B50 mRNA Expression, and Cisplatin and ORG 2766 Treatment

The mean ganglion cell diameter was 29 μm. In age-matched controls no correlation between cell size and B50 mRNA expression was observed. Treatment with either cisplatin + saline or cisplatin + ORG 2766 did not influence cell size (ANOVA: $F_{8,49} = 0.61$, ns).

Effects of Cisplatin/Saline and Cisplatin/ORG 2766 Treatment on B50 mRNA Expression

Examples of B50 mRNA distribution in DRGs are shown in Figure 1. About 50% of the neurons in the age-matched control group expressed significant amounts of B50 mRNA, whereas almost all neurons in the group treated with 14 mg/kg cisplatin were positive for B50 (Fig. 2). Mean B50 mRNA hybridization signals for the age-matched control and cisplatin/saline-treated groups are shown in Figure 3. Differences in B50 mRNA expression did arise between any cisplatin- and saline-treated groups (ANOVA: $F_{4,30} = 5.2487$, $P < 0.0025$). Although hybridization signal in the group treated with 2 mg cisplatin/kg seemed higher that in the 0, 1, and 4 mg/kg groups (Figs. 3, 4), only in the group treated with 14 mg cisplatin/kg was B50 mRNA expression significantly higher than in the age-matched control group (Student-Newman-Keuls test, $P < 0.05$). Mean B50 mRNA expression in peptide-cotreated animals was not different from that in cisplatin/saline-treated animals at any of the time points studied (Student's t-test) (Fig. 4), so there seems to be no effect of peptide treatment on B50 mRNA expression in all DRG cells. As cisplatin is known to affect primarily thick myelinated sensory fibers (Thompson et al., 1984) belonging to neurons with large cell bodies, it is possible that changes in B50 expression initially remain restricted to large cells. This hypothesis was tested by determining the ratio of mean hybridization signal in large cells (diameter > mean) compared to small cells (diameter < mean). This ratio was slightly (but not significantly) higher in saline- vs. ORG 2766-treated groups for cumulative cisplatin doses of 1, 2, and 4 mg/kg, but following a cumulative cisplatin dose of 14 mg/kg, this ratio significantly decreased (Student's t-test: $t = 3.94$, $df = 6$, $P < 0.01$) in the saline-cotreated group as compared to the ORG 2766-cotreated group (Fig. 5). The number of large cells compared to that of small cells was similar for all groups.

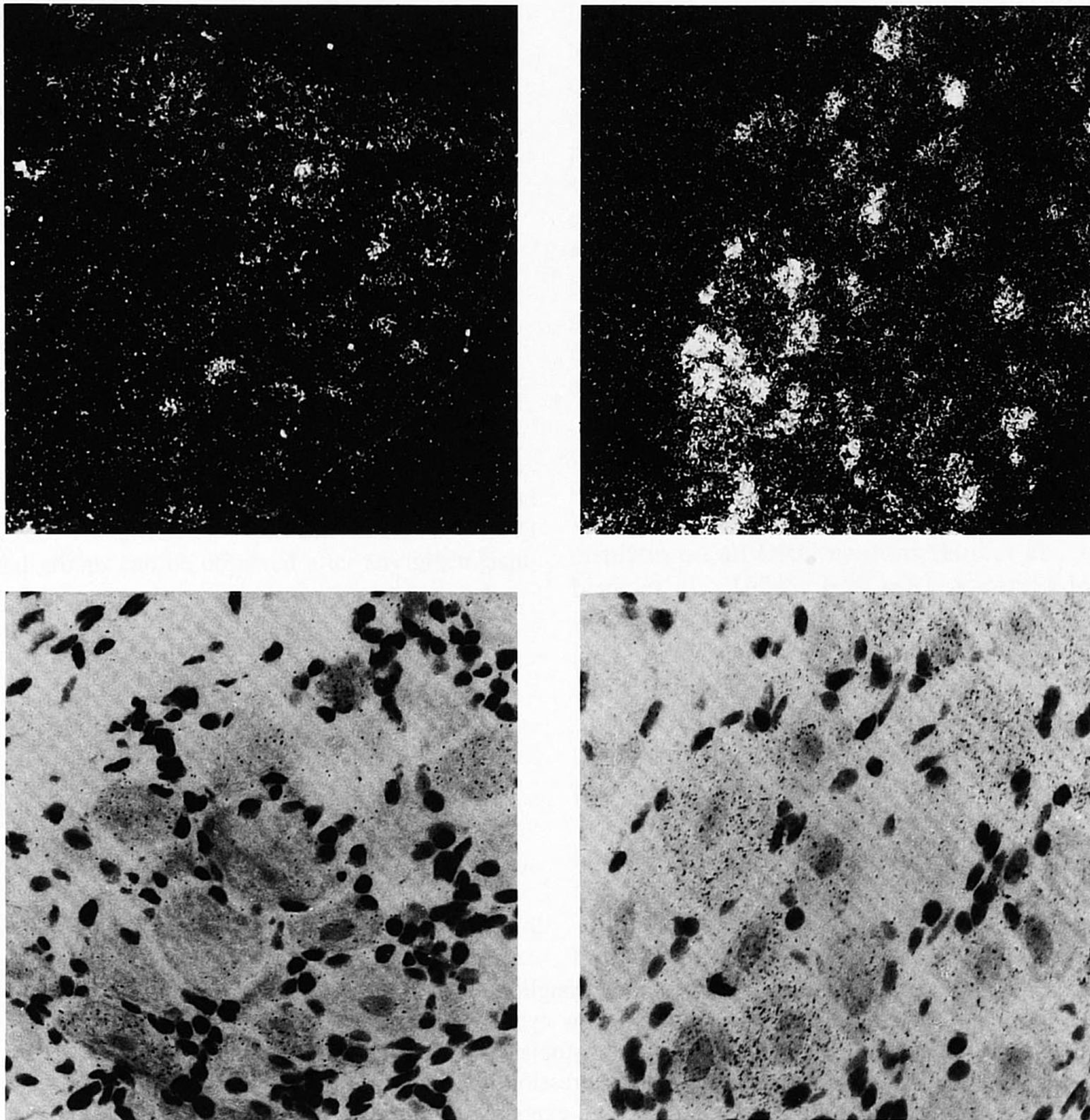

Fig. 1. Darkfield (**top**) and brightfield (**bottom**) photographs of B50 hybridization signal of a DRG (6 μm thick) of an age-control (**left**) and 14 mg cisplatin/saline-treated (**right**) animal. Darkfield, ×90; brightfield, ×360.

DISCUSSION

In this study we demonstrate an increase of B50 mRNA expression in DRG sensory neurons due to treatment with the neurotoxic drug cisplatin. Furthermore, cotreatment with a neurotrophic peptide enhanced this increase in B50 mRNA expression in a subgroup of sensory neurons, compared to saline-treated animals, in the more advanced stages of neurointoxication.

The observation that in adult animals 50% of ganglion cells expressed B50 mRNA is consistant with that of Verge et al. (1990). Low cumulative doses of cisplatin did not affect B50 mRNA expression, but enhanced B50 mRNA expression was observed during the late stage of intoxication (14 mg/kg), when a sensory neuropathy with a significant decrease in sensory nerve conduction velocity, as compared to age-matched controls, was present (De Koning et al., 1987, 1988; Hamers et al., 1991a,b, 1993; Gerritsen van der Hoop et al., 1988, 1994). With respect to the mechanism of action underlying cisplatin neurotoxicity, little is known. Cisplatin's antitumor activity results from its ability to form adducts with guanine and cytosine in DNA (both inter- and intrastrand). In dividing cells this interferes with replication, but in nondividing cells such as neurons it negatively affects transcription (Corda et al., 1992) and thus diminishes protein synthesis. This could well be the reason why cisplatin primarily affects thick myelinated fiber modalities (large sensory neurons that rely most on unimpaired protein synthesis) clinically (Gerritsen van der Hoop et al., 1990, 1994; Hamers et al., 1991b). Nonetheless, in

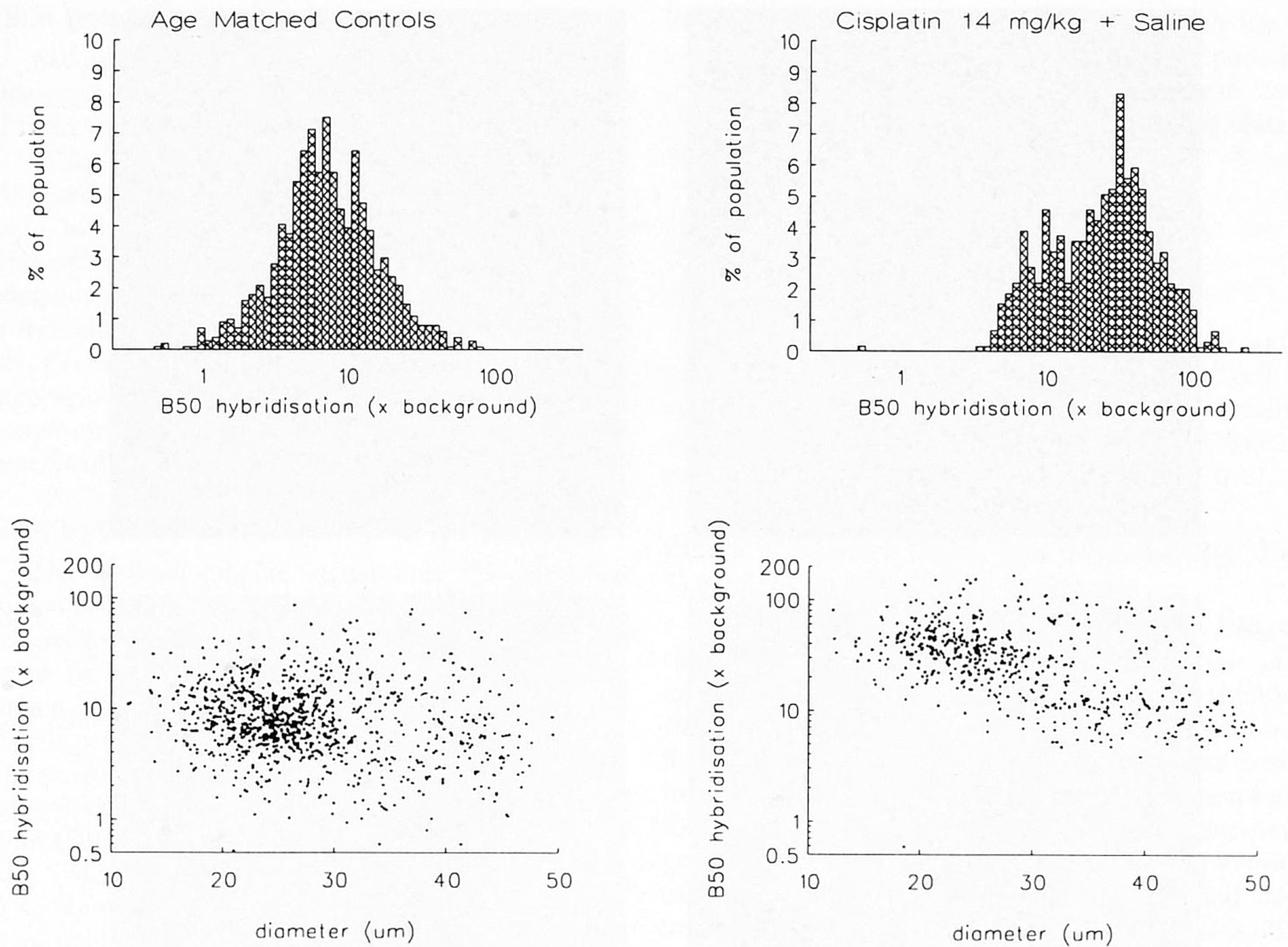

Fig. 2. Diagrams presenting data from all ganglion cells in age-control and 14 mg cisplatin/saline-treated groups. Histograms (**top**) show cytoplasmic B50 labelling density for the two groups. Scatter diagrams (**bottom**) display cytoplasmic B50 hybridization signal as function of cell size. The shift towards higher B50 expression is evident in the 14 mg/saline group. No correlation exists between cell size and B50 expression in the age-control group. A negative correlation is observed for B50 expression and cell size after a cumulative dose of 14 mg/kg.

vitro studies on fetal DRG have shown that cisplatin can be toxic to all types of DRG sensory neurons; this toxicity can be diminished by ORG 2766 coadministration (Hol et al., 1994; Windebank et al., 1994).

Our data indicate that B50 mRNA expression in DRG sensory neurons is upregulated in response to cisplatin-induced neurotoxic damage, as it is during nerve regeneration following mechanical lesions, but not before a cumulative cisplatin dose that is known to induce functional deficits has been administered. A similar temporal correlation has been observed for B50 protein content in rat sciatic nerve axons during acrylamide-induced neuropathy (Bisby and Redshaw, 1987): the onset of acrylamide-induced neuropathy seen after 4–6 injections of acrylamide was followed by an increment of B50 protein synthesis. These findings contrast with the very rapid induction of B50 mRNA expression after mechan-

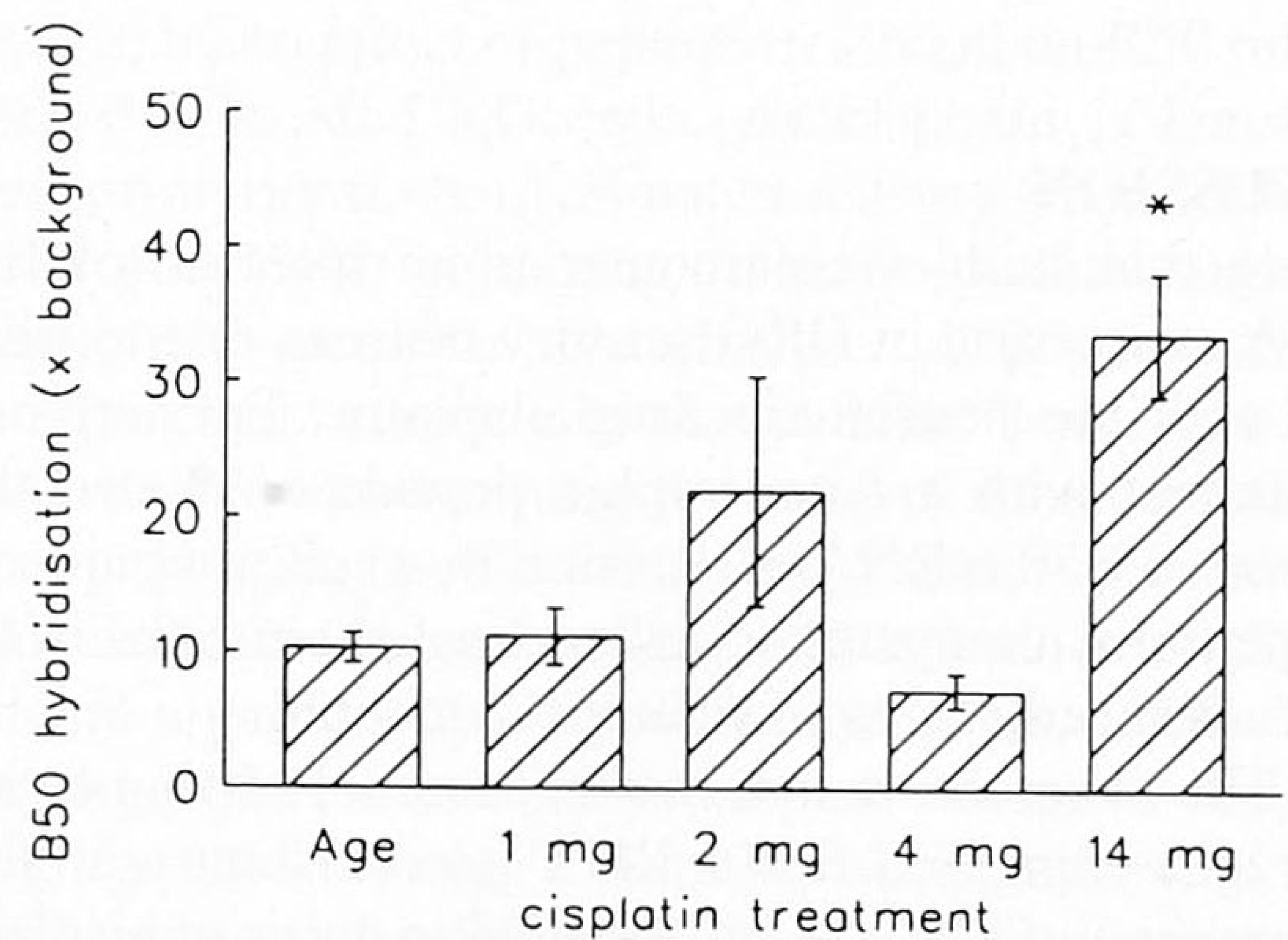

Fig. 3. Mean cytoplasmic B50 expression signal (times background) per cisplatin treatment group (± SEM). Only in the cisplatin 14 mg/kg group is mean B50 expression significantly different from age-controls (*$P < 0.05$).

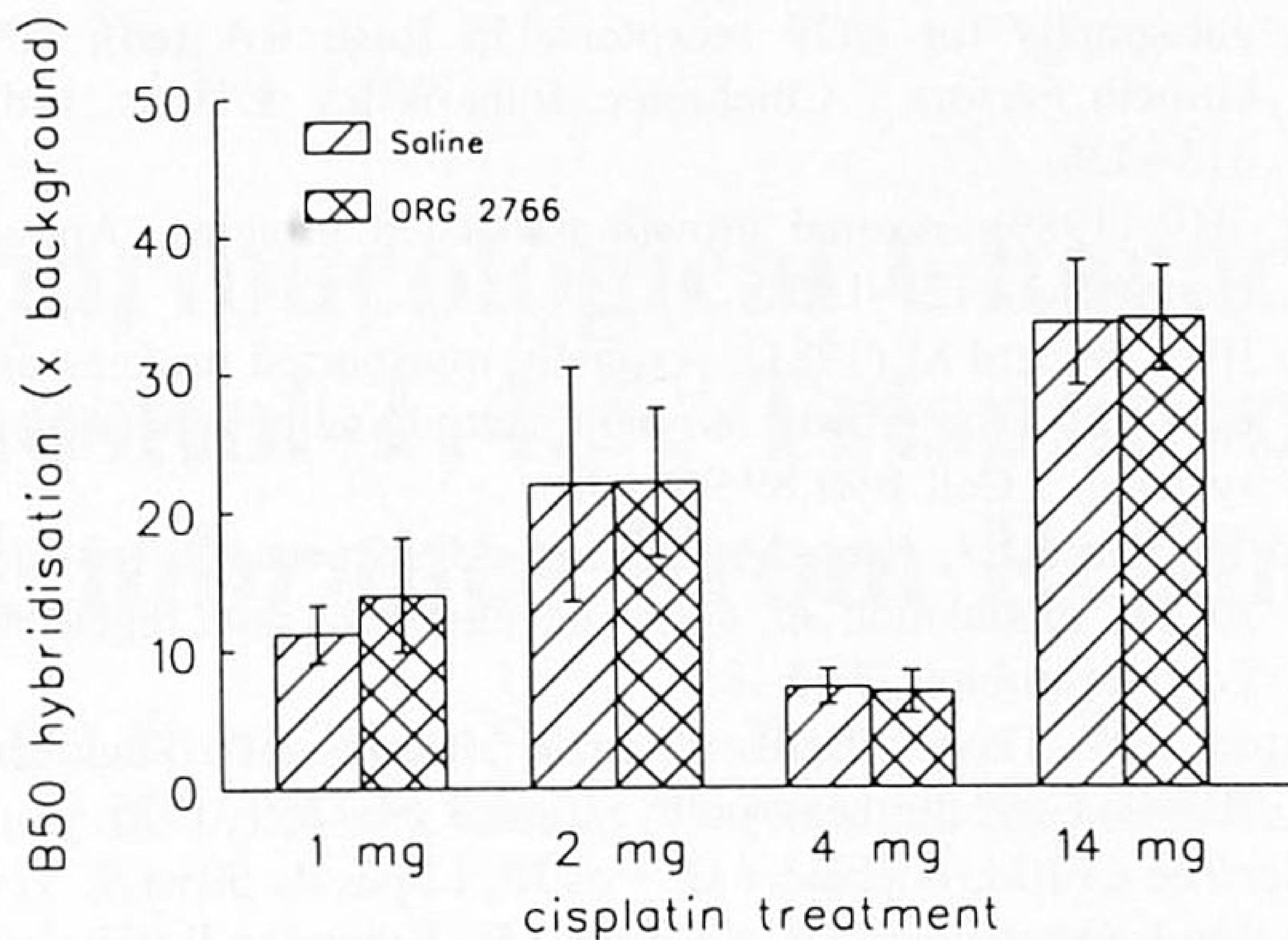

Fig. 4. Mean cytoplasmic B50 expression signal (times background) per cisplatin treatment group cotreated with saline and with ORG 2766. No differences between saline- and ORG 2766-cotreated groups can be observed after any given cisplatin dose.

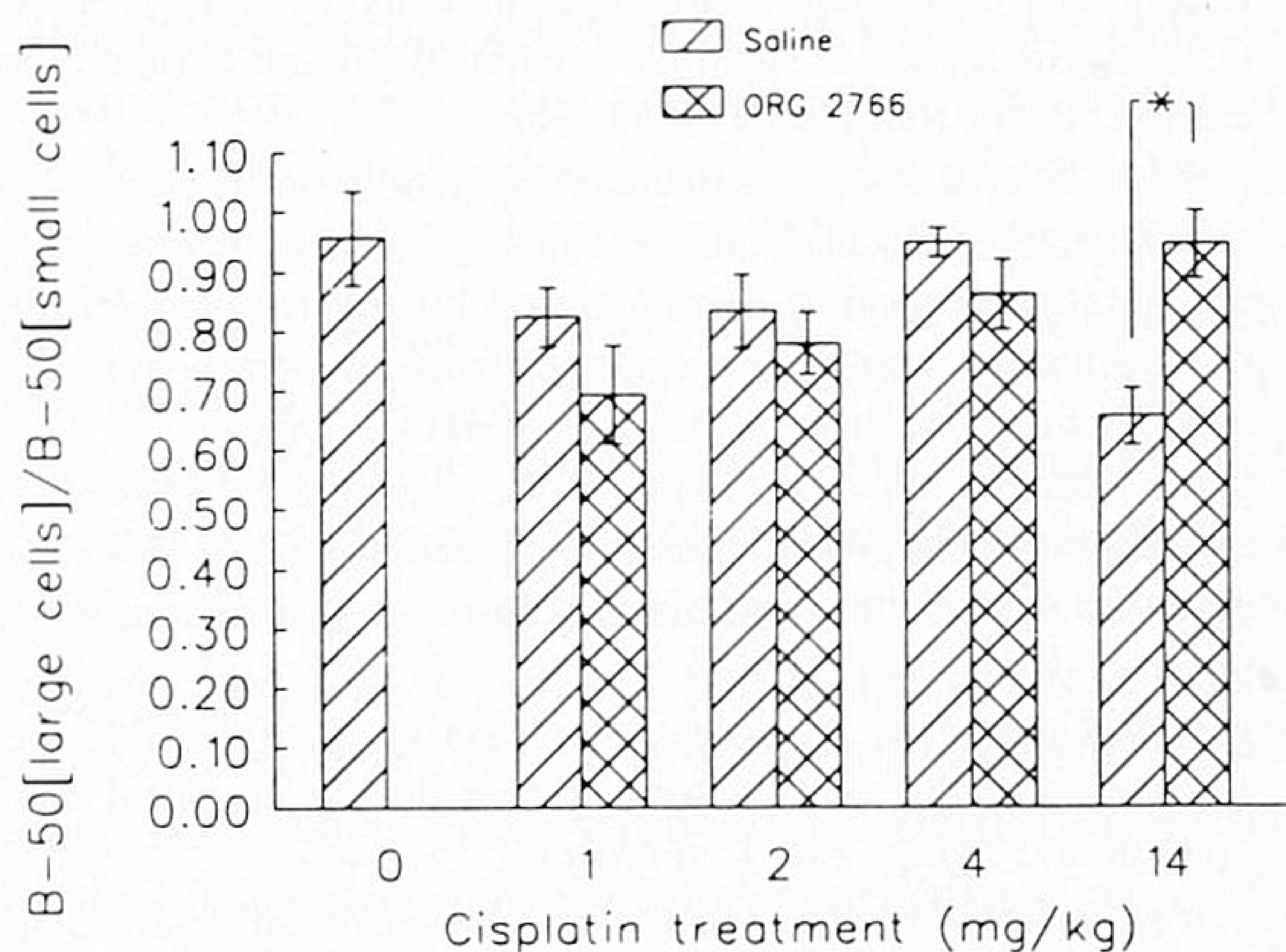

Fig. 5. Ratio of B50 mRNA expression in large cells (diameter > 29 μm) to B50 mRNA expression in small cells (diameter < 29 μm). At a cumulative cisplatin dose of 14 mg/kg, this ratio is significantly lower in the saline-cotreated group as compared with the ORG 2766-cotreated group.

ical nerve lesions (Van der Zee et al., 1989; Verge et al., 1990; Woolf et al., 1990). The three models are similar in that B50 expression is only enhanced when definite neuronal damage, as indicated by functional deficits, is present.

It has been hypothesized that neuropathies develop when there exists an imbalance between degenerative and regenerative influences, and that treatment with neurotrophic substances, such as ORG 2766, would push this balance towards regeneration (Gispen, 1990). This hypothesis is supported by the recent observation of Dyer et al. (1995) that ORG 2766 upregulates expression of the low-affinity nerve growth factor (NGF) receptor and leads to release of (nonneurotrophin) neurotrophic activity that potentiates the action of NGF on neurons. Potentiation of NGF neurotrophic effects by ORG 2766 could certainly be of importance in our model, as Apfel et al. (1991) described beneficial effects of NGF in cisplatin-induced neuropathy in mice. In the intact nervous system ORG 2766 has been shown not to influence B50 expression (Van der Zee et al., 1989), but in the diseased nervous system, the neurotrophic action of the peptide might well lead to further enhancement of B50 (as a protein important in neurite outgrowth and nerve regeneration) expression above endogenously-stimulated B50 expression.

The observation that B50 mRNA expression increased in both large and small sensory neurons after a cumulative dose of 14 mg/kg cisplatin (Fig. 2) is in line with data from in vitro studies indicating toxic action of cisplatin on all DRG neurons (Hol et al., 1994; Windebank et al., 1994). B50 mRNA expression, however, showed relatively lower increase in the larger neurons in DRG of animals cotreated with saline than in those of animals cotreated with ORG 2766 at this time point (Fig. 5). This might be due to the inability of these neurons to maximally maintain their regenerative measures without external trophic support.

In conclusion, B50 mRNA expression is upregulated in the later stage of cisplatin intoxication, around the moment when functional deficits can be measured. This observation lends strength to the hypothesis that both degenerative and regenerative mechanisms are competing during the induction of a neuropathy. At this stage, differences in B50 mRNA content in the subpopulation of larger sensory neurons can be found in the group cotreated with ORG 2766 as compared with the saline-cotreated group. Slight as it is, the latter observation might nonetheless be another manifestation of the neuroprotective effect of this peptide.

REFERENCES

Apfel SC, Lipton RB, Arezzo JC, Kessler JA (1991): Nerve growth factor prevents toxic neuropathy in mice. Ann Neurol 29:87–90.

Bär PRD, Schrama LH, Gispen WH (1990): Neurotrophic effects of the ACTH/MSH-like peptides in the peripheral nervous system. In De Wied D (ed): "Neuropeptide Concept." Amserdam: Elsevier Science Publishers BV, pp 175–211.

Basi GS, Jacobson RD, Virag I, Schilling J, Skene JHP (1987): Primary structure and transcriptional regulation of GAP-43, a protein associated with nerve growth. Cell 49:785–791.

Bisby MA, Redshaw JD (1987): Acrylamide neuropathy: Changes in the composition of proteins of fast axonal transport resemble those observed in regenerating axons. J Neurochem 48:924–928.

Chong MS, Reynolds ML, Irwin N, Coggeshall RE, Emson PC, Benowitz LI (1994): GAP-43 expression in primary sensory neurons following central axotomy. J Neurosci 14:4375–4384.

Corda Y, Anin MA, Leng M, Job D (1992): RNA polymerases react differently at d(ApG) and d(GpG) adducts in DNA modified by cis-diamminedichloroplatinum(II). Biochemistry 31:1904–1908.

Curtis R, Stewart HJS, Hall SM, Wilkin GP, Mirsky R, Jessen KR (1992): GAP-43 is expressed by nonmyelin-forming Schwann cells of the peripheral nervous system. J Cell Biol 116:1455–1464.

De Koning P, Neijt JP, Jennekens FGI, Gispen WH (1987): Evaluation of cis-diamminedichloroplatinum(II) (cisplatin) neurotoxicity in rats. Toxicol Appl Pharmacol 89:81–87.

De Koning P, Neijt JP, Jennekens FGI, Gispen WH (1988): ORG 2766 protects from cisplatin-induced neurotoxicity in rats. Exp Neurol 97:746–750.

Dyer JK, Philipsen HLA, Tonnaer JADM, Hermkens PHH, Haynes LW (1995): Melanocortin analogue Org2766 binds to rat Schwann cells, upregulates NGF low-affinity receptor p75, and releases neurotrophic activity. Peptides 16:515–522.

Elderson A, Gerritsen van der Hoop R, Haanstra W, Neijt JP, Gispen WH, Jennekens FGI (1989): Vibration perception and thermoperception as quantitative measurements in the monitoring of cisplatin-induced neurotoxicity. J Neurol Sci 93:167–174.

Gerritsen van der Hoop R, De Koning P, Boven E, Neijt JP, Jennekens FGI, Gispen WH (1988): Efficacy of the neuropeptide ORG 2766 in the prevention and treatment of cisplatin-induced neurotoxicity in rats. Eur J Cancer 24:637–642.

Gerritsen van der Hoop R, Van Houwelingen JC, Van der Burg MEL, Ten Bokkel Huinink WW, Neijt JP (1990): The incidence of neuropathy in 395 ovarian cancer patients treated with or without cisplatin. Cancer 66:1697–1702.

Gerritsen van der Hoop R, Hamers FPT, Neijt JP, Veldman H, Gispen WH, Jennekens FGI (1994): Protection against cisplatin induced neurotoxicity by ORG 2766: histological and electrophysiological evidence. J Neurol Sci 126:109–115.

Gispen WH (1990): Therapeutic potential for melanocortins in peripheral nerve disease. Trends Pharmacol Sci 11:221–222.

Hamers FPT, Gerritsen van der Hoop R, Steerenburg PA, Neijt JP, Gispen WH (1991a): Putative neurotrophic factors in the protection of cisplatin-induced peripheral neuropathy in rats. Toxicol Appl Pharmacol 111:514–522.

Hamers FPT, Gispen WH, Neijt JP (1991b): Neurotoxic side-effects of cisplatin. Eur J Cancer 27:372–376.

Hamers FPT, Pette C, Bravenboer B, Vecht CJ, Neijt JP, Gispen WH (1993): Cisplatin-induced neuropathy in mature rats: Effects of the melanocortin-like peptide ORG 2766. Cancer Chemother Pharmacol 32:162–166.

Hol EM, Mandys V, Sodaar P, Gispen WH, Bär PRD (1994): Protection by an ACTH4-9 analogue against the toxic effects of cisplatin and taxol on sensory neurons and glial cells in vitro. J Neurosci Res 39:178–185.

Neve RL, Perrone-Bizzozero NI, Finklestein S, Zwiers H, Bird E, Kurnit DM, Benowitz LI (1987): The neuronal growth-associated protein GAP 43 (B-50, F1): Neuronal specificity, developmental regulation and regional distribution of the human and rat mRNA. Mol Brain Res 2:177–183.

Oestreicher AB, Dekker LV, Gispen WH (1986): A radioimmunoassay for the phosphoprotein B-50: Distribution in rat brain. J Neurochem 46:1366–1369.

Plantinga LC, Verhaagen J, Edwards PM, Hol EM, Bär PRD, Gispen WH (1993): The expression of B-50/GAP-43 in Schwann cells is upregulated in degenerating peripheral nerve stumps following nerve injury. Brain Res 602:69–76.

Richardson PM, Verge VMK, Riopelle RJ (1989): Quantitative radioautography for NGF receptors. In Rush RA (ed): "Nerve Growth Factors." Chichester: John Wiley & Sons, Ltd., pp 315–326.

Skene JHP (1989): Axonal growth-associated proteins. Annu Rev Neurosci 12:127–156.

Skene JHP, Willard M (1981): Axonally transported proteins associated with axon growth in rabbit central and peripheral nervous systems. J Cell Biol 89:96–103.

Strand FL, Rose KJ, King JA, Segarra AC, Zuccarelli LA (1989): ACTH modulation of nerve development and regeneration. Prog Neurobiol 33:45–85.

Thompson SW, Davis LE, Kornfeld M, Hilgers RD, Standefer JC (1984): Cisplatin neuropathy. Cancer 54:1269–1275.

Van der Zee CEEM, Nielander H, Vos JP, Lopes da Silva S, Verhaagen J, Oestreicher AB, Schrama LH, Schotman P, Gispen WH (1989): Expression of growth associated protein B-50 (GAP-43) in dorsal root ganglia and sciatic nerve during regenerative sprouting. J Neurosci 10:3505–3512.

Verge VMK, Tetzlaff W, Richardson PM, Bisby MA (1990): Correlation between GAP43 and nerve growth factor receptors in rat sensory neurons. J Neurosci 10:926–934.

Verhaagen J, Van Hooff COM, Edwards PM, De Graan PNE, Oestreicher AB, Schotman P, Jennekens FGI, Gispen WH (1986): The kinase C substrate protein B-50 and axonal regeneration. Brain Res Bull 17:737–741.

Verhaagen J, Edwards PM, Jennekens FGI, Schotman P, Gispen WH (1987): Early effects of an ACTH(4–9) analog (Org 2766) on regenerative sprouting demonstrated by the use of neurofilament binding antibodies isolated from a serum raised by α-MSH immunization. Brain Res 404:142–150.

Verhaagen J, Oestreicher AB, Gispen WH, Margolis FL (1989): The expression of the growth associated protein B-50/GAP43 in the olfactory system of neonatal and adult rats. J Neurosci 9:683–691.

Verhaagen J, Greer CA, Margolis FL (1990a): B-50/GAP43 gene expression in the rat olfactory system during postnatal development and aging. Eur J Neurosci 2:397–407.

Verhaagen J, Oestreicher AB, Grillo M, Khew-Goodall Y-S, Gispen WH, Margolis FL (1990b): Neuroplasticity in the olfactory system: Differential effects of central and peripheral lesions of the primary olfactory pathway on the expression of B-50/GAP-43 and the olfactory marker protein. J Neurosci Res 26: 31–44.

Verhaagen J, Zhang Y, Hamers FPT, Gispen WH (1993): Elevated expression of B-50 (GAP-43)-mRNA in a subpopulation of olfactory bulb mitral cells following axotomy. J Neurosci Res 35:162–169.

Windebank AJ, Smith AG, Russell JW (1994): The effect of nerve growth factor, ciliary neurotrophic factor, and ACTH analogs on cisplatin neurotoxicity in vitro. Neurology 44:488–494.

Woolf CJ, Reynolds ML, Molander C, O'Brien C, Lindsay RM, Benowitz LI (1990): The growth-associated protein GAP-43 appears in dorsal root ganglion cells and in the dorsal horn of the rat spinal cord following peripheral nerve injury. Neuroscience 34:465–478.

Yankner BA, Benowitz LI, Villa Komaroff L, Neve RL (1990): Transfection of PC12 cells with the human GAP-43 gene: Effects on neuriteoutgrowth and regeneration. Brain Res Mol Brain Res 7:39–44.

Zwiers H, Veldhuis HD, Schotman P, Gispen WH (1976): ACTH, cyclic nucleotides, and brain protein phosphorylation in vitro. Neurochem Res 1:669–677.