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Annotation

Neurotoxic acrylamide and neurotrophic melanocortin peptides—can contrasting actions provide clues about modes of action?

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Experimental acrylamide neuropathy has been studied as a model of degenerative neurological disorders of the 'dying-back' type for over 30 years. Functional, histological, ultrastructural, electrophysiological and biochemical aspects of acrylamide neuropathy have been described and several hypotheses concerning the mode of action proposed. However, the mechanism whereby acrylamide causes axonal degeneration and inhibits nerve sprouting remains unknown. By analogy with agonist/antagonist comparisons used by the pharmacologist, we have reconsidered the acrylamide problem in the light of the opposite effects summarized in Table 1, of neurotrophic peptides related to ACTH/MSH (collectively termed melanocortins). The contrasting effects on sprouting and the eventual quality of repair of mechanically lesioned nerves have suggested a mechanism whereby sprouting may regulate perikaryal adjustments to injury. We have also posed the question as to whether a common biochemical mechanism, namely selective proteolysis of neurofilament protein may underlie the opposing effects of acrylamide and melanocortins on nerve sprouting. This possibility implies a hitherto unknown role for neurofilament protein turnover in neuronal maintenance and repair, a suggestion that may provoke further research and discussion.

Keywords: acrylamide, melanocortins, axonal degeneration, nerve sprouting, neurotoxins, neurotrophic agents

INTRODUCTION

Nerve cells depend for their proper function on correct connections with appropriate incoming synapses on dendrites and with target cells at distant axon terminals. Maintenance of the structural and functional integrity of axons, terminals and, to a lesser extent, dendrites is

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Table 1. Contrasting effects of acrylamide and melanocortins on various aspects of post-lesion nerve function

Phenomenon	*Acrylamide*	*Melanocortins*
Functional recovery	Retarded	Accelerated
Number of sprouts	Decreased	Increased
Conduction velocity	Decreased	Increased
Neurofilament turnover	Proteolysis inhibited	Mimic proteolytic product?

dependent on the supply of materials synthesized in the perikaryon and delivered by transport systems which carry diverse components in varying amounts at heterogeneous rates to different destinations. Furthermore, harmonization of the function of the entire cellular unit depends on the flow of information between different regions. This information comprises not only electrical signals but also chemical signals carried by the axoplasmic transport systems. At the input and output regions, cellular connections (synapses) are not rigidly fixed but can be retracted and regrown in response to changing local environments. The flexibility of the system implies a degree of instability which will result in an inherent vulnerability to damage.

To date we understand little about the biological systems involved in maintenance of neuronal integrity and repair. Insight into the problem is coming from two distinct areas of research, namely studies on toxins, which cause neuronal integrity to fail, and studies on trophic agents which support nerve survival and repair. Studies on neurotoxins, often originally discovered as occupational or environmental hazards, have been largely aimed at understanding the processes involved in the lesion, with the hope that the findings will be generally applicable to problems of neuronal damage and repair. Thus, experimental acrylamide neuropathy has been used extensively as a model of degenerative neurological disorders of the 'dying back' type (Cavanagh, 1964). Since the discovery of nerve growth factor (NGF) in 1951 (Levi-Montalcini & Hamburger, 1951), several agents which promote the survival and growth of neurons have been described (Lander, 1987; Barde, 1989) but understanding of the physiological role of these agents and their mode of action is still in its infancy.

In this review, we compare the effects of the neurotoxin acrylamide, which causes axonal degeneration and inhibits nerve repair, with the actions of neuropeptides related to ACTH/MSH (collectively termed melanocortins), which enhance axonal repair. Such a comparison may provide insights into the critical processes affected by these contrasting agents (Table 1). For recent reviews of acrylamide neuropathy and the neurotrophic effects of melanocortins the reader is referred to Miller and Spencer (1985) and Strand *et al.* (1989).

ACRYLAMIDE NEUROPATHY

Distribution of the lesion and ultrastructural changes

The predominant characteristic of acrylamide neuropathy is axonal degeneration which commences at or close to the terminal and progresses proximally (Cavanagh, 1982; Miller & Spencer, 1985). Not all nerves are equally susceptible to damage from acrylamide. In general, long, large diameter nerves are more sensitive, and sensory nerves are more sensitive than motor nerves (Sumner, 1980; Cavanagh, 1982). The selectivity cannot be explained by

differential accessibility to the toxin as acrylamide given by any route rapidly distributes in the total body water and binds equally to proteins in different regions of the nervous system (Miller & Spencer, 1985). Distal regions of long fibres might be predicted to be the most susceptible when the supply of materials required for normal maintenance or repair becomes inadequate due to compromise of either the cell body metabolism or the transport system or because of distal damage. Several studies have shown that the distribution of initial changes does not correlate with the final pattern of axonal degeneration (Jennekens *et al.*, 1979; Cavanagh, 1982) and it has been suggested (Jennekens *et al.*, 1979) that disparate regenerative capacities may play a role in the selective vulnerability of neurons.

Functional neuropathy and axonal degeneration are only seen after repeated doses of acrylamide. The same total dose will result in a neuropathy when given in different dosing regimes but cannot be given in a single dose as this is acutely lethal. Those who are searching for an initial site of action must look either at early times after a single dose, which will not produce a neuropathy, or use chronic dosing regimes in which initial toxic damage is compounded by reactions to the damage. The earliest ultrastructural changes resulting from repeated dosing with acrylamide are accumulations of filaments and membranous materials in terminals and preterminal nodes of Ranvier (Suzuki & Pfaff, 1973; Cavanagh, 1982; Jennekens *et al.*, 1979) observed around the time that neurological symptoms become evident. Apart from dramatic changes in cerebellar Purkinje cells of unknown relationship to other toxic processes, the changes observed in the cell bodies are those one would expect in response to injury (Cavanagh, 1982; Sterman, 1983), even though they occur before there is detectable axonal degeneration. This suggests that the primary toxic action causes both the peripheral terminal accumulations and initiation of damage-induced reactions in the cell body. Only one report (Gold, Griffin & Price, 1985) shows morphological changes after a single dose of acrylamide but the nerves were examined 7 days after dosing, so reactive rather than causative changes cannot be excluded. In this study, the neurofilamentous accumulations observed in proximal but not distal regions of large diameter fibres were attributed to a modest decrease in the rate of slow axoplasmic transport which, in itself, would be insufficient to lead to axonal degeneration.

Functional abnormalities

The earliest neurological signs of acrylamide intoxication in experimental animals are disorders of gait and limb position (Spencer & Schaumburg, 1974; Cavanagh, 1982) indicative of impaired proprioception. Electrophysiological studies on peripheral nerves have confirmed the preferential loss of rapidly conducting sensory nerve fibres (Fullerton & Barnes, 1966; Hopkins & Gilliatt, 1971) shown histologically. These include the Ia fibres innervating the muscle spindles and the early loss of response in these fibres (Lowndes *et al.*, 1978) is probably responsible for the abnormalities in gait and limb positioning, although the role of Purkinje cell damage has yet to be determined. Decreases in maximal conduction velocities measured in mixed peripheral nerves have been attributed to selective loss of large diameter, rapidly conducting fibres (Fullerton and Barnes, 1966; Hopkins & Gilliatt, 1971). Using the H-reflex to measure conduction velocities selectively in Ia afferents, we observed reduced conduction velocities as a result of acrylamide intoxication (Figure 1). Our experiments do not preclude a preferential loss of the fastest conducting among the Ia fibres but the time course of the changes does not support this explanation. The decrease in conduction velocity occurs very late in the recovery phase after the neurological signs, which correlate with the loss of Ia afferents (Lowndes *et al.*, 1978), have returned to normal. Return of Ia fibre function indicates that the

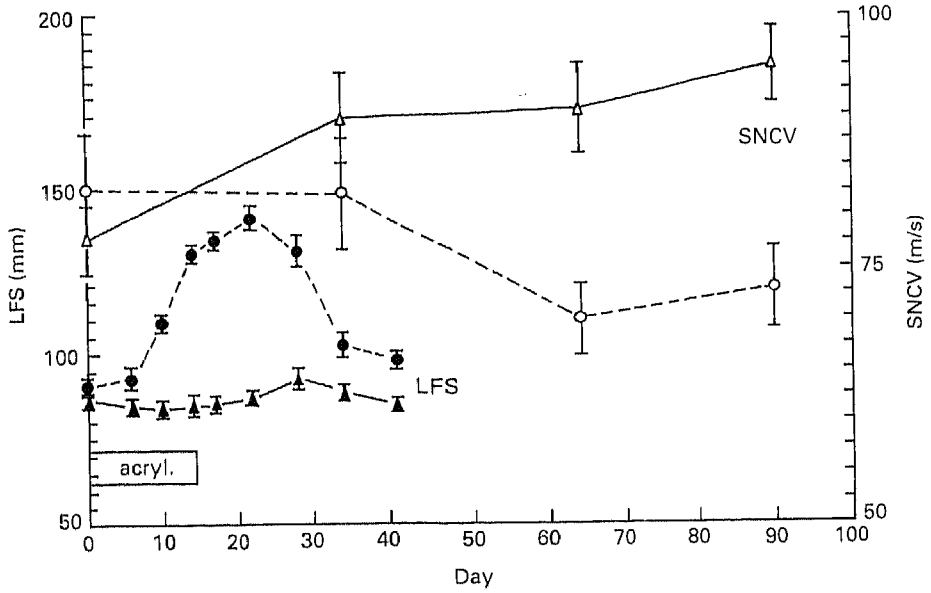

Figure 1. Disparate time courses of deficits in limb positioning and sensory nerve conduction velocity in acrylamide neuropathy. Rats (○,●) were dosed with acrylamide (50 mg/kg, i.p.) every 48 h during the period shown (total dose 250 mg/kg) or with saline (△,▲). The limb position on landing from a fall (LFS, ●,▲) and H-related sensory nerve conduction velocity in the tibial/sciatic nerve (SNCV, ○,△) are shown. The values given are the mean ± SEM of eight rats in the acrylamide group and 12 rats in the control group.

damaged axons have regenerated and formed the appropriate functional terminals. The observation that conduction velocity decreases subsequent to this regeneration indicates that the decrease must represent a phenomenon other than axonal degeneration. The possibility that the reduced conduction velocity results from a disturbance of regenerative responses is discussed more fully later.

Axoplasmic transport

Conflicting reports of changes in the various categories of axoplasmic transport reflect the difficulties in interpreting this type of data in a progressive condition where causative and reactive changes are difficult to separate. Sickles (Sickles, 1989a) found a marked (40–50%) reduction in the *quantity* of labelled material rapidly transported from dorsal root ganglia within 3 h of a single dose of acrylamide. Protein synthesis in the ganglia was not impaired and only a modest (10–20%) reduction in the *rate* of transport was seen. Similar effects were observed following dosing with 2,5-hexanedione (2,5-HD) (Sickles, 1989b), which also causes axonal degeneration. Non-neurotoxic analogues of acrylamide or of 2,5-HD did not reduce the quantity of rapidly transported material. Nor did β,β′-iminodipropionitrile (IDPN), a neurotoxin which causes proximal neurofilamentous accumulations but not axonal degeneration. Using a single dose regime, marked reductions in retrograde transport of NGF (Miller *et al.*, 1983) and tetanus toxin (Miller & Spencer, 1984) have also been reported. Decreases in retrograde transport of both proteins and glycoproteins also occur early in the course of chronic intoxication, before the first signs of functional or ultrastructural abnormalities (Jakobsen &

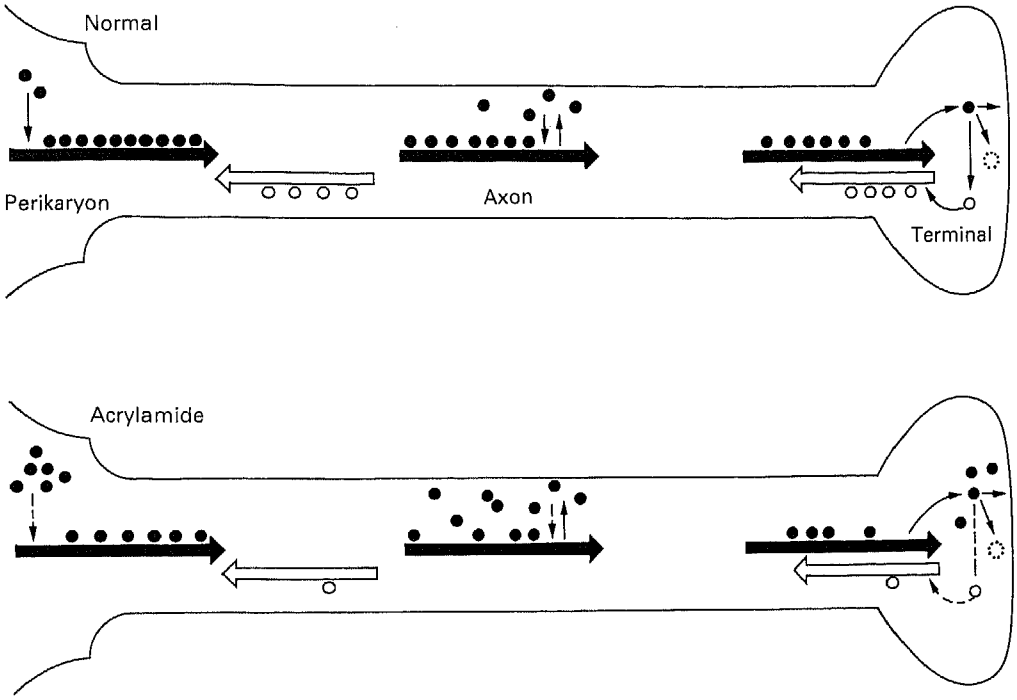

Figure 2. Deficits in loading on to transport systems may explain many of the abnormalities seen in acrylamide neuropathy. In the acrylamide-exposed nerve, less material is loaded on to the anterograde transport system leading to a decreased amount of transported material compared to normal. In the axon, off-loading is normal but re-loading from the stationary phase is impaired, leading to a shift from the transported to the stationary phase. At the terminal, although a reduced amount of material arrives, its subsequent removal is compromised by impaired processing and/or loading on to the retrograde transport system. Retrograde transport is thus reduced and at the terminal, supply exceeds demand and organelles accumulate.

Sidenius, 1983). Taken together, these data suggest direct action of acrylamide on the rapid transport systems is a key step in the initiation of axonal degeneration. Distal degeneration could thus be explained by a failure of supply of essential materials. However, the acrylamide-induced build-up of organelles in preterminal and terminal axons prior to apparent nerve dysfunction (Cavanagh, 1982) suggests that there is an excess, rather than a deficit, in supply. Distal accumulations of rapidly transported proteins have also been reported to occur prior to axonal degeneration (Souyri, Chretien & Droz, 1981). These apparent contradictory findings can be reconciled if the loading of materials on to the transport systems, rather than the systems themselves, is impaired. Newly synthesized materials associate with the anterograde system in or close to their site of synthesis in the perikaryon; in the axon, a complex exchange of organelles between the transported and stationary phase occurs; at the distal end, materials delivered are removed by secretion, degradation or loaded on to the retrograde systems. An effect of acrylamide on the loading system would result in both a decrease in the amount of label transported and distal accumulation (Figure 2). As most of the rapidly transported proteins participate in the stationary/mobile exchange occurring in transit along the axon (Mũnoz-Martinez, 1982) the scheme shown in Figure 2 would also explain the shift from the transported to the stationary phases reported for glycoproteins (Harry *et al.*, 1989) and some forms of the enzyme acetyl-

Table 2. Comparison of the neurofilament accumulation, neurofilament transport inhibition and axonal degeneration caused by four different neurotoxins

Toxin	*Site of NF accumulation*	*Site of severe inhibition of NF transport*	*Axon degeneration*
Acrylamide	Distal	—	Yes
2,5-HD	Proximal side of distal nodes	Distal nodes	Yes
IDPN	Proximal	Proximal→distal	No
3,4-HD	Proximal	Proximal→distal	Yes

2,5-HD, 2,5-hexanedione.
IDPN, β,β'iminiodipropionitrile.
3,4-HD, 3,4-dimethyl,2,5-hexanedione.

cholinesterase (Couraud *et al.*, 1982). It is, for obvious technical reasons, not possible to determine whether the same rapid effect of acrylamide occurs in the slow transport system. The modest reduction in the slow component recorded 6 days after a single dose of acrylamide (Gold *et al.*, 1985) could well include reactive alterations. Chronic acrylamide administration resulting in changes in fast and slow axonal transport are qualitatively the same as those induced by axotomy (Gold *et al.*, 1985; Bisby & Redshaw, 1987).

A failure in the loading of materials on to the retrograde and anterograde transport may explain the ultrastructural abormalities observed in the early stages of acrylamide neuropathy. The possible biochemical lesion which causes this abnormality and the relationship between the altered transport and the breakdown of axonal integrity are discussed further below.

Biochemical lesions

Acrylamide is a moderately reactive chemical and binds covalently to biological sulphydryl and amine groups (Miller & Spencer, 1985). Many enzymes and biological processes are dependent on thiol groups and would be expected to be affected by acrylamide, but the relevance of these effects to the axonal degeneration needs to be shown. A compromise by acrylamide of the metabolic capacity of the axon was an attractive hypothesis since several of the enzymes are thiol-dependent and replacement of inhibited enzymes would require axoplasmic transport, which is itself energy dependent. Although inhibition of enzymes in mitochondria and the glycolytic pathway have been shown (Howland, 1985; Sickles, 1987), it seems unlikely that this is a primary cause of acrylamide neuropathy. Rapid axoplasmic transport is reduced when the energy reserves (ATP and CP) in the axon are still high (Sickles & Pearson, 1987) and reduced glucose utilization is not seen in acrylamide-intoxicated rats (Hashimoto & Aldridge, 1970).

The prominence of neurofilamentous accumulations as an early ultrastructural abnormality in a number of toxic neuropathies and human neurological disorders have prompted several groups to propose that the axonal cytoskeleton may be a primary site of damage. As is shown in Table 2, in general, accumulation of neurofilaments occurs in the region from which their removal by transport is impaired (Anthony, Giangaspero & Graham, 1983; Griffin, Price &

Hoffman, 1983; Griffin *et al.*, 1984; Gold *et al.*, 1985). This indicates that distal neurofilament turnover may be impaired in acrylamide neuropathy.

Accumulations of neurofilaments do not necessarily cause axonal degeneration. Proximal accumulations of neurofilaments are caused by both IDPN and 3,4-dimethyl-2,5-hexanedione (3,4-HD) but distal Wallerian degeneration results only in the latter case (Anthony *et al.*, 1983; Griffin *et al.*, 1983). Comparison of ultrastructural changes in different nerves indicates that neurofilamentous accumulations resulting from hexacarbon treatment are widespread, but axonal degeneration only results when the geometry of the nerve is such that the mass of neurofilaments causes a serious blockage of distal nodes of Ranvier (Jones & Cavanagh, 1983). In these susceptible nerves, the neurofilaments are no longer able to reach the terminal region where proteolytic removal normally occurs.

Several lines of evidence suggest a direct effect of 2,5-HD and related compounds on the neurofilament proteins. Cross-linking of neurofilaments by these agents has been shown to occur both *in vitro* (Graham *et al.*, 1985) and *in vivo* (Lapadula *et al.*, 1986). Ultrastructural studies of acrylamide neuropathy do not indicate that distal nodes of Ranvier become plugged with neurofilaments, as in the case with 2,5-HD. Furthermore, although acrylamide binds directly to neurofilaments (Lapadula *et al.*, 1989) there is no evidence for cross-linking. However, the similarity of the effect of acrylamide and 2,5-HD on intermediate filament integrity in various cell types (Sager & Matheson, 1988) suggests other actions of acrylamide on neurofilaments may result in similar structural changes. *In vitro* phosphorylation of neurofilaments was altered in the spinal cord of rats in which neurological symptoms were already apparent (Howland & Alli, 1986), although immunocytochemical studies indicated that in neuronal perikarya changes in neurofilament phosphorylation occurred late and were consistent with a reaction to injury (Gold *et al.*, 1988). Inhibition of neurofilament degradation *in vitro* by calcium-activated neutral protease (CANP) has also been reported both for acrylamide and hexanedione, whereas non-neurotoxic analogues were inactive (Tanii, Hayashi & Hashimoto, 1988). These observations may be related since it is known that phosphorylated neurofilaments are less susceptible to proteolytic digestion (Pant, 1988). Both phosphorylation and proteolytic cleavage are important in the structure and function of neurofilaments (Schlaepfer, 1987). Inhibition of CANP by the specific inhibitor leupeptin causes neurofilament accumulation in terminals (Roots, 1983), indicating that cleavage by this enzyme is responsible for at least some of the disposal of neurofilaments delivered by axoplasmic flow. The subsequent fate and possible function of the proteolytic fragments is unknown but both Schlaepfer (Schlaepfer, 1987) and our group (Edwards *et al.*, 1984) have suggested that they may be signals involved in the chemical communication between distal regions of the nerve and the perikaryon. Leupeptin also blocks the anterograde-to-retrograde conversion of axonally transported vesicles (Sahenk & Lasek, 1988). It is difficult to deduce from the *in vivo/vitro* studies in chronically intoxicated animals whether neurofilament phosphorylation and proteolysis represent initial sites of action that subsequently lead to neurofilamentous accumulation, inhibition of retrograde transport and axonal degeneration.

One other direct action of acrylamide deserves consideration. Rapid inhibition of brain transglutaminase results from a single dose of acrylamide (Bergamini & Signorini, 1990). Transglutaminase, a calcium-activated thiol enzyme, is also inhibited by acrylamide *in vitro* (Bergamini & Signorini, 1988). Cytoskeletal and membrane proteins are preferred substrates and a role in vesicle motility has been proposed (Pastuszko *et al.*, 1986). Thus it may be involved in loading of the rapidly transported materials on to axoplasmic transport systems, inhibition of which would produce many of the observed consequences of acrylamide neuropathy.

From whatever cause, direct effects of acrylamide on the neurofilaments implies that the distal accumulations are not simply a manifestation of a generalized malfunction of transport but rather may be a cause of these abnormalities. The effects of the toxin on neuronal sprouting and repair, which are not easily explained by a transport deficit, may also be mediated by an initial disturbance of neurofilament metabolism.

ACRYLAMIDE AND REPAIR

The first observation that acrylamide impairs axonal repair was made by Morgan-Hughes and co-workers (Morgan-Hughes, Sinclair & Durston, 1974). A number of abnormalities were noted: extensive dying-back from the site of the crush, delayed functional recovery, reduced number and diameter of regenerating fibres and reduced conduction velocity. The axonal degeneration seen proximal to a ligation (Cavanagh & Gysbers, 1980) is not a non-specific toxic effect, since no retrograde degeneration was found with the neurotoxins isoniazid and 2,5-hexanedione. Retrograde degeneration was equally severe when the ligature was placed proximally or distally on the nerve (Sharer & Lowndes, 1985). This suggests that arguments for selective susceptibility of unlesioned nerves to acrylamide, based on the distance of terminals of long axons from the cell body, are invalid. Ultrastructural changes indicated an excessive accumulation of membranous organelles but neurofilamentous accumulations were not noted (Cavanagh & Gysbers, 1981). In spite of this 'dying-back', nerves are later able to regenerate, as had been noted in previous studies in chronic acrylamide treatment without mechanical lesions (Fullerton & Barnes, 1966; Suzuki & Pfaff, 1973). However, the regenerative sprouts are highly abnormal and swollen with neurofilamentous accumulations and membranous structures (Griffin, Price & Drachman, 1977; Cavanagh & Gysbers, 1980). The impaired outgrowth is not the result of inadequate supply of materials by axoplasmic system, as might be suggested by the reduced rapid transport discussed above. Conversely, the regenerating tips have been shown to contain more than normal amounts of rapidly transported organelles (Griffin *et al.*, 1977) although their outgrowth was much reduced. The inhibitory effect has since been shown to be a generalized one on sprouting that is independent of axonal damage. The effect of acrylamide on sprouting, which occurs spontaneously in motor nerve terminals and is greatly exaggerated following local treatment with botulinum toxin or partial denervation, has been studied in the rat sternocostal muscle (Kemplay & Cavanagh, 1984). Using this model, a marked inhibition of spontaneous and induced sprouting within 24 h of a single dose of acrylamide has been shown. This effect is long-lasting and can be detected as long as 31 days after dosing. Furthermore, inhibition of sprouting and retraction of preformed sprouts was also observed after acrylamide treatment *in vitro*. This would indicate a rapid and direct local effect on neurites which does not involve the cell bodies. Inhibition of neurite outgrowth and destruction of preformed neurites has also been described in retinal explant cultures (Tanii, Kato & Hashimoto, 1987). A direct effect on sprouting may underlie the reduced repair capacity. Other neuronal responses to injury appear to be normal. Thus the early and marked changes in the cell bodies of acrylamide-treated animals are similar to those seen following mechanical injury (Cavanagh, 1982; Sterman, 1983). Changes in perikaryal neurofilaments (Gold *et al.*, 1988) and in the synthesis and rapid transport of the growth-associated protein GAP43/B50 are also the same in acrylamide-treated and mechanically injured nerves (Bisby & Redshaw, 1987). Following injury to a nerve, the diversity of reactions set in motion may be triggered by different signals generated as a result of the damage. The early lesion caused by acrylamide, which inhibits sprouting and impairs exchange of materials with the transport system may also generate the signal for perikaryal responses.

This would explain how such responses have been observed prior to any morphological evidence of injury (Cavanagh, 1982).

One striking effect of acrylamide on neuronal repair reported by Morgan-Hughes was that the conduction velocities of the regenerated fibres were greatly reduced in comparison with the non-intoxicated animals (Morgan-Hughes *et al.*, 1974). This reduction cannot be explained on the basis of selective loss of large diameter fibres as all the regenerating fibres will be of small diameter. This supports our suggestion that in nerves recovering from injury, whether caused mechanically or by the toxin itself, acrylamide caused reduced conduction velocities by an effect on repair processes which results in reduced axonal diameter. The finding that neurotrophic peptides had the opposite effect both on regenerative sprouting and on final conduction velocities suggests that these two phenomena may be related.

MELANOCORTINS AND REPAIR

Melanocortins are peptides related to ACTH and α-MSH which exert effects on various aspects of neural function that have collectively been described as an enhancement of neural plasticity. The neurotrophic actions of these peptides has recently been reviewed (Strand *et al.*, 1989) and only those aspects relevant to the consideration of acrylamide neuropathy will be discussed here. Melanocortins accelerate recovery of peripheral nerve function in experimental animals following a crush or cut lesion (Bijlsma *et al.*, 1983) and enhance collateral sprouting after partial denervation (De Koning *et al.*, 1989). The most marked effect of the peptide is a large increase in the number of regenerative sprouts that are formed early in the repair process (Verhaagen, 1987), even after a single dose of the peptide. Increased sprouting is the direct opposite of what is seen in acrylamide neuropathy. Interestingly, another phenomenon is also opposite. Following nerve crush, conduction velocities are normally reduced for long periods after return of function. This late deficit is reversed by treatment with melanocortins (De Koning & Gispen, 1987) and normalization of conduction velocities is seen even though the peptides are only given during the early sprouting phase (Figure 3). Indeed, the beneficial effect of the peptides is lost if initiation of treatment is delayed for one week after injury (Edwards *et al.*, 1984). Does the early effect of peptides on the number of sprouts finally result in an increased conduction velocity? This would be in keeping with the opposite effects of acrylamide—a decreased initial sprouting leading to a delayed reduction in conduction velocity. A possible explanation for a causal relationship between numbers of regenerative sprouts and eventual conduction velocity is proposed below. The mechanism of action whereby neuropeptides increase the number of sprouts in response to injury is as much of a mystery as the cause of acrylamide-induced reduction. Axoplasmic transport is not altered (Crescitelli, Strand & Keim, 1989) and changes in the synthesis and transport of the growth-associated protein GAP43/B50 are in keeping with the increased number of sprouts (Van der Zee *et al.*, 1989) and thus could be the result of the increased outgrowth. Melanocortins are active when applied at the site of the nerve injury (Edwards *et al.*, 1986) and may act by mimicking locally-produced signals that initiate sprouting following injury. Injured nerve extracts contain neurotrophic factors (Politis & Spencer, 1983) and MSH-like biological activity (Edwards *et al.*, 1984). The chemical identity of these agents is unknown but it is possible that one of the neurotrophic factors is responsible for the MSH-like activity and is the physiological sprouting stimulus mimicked by exogenous melanocortins. The source of this 'ensproutin' in unknown. One of the earliest changes detected in degenerating nerves is a selective proteolysis of the neurofilaments by a calcium-activated neutral protease. The presence, within the middle size neurofilament protein, of an epitope that

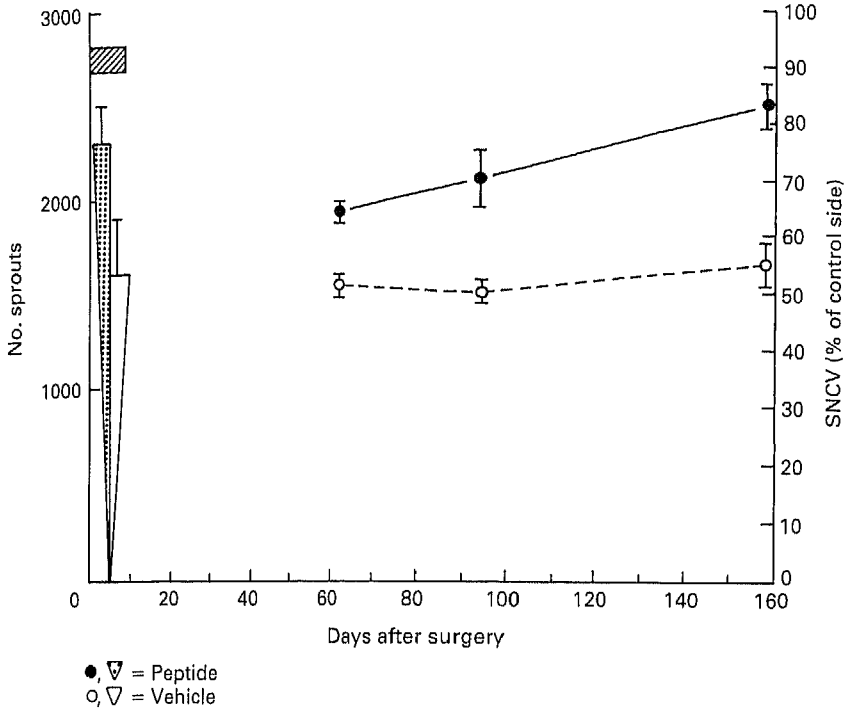

Figure 3. Treatment with melanocortin peptides during the first week after crush injury results in rapid stimulation of sprouting and subsequent increased sensory nerve conduction velocity. Peptide or vehicle was administered during the period shown by the bar, every 48 h from the day of crush injury of the sciatic nerve. The total number of sprouts at the distal border of the crush (▨, □) was measured 4 days after surgery. The H-related sensory nerve conduction velocity in the tibial/sciatic nerve (●, ○) was measurable from 50 days after surgery. The data are taken from Verhaagen *et al.* (1987) and De Koning and Gispen (1987).

cross-reacts with sera raised against MSH led to the suggestion that 'ensproutin' could be released from NFM by selective proteolysis (Edwards *et al.*, 1984). Inhibition of neurofilament degradation by acrylamide would, in this model, lead to a decreased production of 'ensproutin' and an inhibition of sprouting. Attempts to demonstrate the formation of this peptide have not yet been successful and direct evidence for the model is still sought.

REGENERATIVE SPROUTING AND CONDUCTION VELOCITIES

One of the causes of reduced conduction velocity in regenerated nerves is the decreased diameter of both the regenerated nerve axons and the nerve proximal to the injury site. The proximal decrease is associated with a decreased synthesis and transport of neurofilament proteins (Hoffman, Griffin & Price, 1984; Goldstein *et al.*, 1988). While a proximal/distal distinction is less clear in acrylamide neuropathy, a similar phenomenon of reduced conduction velocity, noted earlier, is associated with decreased axon diameter and neurofilament transport (Gold *et al.*, 1985). The mechanism of inducing this down-regulation is unknown but events occurring at the transition between proximal and regenerating nerves may be crucial. At this region, there is a huge change in the volume of axoplasm between the parent axon and the thin regenerating

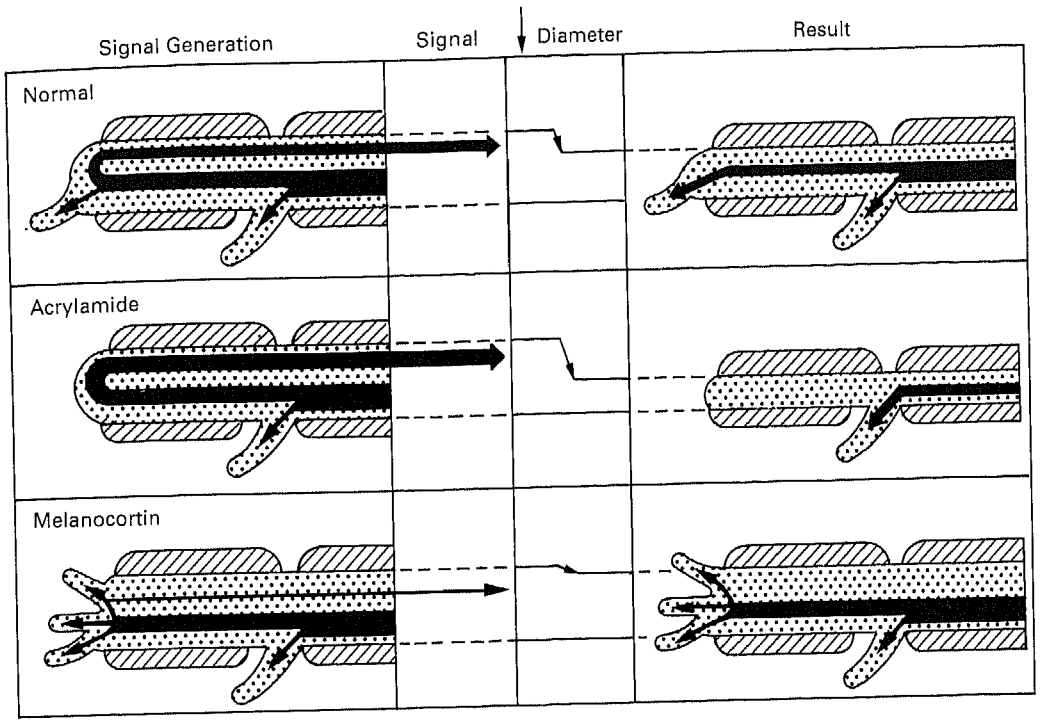

Figure 4. The number of regenerating sprouts may determine the magnitude of signals reaching the perikaryon following turn-around at the parent/sprout transition. In a normal regenerating nerve, the differential between parent and combined sprout volume results in turn-around of material arriving at the transition. The returning material may act as a signal for the down-regulation of perikaryal neurofilament synthesis and transport leading to decreased diameter in the parent axon. Acrylamide reduces the number of sprouts and the parent/combined sprout differential is therefore greater. The resulting increased turn-around sends a larger signal to the perikaryon, causing exaggerated down-regulation and more pronounced decrease in parent axon diameter. Melanocortins increase the number of sprouts, reducing the parent/combined sprout differential and hence the size of the down-regulatory signal so the final parent diameter is larger.

sprouts. Proximal to the transition, organelles and rapidly-transported materials accumulate and retrograde transport increases due to a higher turn-around (Chan, Smith & Snyder, 1989). The increased returning flow from the parent/sprout transition may act as the signal to the perikaryon to reduce neurofilament supply, thereby decreasing the proximal diameter and thus the magnitude of the differential at the transition. Retrograde flow is certainly important in triggering perikaryal responses to injury, since its blockade delays the onset of morphologic and metabolic changes induced by axotomy (Singer, Mehler & Fernandez, 1982). In acrylamide-treated nerves, there are less sprouts and the differential between parent and combined sprout volume will be greater and the amount of material returned will be greater (Figure 4). This may represent a larger signal to the perikaryon which responds with an exaggerated decrease in neurofilament supply and eventually a more profound reduction in conduction velocity. The converse is true of melanocortins, which cause increased sprouting. This model would therefore explain the opposing effect of the two agents on two apparently unrelated phenomena, namely initial sprouting and final conduction velocity.

SUMMARY

Acrylamide has long been a subject of interest and many of the ideas about its toxic action proposed in this review are not new. The particular aim was to reassess these ideas in the light of the apparent opposing effects of the neurotoxin acrylamide and neurotrophic melanocortins. These contrasts, summarized in Table 1, have suggested a mechanism whereby sprouting may regulate perikaryal adjustments to injury. The possibility that acrylamide may reduce formation of a neurofilament-derived sprouting stimulus whereas melanocortins mimic it, is attractive, but in the absence of direct evidence for the proposed 'ensproutin', remains speculative.

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