Short Communication

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Exogenous heat shock cognate protein Hsc70 prevents axotomy-induced death of spinal sensory neurons

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Abstract Elevation of intracellular heat shock protein (Hsp)70 increases resistance of cells to many physical and metabolic insults. We tested the hypothesis that treatment with Hsc70 can also produce that effect, using the model of axotomy-induced neuronal death in the neonatal mouse. The sciatic nerve was sectioned and in some animals purified bovine brain Hsc70 was applied to the proximal end of the nerve immediately thereafter and again 3 days later. Seven days postaxotomy, the surviving sensory neurons of the lumbar dorsal root ganglion (DRG) and motoneurons of the lumbar ventral spinal cord were counted to assess cell death. Axotomy induced the death of approximately 33% of DRG neurons and 50% of motoneurons, when examined 7 days postinjury. Application of exogenous Hsc70 prevented axotomy-induced death of virtually all sensory neurons, but did not significantly alter motoneuron death. Thus, Hsc70 may prove to be useful in the repair of peripheral sensory nerve damage.

INTRODUCTION

The class of proteins known as heat shock proteins (Hsps), which in many systems appear to be linked to cell survival following acute metabolic stress, were first described in the brain after traumatic injury by Currie and White (1981). This observation remained a curiosity until the late 1980s, when it became clear that many different types of trauma to the nervous system enhanced production of inducible Hsp70 (Mayer and Brown 1994). Furthermore, prior elevation of Hsp70 induced by a conditioning stress correlated with increased survival of neural cells following a severe stress (Barbe et al 1988; Khan and Sotelo 1989; Kirino et al 1991; Lowenstein et al 1991, Nowak and Jacewicz 1994; Tytell et al 1994).

Previously, we had found that Hsc70 made by glial sheath cells was transferred into the axon in the squid giant axon (Tytell et al 1986) and this observation provided the rationale for hypothesizing that a neuron might not have to make its own Hsc70, but perhaps could be protected by the protein supplied to it by the glia or other extraneuronal sources. In this paper, we test this hypothesis using the well established model in which spinal sensory (dorsal root ganglion, DRG) and motor neurons die in the neonatal mammal after transection of their peripheral projections (Schmalbruch 1984; Crews and Wigston 1991; Snider et al 1992; Li et al 1994). The results show that treatment with exogenous Hsc70 significantly prevented spinal sensory, but not motor, neurons from axotomy-induced death. Furthermore, Hsc70 was retrogradely transported by both sensory and motor neurons. These data suggest that Hsc70 may be useful as a cytoprotective agent in the nervous system, especially for sensory neurons, following injury.

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RESULTS AND DISCUSSION

Unilateral transection of the sciatic nerve was performed in 5-day-old mice and a Gelfoam pad containing 5 µl of either saline only or saline plus 5, 25, or 75 µg of purified bovine brain Hsc70 was applied to the proximal stump of

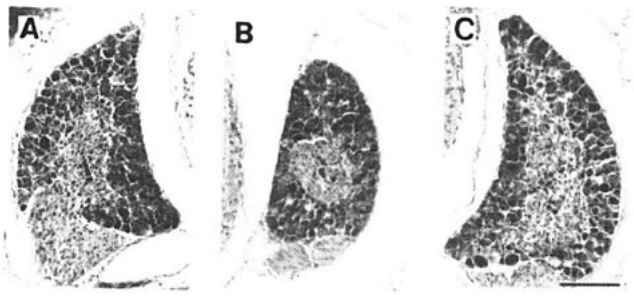

Fig. 1 Photomicrographs of hematoxylin/eosin-stained dorsal root ganglia (DRG) from control (A), axotomized and saline-treated (B) and axotomized and 75 μg Hsc70–treated (C) mice. Note the overall sizes and the number of neuronal somata in the control (A) and Hsc70–treated (C) DRG are similar, whereas the DRG from the saline-treated axotomized mouse (B) is much smaller because of the loss of neurons. Scale bar = 100 μm.
Methods: Neonatal BalB/cByJ mice (Jackson Laboratory, Bar Harbor, ME) were anesthetized by hypothermia and unilateral sciatic nerve section at mid-thigh level was performed on postnatal day 5. A small length (about 2–3 mm) of the nerve was removed to prevent reinnervation. A 1–2 mm^3 piece of Gelfoam (Upjohn Co., Kalamazoo, MI), presoaked in 5 μl of a solution of purified bovine brain Hsc70 in saline (0.9% NaCl, pH 7.2), at a concentration of 1, 5 or 15 μg/μl, was apposed to the proximal nerve stump. Four days after the operation, treated animals were injected in the musculature surrounding the lesion site with 5 μl of the appropriate concentration of Hsc70 solution; they were killed 3 days later (7 days postaxotomy). Axotomized control animals received the same treatment paradigm with vehicle. All animals were killed with an overdose of ether. Whole lumbar spinal cords with attached vertebra and dorsal root ganglia were dissected and fixed by immersion in Bouin's fixative for 2–3 weeks to decalcify the bone. Tissues were embedded in paraffin and 12-μm-thick serial sections were cut using a rotary microtome and stained with hematoxylin and eosin. Hsc70 was purified from bovine brain in a manner similar to that of Schlossman et al (1984), as modified by Schmid and Rothman (1985). In brief, a brain cytosol preparation was fractionated by DEAE ion exchange chromatography followed by ATP affinity chromatography, which takes advantage of the high affinity of native Hsc70 for ATP. The protein eluted from the ATP affinity column consists mainly of the constitutive form (Hsc70), with about 5% being the inducible form (Hsp70). The purity of the Hsc70 used for these experiments was confirmed by one-dimensional polyacrylamide gel electrophoresis (not shown).

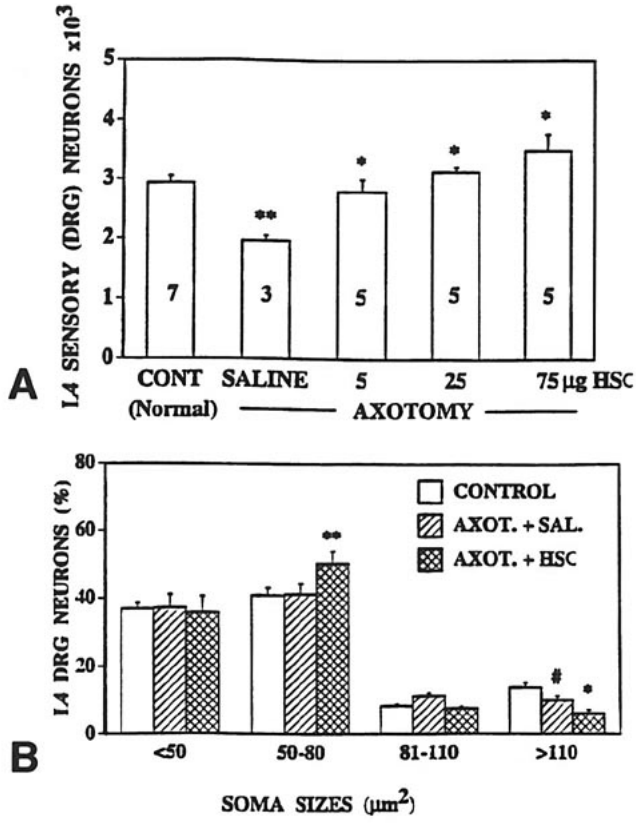

Fig. 2 Surviving neuron numbers (A) and proportions of neurons as a function of soma sizes (B) in L4 DRG of 12-day-old mice following unilateral axotomy on day 5 and treatment with either saline (SAL) or different concentrations of Hsc70. Animals were treated as described in the legend to Figure 1. (A) Numbers within each bar indicate mice per group; $^*P < 0.05$ compared to saline; $^{**}P < 0.01$ compared to intact control. (B) Axotomy caused a preferential loss of neurons with the largest nuclei (> 110 μm^2; $^*P < 0.05$). However, when 75 μg Hsc70 was administered following axotomy, this class of neurons survived, but showed a decrease in soma size which led to a increase in proportion of neurons with nuclei of 50–80 μm^2 ($^{**}P < 0.01$). For each group in this analysis, 5 animals and a total of 580–870 cells were examined.
Methods: Neuronal cell counts were made blindly on every 5th section in the fourth lumbar (L4) dorsal root ganglion (for sensory neurons) or in the lateral motor column of L4 spinal cord (for motoneurons) according to a method described previously (Li et al 1994). Briefly, only cells with a large nucleus containing at least one distinct nucleolus and a well-stained cytoplasm were counted. Using these rather stringent criteria, we have found previously that less than 1% of the cells appear on two successive sections and, therefore, only an insignificant number of cells would be counted twice (see Li et al 1994). Statistical comparisons were made using Student's *t*-test.

the nerve, after which the wound was sutured closed. Four days later an intramuscular injection of 5 μl of saline only or saline plus the same dose of Hsc70 was made at the wound site. Three days after the injection (i.e. 7 days postaxotomy or 12 days postnatal), all mice were killed by an overdose of ether and their spinal cords and DRGs were collected for histological analysis.

In the mice that received saline only, there was an apparent decrease in the size of lumbar segment (L)4 DRG on the ipsilateral side (Fig. 1B), compared to the contralateral intact DRG (Fig. 1A). This shrinkage of the DRG was a result of a 33% decrease in the number of DRG neurons after axotomy and treatment with saline (Fig. 2A). In contrast, treatment with Hsc70 prevented atrophy induced by axotomy (Fig. 1C) and those animals showed significantly more surviving DRG neurons at all three doses of Hsc70 tested, compared to those treated with saline (Fig. 2A). In fact, the two highest doses of Hsc70 (25 and 75 μg) prevented the death of virtually all axotomized DRG neurons (Fig. 2A).

The DRG is comprised of neurons with a range of diameters and there is some evidence that the size of the neuron is related to its target, with the larger ones innervating muscle spindle fibers and the smaller ones

innervating touch and pain receptors in the skin (e.g. Brown 1981; Willis and Coggeshall 1991). To determine whether the protective effect of Hsc70 was selective for a particular size class of DRG neurons, we evaluated the soma areas of the surviving neurons in comparison to uninjured and saline-treated controls. Figure 2B shows that axotomy produced a selective loss of the largest DRG neurons (somal area > 110 μm^2) and that Hsc70 treatment did not prevent the decrease in that size class of neurons. However, only in the Hsc70-treated axotomized mice was the decrease in the number of the largest DRG neurons associated with a significant increase in the number of smaller DRG neurons (somal area = 50–80 μm^2) (Fig. 2B). This shift in the size distribution of DRG neurons with Hsc70 treatment following axotomy suggests that Hsc70 rescued the largest neurons from axotomy-induced death, but did not prevent them from decreasing in size after the injury.

Like sensory neurons, L4 spinal motoneurons undergo massive cell death following sciatic nerve section in the neonatal mouse (e.g. Li et al 1994). In the same animals examined for DRG neuron survival, we also determined whether the Hsc70 altered the survival of motoneurons. As shown in Figure 3, axotomy followed by saline treatment led to a decrease in L4 motoneuron numbers by 50% ($P < 0.01$) when compared to contralateral controls. Treatment with Hsc70 did not significantly alter this outcome (Fig. 3), in contrast to its effect on DRG neurons. Neither did Hsc70 significantly alter atrophy of axotomized motoneurons (not shown). Although there was a trend toward increased survival of motoneurons in the mice which received 5 or 25 μg Hsc70, the highest dose of Hsc70 (75 μg) paradoxically exacerbated the death of axotomized motoneurons relative to the lower two doses of Hsc70 ($P < 0.01$). A similar negative effect of Hsc70 at higher doses has been observed previously in cultured smooth muscle cells subjected to nutrient deprivation stress (Johnson et al 1995) and suggests that, for some types of cells, there may be a concentration of Hsc70 which interferes with, rather than supports, cell function. The protective effect of Hsc70 on axotomized sensory neurons was not simply a non-specific response of the DRG neurons to the administration of a protein. In other work in this laboratory, 5 μg of glial cell line-derived neurotrophic factor (GDNF) was administered to the axotomy site in the same manner as for Hsc70. GDNF, made by Schwann cells and astrocytes, is a member of the transforming growth factor β superfamily with marked survival and growth-promoting effects on spinal and brainstem motoneurons. In contrast with Hsc70, GDNF had no effect on the postaxotomy death of DRG neurons (Li et al 1995), but did significantly promote the survival of the spinal motoneurons (Oppenheim et al 1995). Additionally, it has been shown that a number of other

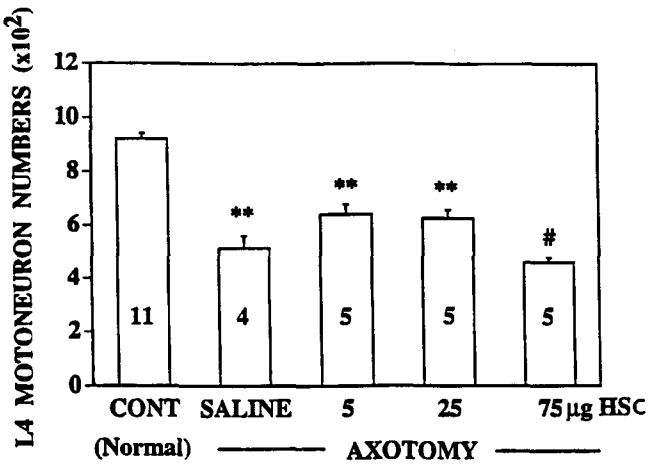

Fig. 3 Motoneuron numbers (means ± SEM) in the lateral motor column of L4 spinal cords from 12-day-old mice following axotomy on day 5 and treatment with either saline or different doses of Hsc70. Compared to the intact control group (CONT), all axotomy groups had significantly fewer surviving motoneurons (**$P < 0.01$) and the differences between the 5 and 25 μg Hsc70–treated groups and the saline-treated one were not significant. However, treatment with the highest dose (75 μg) of Hsc70 led to a greater loss of motoneurons than that seen with the two lower doses of Hsc70 (#$P < 0.01$). Numbers in bars represent the number of animals. Cell counts were performed as described in the legend to Figure 2.

trophic agents promote the survival of motoneurons without affecting that of sensory neurons (e.g. Oppenheim et al 1993), although both neuronal cell types retrogradely transport these agents (e.g. Yan et al 1993; Yan and Matheson 1995). These observations support the specificity of the effect of the exogenously applied Hsc70 on DRG neurons.

To verify that the administered Hsc70 was taken up and retrogradely transported by either, or both, sensory and motor neurons following axotomy, another experiment was performed in which biotin-labeled Hsc70 (b-Hsc70) was administered to the axotomy site. Then the presence of b-Hsc70 in the proximal portion of the sciatic nerve and in the neuronal somata of the DRG and the corresponding segment of lumbar spinal cord was evaluated by polyacrylamide gel electrophoresis, followed by Western blotting of samples collected 1, 6 and 24 h postaxotomy. As shown in Figure 4, the b-Hsc70 was taken up by the sciatic nerve, DRG and spinal cord. The fact that the b-Hsc70 was most prominent in the nerve only at the earliest collection time (1 h) suggests that it was not only taken up, but also cleared from the nerve via retrograde transport to the DRG and spinal motoneurons. Although b-Hsc70 also was detected in the DRG and spinal cord at 1 h postinjection, this fact does not conflict with the idea of retrograde transport of the protein because the average rate of that process (about 10 mm/h), would cover the distance from the axotomy

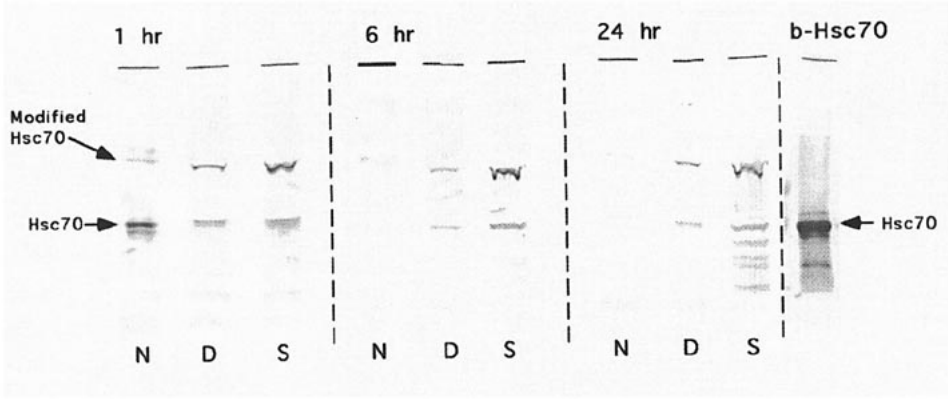

Fig. 4 Representative Western blots of samples of the proximal nerves (N), the ipsilateral DRG (D) and spinal cord (S) following axotomy and treatment with biotinylated Hsc70 (b-Hsc70). Samples were analyzed 1, 6 or 24 h later. At 1 h after treatment, b-Hsc70 was prominent in the sciatic nerve, but was absent at the later times, suggesting that it was retrogradely transported. The DRG and spinal cord contained b-Hsc70 at all time points, implying that the protein was retained for at least 24 h in the neuron somata. In the spinal cord at 24 h, the presence of lower molecular weight, biotin-positive bands suggest that some of the b-Hsc70 had been degraded. An unidentified biotin-positive band of about 120 kDa was also detected at 1 h in the nerve and at all three times in the DRG and spinal cord, suggesting that a portion of the b-Hsc70 was covalently linked to one or more other proteins in the tissue. The last lane on the right is a sample of the stock solution of b-Hsc70 that was administered to the axotomy site and shows that it was mostly intact Hsc70, but did contain a minor amount of a lower molecular weight contaminant.
Methods: Biotin was conjugated to 200 μg of Hsc70 using the biotin-XX succinimidyl ester reagent (Molecular Probes Inc., Eugene, OR) according to the manufacturer's specifications. Unconjugated biotin was separated from the biotinylated Hsc70 (b-Hsc70) by ultrafiltration and washing the protein twice using a 10 kDa molecular weight cut-off membrane filter in a 1.5 ml centrifuge (type UFC3 LGC, Millipore Corp., Bedford, MA). Following axotomy on day 5, mice were treated with Gelfoam pieces containing b-Hsc70 as described in the legend to Figure 1. Proximal sciatic nerve, L4 DRG and L4 spinal cord samples were dissected out at different time intervals (1, 6 or 24 h post-treatment) and homogenized in SUB electrophoresis solution (1% SDS, 8 M urea, 2% β-mercaptoethanol; 100 μl for the nerve and DRG and 400 μl for the spinal cord), centrifuged at 10 000 x g for 5 min, and then 5 or 10 μl samples of each were loaded a 0.75 mm-thick 10% acrylamide minigel. The samples were electrophoresed and then electroblotted onto nitrocellulose. The nitrocellulose sheet was then treated with avidin–biotin–horseradish peroxidase conjugate (Vector Laboratories, Burlingame, CA) and bound HRP was detected by reacting the blot with diaminobenzidene and hydrogen peroxide according to routine immunoblot procedures recommended by the manufacturer. The line at the top of each lane marks the top of each blot and was used for alignment. Two mice were examined for each time interval.

site to the spinal cord within that time (Grafstein and Forman 1980). The alternative possibility that the b-Hsc70 appearing in the DRG and spinal cord arrived there by diffusion through the extracellular space or via the vasculature is considered unlikely because one would then have to further presume that the neurons were able to concentrate the protein as it was being diluted throughout the animal's body in the case of the DRG and that the protein passed through the blood–brain barrier in the case of the spinal cord. Furthermore, this analysis suggested that a portion of the b-Hsc70 was covalently modified so that it ran on the gel at about 120 kDa (Fig. 4, arrow indicating modified Hsc70). This modification of the b-Hsc70 probably occurred after its uptake, since no higher molecular weight components were detectable in the b-Hsc70 administered to the mice (Fig. 4, furthest right lane). Furthermore, the modified b-Hsc70 appeared to be produced predominantly in the neuronal somata, as it was only weakly detected in the 1-h nerve sample in contrast to its prominence in the DRG and spinal cord samples at all three collection times (Fig. 4). Another indication that the b-Hsc70 taken up by the neural tissue was subject to metabolism was provided by the appearance in the 24-h spinal cord sample of a number of lower molecular weight biotinylated polypeptides, probably reflecting degradation of the b-Hsc70. However, the mechanisms involved in the modification of b-Hsc70 to produce a 120 kDa band are still unclear.

Our results strongly support the hypothesis that at least one class of neurons, sensory cells, become more resistant to potentially lethal trauma when their endogenous Hsc70 is supplemented by the administration of exogenous Hsc70. The fact that the exogenous Hsc70 was prepared from a different species is likely to be of minor importance because of the high degree of conservation of Hsc70 structure across species (Morimoto et al 1990). It is not known why motoneuron survival was not significantly increased following treatment with Hsc70, but the distinction in the response of these two classes of neurons is not surprising, since they respond differently to a variety of neurotrophic factors (e.g. Oppenheim et al 1993). The trend towards increased motoneuron survival with the lower doses of Hsc70 (Fig. 3) suggests that a significant protective effect might be elicited using doses and/or schedules of administration different from those used here and will be evaluated in future work.

Previous studies of the sciatic nerve have shown that there is an increase in production of either inducible (Hsp70) or constitutive (Hsc70) *endogenous* stress

protein 70 following axonal injury (Tedeshi et al 1993; Edbladh et al 1994). Furthermore, we have observed that there is a substantial amount of Hsc70, and some Hsp70, detectable by immunohistochemistry in uninjured DRG neurons (not shown). Recently, abundant levels of immunoreactive Hsc70 have been reported in human DRG neurons (Kato et al 1995). Together, these observations suggest that basal levels of endogenous Hsc70 are insufficient to maximally protect neurons from the deleterious effects of axotomy. This idea is consistent with the observations in many other model systems that a mild, conditioning stress which elevates endogenous Hsc70 above the basal level reduces the extent of cell death after a subsequent severe stress (Barbe et al 1988; Khan and Sotelo 1989; Kirino et al 1991; Lowenstein et al 1991; Nowak and Jacewicz 1994; Tytell et al 1994). Thus, the amplification of Hsc70 levels, whether by stimulating endogenous production or by providing more of the protein from exogenous sources, improves neuronal resistance to damage. In the sciatic nerve, the potential contribution of extra-neuronal Hsc70 has, in fact, been implicated by Edbladh et al (1994), who have shown that Hsc70 induced in the glial sheath cells at the site of axotomy is taken up by the axons and retrogradely transported to the DRG. However, these results should not be taken to mean that the exogenous Hsc70 functions in an identical manner as that produced endogenously. Other work has indicated that exogenous Hsc70 becomes associated with the cell surface, but is not internalized (Johnson and Tytell 1993) and that it enhances the efflux of calcium ions from neurons in culture (Smith et al 1995). The latter is an especially intriguing observation since increased intracellular calcium is well known to be one of the major factors leading to death of an injured cell.

Although our work has shown that exogenous Hsc70 prolonged the survival of axotomized sensory neurons, further studies must be done to determine if the rescue of those neurons is permanent and whether their capacity for axonal regeneration is also enhanced. Because the stress protein used in the present study consists of 95% Hsc70 and 5% Hsp70 (see legend to Fig. 1), whether the survival-promoting activity on sensory neurons is due to either form of the protein is not known. The use of recombinant forms of the two variants of the 70-kDa stress protein should help answer this question. These studies will also help to confirm whether or not this cytoprotective protein has potential as a therapeutic agent in nervous system injury.

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REFERENCES

Barbe MF, Tytell M, Gower DJ and Welch WJ (1988) Hyperthermia protects against light damage in the rat retina. Science **241**, 1817–1820.

Brown AC (1981) Organization in the Spinal Cord, Springer-Verlag, New York.

Crews LL and Wigston DJ (1991) The dependence of motoneurons on their target muscle during postnatal development of the mouse. J. Neurosci. **10**, 1643–1653.

Currie RW and White FP (1981) Trauma-induced protein in rat tissues: a physiological role for a 'heat shock' protein? Science **214**, 72–73.

Edbladh M, Ekström PAR and Edström A (1994) Retrograde axonal transport of locally synthesized proteins, e.g. actin and heat shock protein 70, in regenerating adult frog sciatic sensory axons. J. Neurosci. Res. **38**, 424–432.

Grafstein B and Forman DS (1980) Intracellular transport in neurons. Physiol. Rev. **60**, 1167–1283.

Johnson AD and Tytell M (1993) Exogenous Hsp-70 becomes cell associated, but not internalized by stress arterial smooth muscle cells. In Vitro Cell. Dev. Biol. **29A**, 807–812.

Johnson AD, Berberian PA, Tytell M and Bond MG (1995) Differential distribution of 70–kD heat shock protein in atherosclerosis–Its potential role in arterial SMC survival. Arterioscler. Thromb. Vasc. Biol. **15**, 27–36.

Kato M, Mizuguchi M and Takashima S (1995) Developmental changes of heat shock protein 73 in human brain. Dev. Brain Res. **86**, 180–186.

Khan NA and Sotelo J (1989) Heat shock stress is deleterious to CNS cultured neurons microinjected with anti-HSP70 antibodies. Biol. Cell **65**, 199–202.

Kirino T, Tsujita Y and Tamura A (1991) Induced tolerance to ischemia in gerbil hippocampal neurons. J. Cereb. Blood Flow Metab. **11**, 299–307.

Li L, Oppenheim RW, Lei M and Houenou LJ (1994) Neurotrophic agents prevent motoneuron death following sciatic nerve section in the neonatal mouse. J. Neurobiol. **25**, 759–766.

Li L, Lo AC, Lei M, Oppenheim RW and Houenou LJ (1995) Effects of neurotrophic factors on the survival of sensory neurons following axotomy in the neonatal mouse. Soc. Neurosci. Abst. 21, 1789.

Lowenstein DH, Chan PH and Miles MF (1991) The stress protein response in cultured neurons: Characterization and evidence for a protective role in excitotoxicity. Neuron **7**, 1053–1060.

Mayer J and Brown I (1994) Heat Shock Proteins in the Nervous System, Academic Press, New York.

Morimoto RI, Tissières A and Georgopoulos C (1990) The stress response, function of the proteins, and perspectives. In: Stress Proteins in Biology and Medicine, eds RI Morimoto, A Tissières and C Georgopoulos, Cold Spring Harbor Laboratory, NY, 1–36.

Nowak TS Jr and Jacewicz M (1994) The heat shock/stress response in focal cerebral ischemia. Brain Pathol. **4**, 67–76.

Oppenheim RW, Prevette D, Haverkamp LJ, Houenou LJ, Yin QW and McManaman (1993) Biological studies of a putative avian muscle-derived neurotrophic factor that prevents naturally occurring motoneuron death in vivo. J. Neurobiol. **24**, 1065–1079.

Oppenheim RW, Houenou LJ, Johnson JE et al (1995) Developing motor neurons rescued from programmed and axotomy-induced cell death by GDNF. Nature **373**, 344–346.

Schlossman DM, Schmid SL, Braell WA and Rothman JE (1984) An enzyme that removes clathrin coats: Purification of an uncoating ATPase. J. Cell Biol. **99**, 723–733.

Schmalbruch H (1984) Motoneuron death after sciatic nerve section in newborn rats. J. Comp. Neurol. **224**, 252–258.

Schmid SL and Rothman JE (1985) Two classes of binding sites for uncoating protein in clathrin triskelions. J. Biol. Chem. **260**, 10050–10056.

Smith PJS, Hammar K and Tytell M (1995) Effects of exogenous heat shock protein (hsp70) on neuronal calcium flux. Biol. Bull. **189**, 209–210.

Snider WD, Elliott JL and Yan Q (1992) Axotomy-induced neuronal death during development. J. Neurobiol. **23**, 1231–1246.

Tedeshi B, Ciavarra RP and Morgan CW (1993) A stress-inducible heat shock protein (HSP68) is expressed in rat DRG following axotomy. Soc. Neurosci. Abstr. **19**, 1486.

Tytell M, Greenberg SG and Lasek RJ (1986) Heat shock-like protein is transferred from glia to axon. Brain Res. **363**, 161–164.

Tytell M, Barbe MF and Brown IR (1994) Induction of heat shock (stress) protein 70 and its mRNA in the normal and light damaged rat retina after whole body hyperthermia. J. Neurosci. Res. **38**, 19–31.

Willis WD and Coggeshall RE (1991) In: *Sensory Mechanisms of the Spinal Cord*, Plenum Press, New York.

Yan Q and Matheson CR (1995) Retrograde transport study of glial cell line-derived neurotrophic factor (GDNF) in adult rat nervous system. Soc. Neurosci. Abst. **21**, 1301.

Yan Q, Elliott JL, Matheson C et al (1993) Influence of neurotrophins on mammalian motoneurons *in vivo*. J. Neurosci. **24**, 1555–1577.