The Pathobiology of Traumatically Induced Axonal Injury in Animals and Humans: A Review of Current Thoughts

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ABSTRACT

This manuscript provides a review of those factors involved in the pathogenesis of traumatically induced axonal injury in both animals and man. The review comments on the issue of primary versus secondary, or delayed, axotomy, pointing to the fact that in cases of experimental traumatic brain injury, secondary, or delayed, axotomy predominates. This review links the process of secondary axotomy to an impairment of axoplasmic transport which is initiated, depending upon the severity of the injury, by either focal cytoskeletal misalignment or axolemmal permeability change with concomitant cytoskeletal collapse. Data are provided to show that these focal axonal changes are related to the focal impairment of axoplasmic transport which, in turn, triggers the progression of reactive axonal change, leading to disconnection. In the context of experimental studies, evidence is also provided to explain the damaging consequences of diffuse axonal injury. The implications of diffuse axonal injury and its attendant deafferentation are considered by noting that with mild injury such deafferentation may lead to an adaptive neuroplastic recovery, whereas in more severe injury a disordered and/or maladaptive neuroplastic re-organization occurs, consistent with the enduring morbidity associated with severe injury. In closing, the review focuses on the implications of the findings made in experimental animals for our understanding of those events ongoing in traumatically brain-injured humans. It is noted that the findings made in experimental animals have been confirmed, in large part, in humans, suggesting the relevance of animal models for continued study of human traumatically induced axonal injury.

Key words: diffuse axonal injury; delayed axotomy; cytoskeletal disruption; axoplasmic transport; deafferentation

INTRODUCTION

Over the course of the past 25 years, it has become increasingly clear that in patients sustaining mild, moderate, and severe forms of traumatic brain injury, axonal damage can occur, and based on the numbers of axons involved and their distribution, varying degrees of morbidity can ensue [Strich, 1956, 1961, 1970; Peerless and Rewcastle, 1967; Nevin, 1967; Oppenheimer, 1968; Tomlinson, 1970; Mitchell and Adams, 1973; Clark, 1974; Adams et al., 1977, 1980, 1982, 1989; Pilz, 1983; Imajo and Roessman, 1984; Simpson et al., 1985; Cordobes et al., 1986; Imajo et al., 1987; Sahuquillo-Barris et al., 1988; Sahuquillo et al., 1989; Blumbergs et al., 1989, 1994, 1995 (this issue); Crooks et al., 1992; Shigemori et al., 1992; Grady et al., 1993; Christman et al., 1994; Gultekin and Smith, 1994; Sheriff et al., 1994a,b,c]. Because of the well-recognized fact that, when observed, axonal damage can be found in multiple foci throughout the brain and brain stem, such ax-
onal damage has been termed diffuse axonal injury (DAI) (Gennarelli et al., 1982; Adams et al., 1982), a designation emphasizing that such injury involves more than focal perturbation to the neuraxis. In the early posttraumatic period (1–2 days), axonal damage has been associated with numerous large reactive axonal swellings, traditionally termed retraction balls, while with more prolonged survival (weeks to months), microglial stars and Wallerian degeneration have been linked to axonal injury (Strich, 1956, 1961, 1970; Peerless and Rewcastle, 1967; Nevin, 1967; Oppenheimer, 1968; Tomlinson, 1970; Mitchell and Adams, 1973; Clark, 1974). While some early workers viewed axonal injury as a phenomenon secondary to other factors, such as edema or elevated intracranial pressure with its attendant ischemic insult (Evans and Scheinker, 1944; Jellinger and Seitelberger, 1970; Jellinger, 1977), Adams and colleagues (1982) provided evidence from a carefully monitored patient population that diffuse axonal injury occurs as a primary response to trauma.

With the initial description of traumatically induced axonal damage, the findings of retraction balls, or reactive axonal swellings, early in the posttraumatic course suggested to many that forces of injury immediately tore axons, causing them to retract and expel their axoplasmic mass (Strich, 1956, 1961, 1970; Peerless and Rewcastle, 1967; Nevin, 1967; Oppenheimer, 1968; Tomlinson, 1970; Mitchell and Adams, 1973; Clark, 1974). More contemporary studies, however, conducted in both experimental animals and humans, have demonstrated that the pathogenesis of traumatically induced axonal damage is more complex than originally posited. In many cases, it involves evolving intraaxonal changes that lead to progressive axonal swelling and detachment over a 12- to 24-h period (Povlishock et al., 1983; Povlishock and Kontos, 1985; Cheng and Povlishock, 1988; Erb and Povlishock, 1988; Maxwell et al., 1988, 1991; Gennarelli et al., 1989; Tomei et al., 1990; Maxwell et al., 1991; Yaghmai and Povlishock, 1992; Grady et al., 1993; Christman et al., 1994; Sherriff, et al., 1994c).

In the following passages, we will review our current understanding of the pathobiology of the axonal damage associated with traumatic brain injury, drawing upon evidence derived from both the laboratory and the clinical setting. For the purpose of organization, we will first consider work conducted in an experimental setting, followed, in turn, by a consideration of human findings.

**EXPERIMENTAL STUDIES**

To date, the majority of studies conducted in experimental animals have utilized models of traumatic brain injury that, although capable of producing scattered axonal damage, do not produce the full spectrum of diffuse axonal injury as seen in traumatically brain-injured humans. Although beyond the scope of the current communication, it appears that only those models using subhuman primates subjected to angular acceleration replicate the full spectrum of human diffuse axonal injury. Many of the other currently used animal models, such as fluid percussion and cortical impaction, produce less widespread and more focally confined axonal damage (for review, see Gennarelli, 1994, and Povlishock et al., 1994). Because of the technical and fiscal limitations associated with the use of subhuman primates, most of the descriptive work on the pathobiology of traumatically induced axonal injury has been based upon data obtained from fluid percussion models utilizing cats, rats, and pigs. More recently, studies conducted in these models have been supplemented by isolated nerve stretch models utilizing the optic nerve (Maxwell et al., 1991; Tomei et al., 1990). Resulting from this variety of approaches has been an increased understanding of those factors involved in the genesis of traumatically induced axonal damage. This review will focus on the evidence for primary disruption of the axon cylinder, termed primary axotomy, versus the evidence for delayed or progressive alterations of the axon cylinder, termed delayed, or secondary, axotomy. In consideration of primary versus delayed axotomy, this review will consider the potential factors involved in their pathogenesis as well as their overall implications for the injured brain.
Evidence for Delayed or Secondary Axotomy

Laboratory efforts focusing on the pathobiology of diffuse axonal injury first unmasked the phenomenon of delayed axotomy early in the 1980s. Specifically, at that time, work conducted in our laboratory described the sequence of delayed axotomy in experimental animals subjected to mild traumatic brain injury (Povlishock et al., 1983). In these animals, anterograde tracers were used to fill neurons and their projection systems. The use of these anterograde tracers allowed us to assess whether the forces of injury immediately tore the axons, with an extrusion of their axoplasmic contents, or evoked more subtle intraxonally changes leading to a progression of reactive axonal abnormality.

Through this approach, we found no evidence of primary axotomy involving the expulsion of the anterogradely transported protein. Rather, we observed that the traumatic episode triggered a perturbation at one point along the axon’s length, where an impairment in anterograde transport occurred (Povlishock et al., 1983) (Fig. 1). Such impaired anterograde axoplasmic transport was first recognized within an hour of the traumatic episode, where it was associated with a focal accumulation of the anterogradely transported tracer localized within tubular and vesicular profiles of endoplasmic reticum. With increasing posttraumatic survival (2–3 h), this accumulation of the tracer continued concomitant with an increase in tracer-containing organelles, all of which were delivered by anterograde transport. This impairment, or focal block, of the anterograde transport led, over the next several hours, to further accumulation of tracer-containing organelles, which then led to local swelling and expansion of the axonal cylinder. Over time, this led to lobulation of the focal axonal swelling and subsequent disconnection of the axon at 6–12 h postinjury. The process of disconnection was rarely observed in ultrastructural analysis, indicating that it occurs rapidly. During this rapid sequence of disconnection, the proximal and distal segments of the axon pinched off from one another, each becoming sealed by a continuous axolemma and encompassed by an independent myelin sheath. Since our first description of these events within the cat corticospinal, corticoreticular, and cerebellar efferent systems, we have also observed comparable reactive change in the visual system (Cheng and Povlishock, 1988). Further, we observed a comparable sequence of changes in injuries of moderate to severe intensity (Erb and Povlishock, 1988), indicating that this evolving/delayed axotomy is not confined to animals sustaining only the mildest of injuries. Other investigators using different models of traumatic brain injury have confirmed the same repertoire of focal axoplasmic impairment and swelling, thus suggesting that the axonal pathobiology described above was neither injury model—nor species specific (Gennarelli et al., 1989; Maxwell et al., 1988, 1991; Tomei et al., 1990).

Mechanisms Responsible for Traumatically Induced Impaired Axoplasmic Transport

Confident that traumatic brain injury can result in focal impairment of axoplasmic transport leading to axonal swelling and disconnection (i.e., delayed axotomy), we next questioned what factors could be responsible for initiating this impairment in axoplasmic transport. While, conceivably, multiple factors are involved, observations of local neurofilament change occurring in relation to the above-described axon abnormalities (Povlishock et al., 1983; Erb and Povlishock, 1988) suggested to us that a traumatically induced perturbation of the axonal cytoskeleton may underlie the observed impairment of axoplasmic transport that progresses to axonal swelling and disconnection. Our laboratory initiated an assessment of this issue, utilizing well-controlled animal models of traumatic brain injury (Yaghmai and Povlishock, 1992), and in these animal models, the course of reactive axonal change was followed, using antibodies targeted to various neurofilament subunits. While it is beyond the scope of this review to comment on all of the findings obtained through the use of these antibodies, it is important to note that antibodies targeted to the 68-kDa subunit proved most useful in detecting the early neurofilament changes that were associated with the genesis of impaired axoplasmic transport. Specifically, within 15 min of the traumatic episode, focal increases in 68-kDa immunoreactivity could be detected at the light microscopic level. At the electron microscopic level, there was an apparent focal increase in the immunoreactive subunit, which now appeared to lose its linear alignment in relation to the long axis of the axon cylinder (Povlishock, 1992).

Over time (30 min–1 h), this neurofilament misalignment became more striking and was then associated with the accumulation of organelles assumed to represent the initiation of impaired axoplasmic transport (Yaghmai and Povlishock, 1992) (Fig. 1). Precisely how the observed neurofilament abnormalities translate into impaired axoplasmic transport remains a matter of controversy, as axoplasmic transport itself has long been linked with the intraxonally microtubular network (Lasek and Hoffman, 1976; Grafstein and Forman, 1980). It is possible that the misalignment of the neurofilament network also translates into microtubular misalignment, which, in turn, causes impaired axoplasmic transport; however, this issue requires further investigation.

With our observation of an altered intraxonally neurofilament/cytoskeletal network, questions naturally arose as to the potential causative mechanism(s). On one hand, one could posit that the traumatic event mechanically dis-
FIG. 2. This schematic illustration depicts a potential fate of the axon in which focal axolemmal disruption, influx of normally excluded ions, and neurofilamentous compaction (A) result in formation of a reactive axonal swelling (see enlargement B) in a different region of the axon. Specifically, at the site of influx, neurofilamentous compaction and swollen mitochondria are evident (C). Neurofilament compaction is associated with neurofilament sidearm loss (D). Here we speculate that obstructed axonal transport results in upstream axonal enlargement, neurofilamentous misalignment, organelle accumulation, and formation of the typical reactive axonal swelling (E) (compare to Fig. 1). The specific time course for this reactive axonal swelling formation is still under investigation.

rupts the neurofilament/cytoskeleton network, leading to the above-described abnormalities. Alternatively, one could also postulate that the shear and tensile forces of the injury evoke local, intraxonial neurochemical alterations that similarly lead to the above-described change. Lastly, one could argue that the axolemma itself could be concomitantly perturbed, thereby leading to local ionic dysregulation, causing impairment of axoplasmic transport.

To explore these possibilities and to critically assess the potential for axolemmal perturbation in the genesis of the above-described changes, many of these studies were repeated in the presence of extracellular tracers of various molecular weights (Pettus et al., 1994). This approach was employed on the premise that if the axolemma were perturbed by the traumatic episode, then tracers normally confined to the extracellular compartment would pass through the axolemma to reach the intraxonial front. Through this approach it was found that in the case of mild, and in many cases of moderate, traumatic brain injury, the above sequence of neurofilament abnormalities could be seen independent of any alteration in the axolemmal permeability (Pettus et al., 1994). With the injuries of increased severity, however, alterations in axolemmal permeability were seen, and these sites of axolemmal perturbation were associated with an unantici-
pated sequence of neurofilament abnormalities. Specifically, within these sites, the neurofilaments did not become misaligned as seen with milder injuries. Rather, in these foci, the neurofilaments remained linear in their alignment, yet rapidly lost their sidearms, collapsing on one another and becoming compacted (Pettus et al., 1994) (Fig. 2). Surprisingly, these focally compacted neurofilaments remained relatively stable for a several hour period, after which they became misaligned. This misalignment, in turn, triggered disordered axoplasmic transport that appears to correlate with swelling. The overall progression of axonal events involved here, however, has not been fully characterized, and it remains to be seen if this sequence of filament collapse and local swelling is operant in all instances.

Based upon the above set of experiments, it now appears that the pathobiology of delayed or secondary axotomy involves differing patterns of pathogenesis dependent upon the level of injury severity. With mild injury, the primary insult appears to be at the cytoskeletal front involving the neurofilament domain, while with more severe injury alterations in axolemmal permeability occur and interact with local cytoskeletal changes, perhaps driving the subsequent progression of reactive axonal change. In the case of the mild traumatic episode, it cannot be ruled out that the described neurofilament abnormalities are a direct result of mechanical forces acting on the local intraaxonial cytoskeleton. Primary axoplasmic failure without concomitant axolemmal perturbation has been demonstrated in isolated axons subjected to mechanical loading (Gallant, 1992), suggesting such a possibility here. Alternatively, it is possible that the forces associated with a mild traumatic insult evoke local neurochemical changes capable of eliciting neurofilament misalignment. At present, a literature is just beginning to emerge suggesting that the amino-containing head domain of the neurofilament participates in neurofilament assembly and disassembly via activation of second messenger-dependent protein kinase C and protein kinase A, which can prevent assembly as well as promote disassembly (Nixon and Sihag, 1991). The process of assembly and disassembly of neurofilament subunits has been proposed to be ongoing in normal axons (Angelides et al., 1989), and, thus, one wonders if this process could be accelerated, at least in terms of the 68-kDa subunit, in the traumatic condition. Obviously, this issue requires further investigation.

With more severe injury associated with altered axolemmal permeability, the causative factors related to the observed focal neurofilament sidearm loss are unclear. One could posit that with alterations in axolemmal permeability normally excluded ions, such as calcium, enter the intraaxonial compartment and exert damaging ef-
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fects. This possibility has been considered by multiple investigators in multiple models of axonal injury, in which increased intraaxonal calcium has been associated with rapid and immediate dissolution of the related cytoskeleton (Schlaepfer, 1974, 1987; Schlaepfer and Zimmerman, 1985; Balentine and Spector, 1977; Balentine, 1985, 1988; Brown and Eagles, 1986; Badalamente et al., 1986; Adams et al., 1991). In our hands, however, the observed changes in axolemmal permeability were not associated with dissolution of the cytoskeleton. Rather, they involved specific patterns of focal sidearm loss and compaction. This would suggest that although calcium may be involved in the pathobiology seen in these injured axons, it may be acting through previously unsuspected mechanisms. Perhaps, with transient membrane alterations in calcium flux, the neurofilament sidearms initially could be cleaved by calcium-activated proteases, with the then-compacted neurofilaments remaining resistant to further degradation. Whether these events are operant in the case of traumatic brain injury still remains to be determined, yet interesting parallels can be found in a recent study of lamprey axonal transection in which a remarkably similar sequence of neurofilament sidearm loss and compaction occurred in the first 12 h posttransection (Hall and Lee, 1995). Since these studies were conducted using Western blotting and antibodies targeted to the sidearm-specific, as well as rod-specific, neurofilament domains, these studies provided evidence that axonal damage can translate into sidearm loss, followed by rapid neurofilament compaction.

Recently we have extended our studies of severe traumatic brain injury using antibodies targeted to sidearms of 150-kDa neurofilament subunits and the related rod domains, which are not normally visualized in the presence of an intact sidearm. Through this approach, in two different animal models of traumatic brain injury, we observed that severe injury was associated with an immediate focal unmasking of the rod domain due to focal neurofilament sidearm loss (Povlishock et al., 1995). Collectively, all of the above descriptions of traumatologically induced delayed axotomy demonstrate the complexity of those reactive axonal changes ongoing following traumatic brain injury and suggest that injuries of varying severity may trigger different initiating forms of an axonal pathology.

Primary Axotomy

As alluded to in the previous passages, most experimental studies of traumatic brain injury suggest that in mild, moderate, and in many cases severe traumatic brain injury, the above-described sequence of reactive axonal change occurs independent of any overt/primary disruption of the overlying axolemma. As noted above, alterations in axolemmal permeability have been observed; yet these occur without any overt disruption of the axolemma, at least as detected by routine electron microscopic analysis (Pettus et al., 1994; Pettus and Povlishock, 1995). Despite the fact that most reactive axonal changes appear to occur without direct renting of the axolemma, evidence for primary axotomy has been described in the subset of axons subjected to the most severe form of traumatic brain injury. Specifically, Maxwell and colleagues (1993), utilizing the subhuman primate model of angular acceleration, have demonstrated through serial section electron microscopic analysis, the occurrence of discrete foci of axolemmal disruption or renting within minutes of the traumatic episode, suggesting that these axons themselves were mechanically rent at the moment of injury. Typically, at these sites of axolemmal renting, the underlying cytoskeleton underwent rapid dissolution consistent with a rapid influx of Ca$^{2+}$-activated neutral proteases capable of such dramatic cytoskeletal damage. Interestingly, the finding of direct axolemmal renting was observed only in thin caliber, finely myelinated axons suggesting that these axons may be most vulnerable to tearing injury. Taken together with previous work, this study indicates that although delayed or secondary axotomy appears to be the principal form of reactive axonal change, it is impossible to exclude the occurrence of primary axotomy, particularly in the cases of most severe traumatic injury.

CONSEQUENCES OF TRAUMATICALLY INDUCED AXONAL INJURY

Emerging evidence is accumulating that the acute and chronic consequences of the above-described axotomy are downstream synaptic loss followed by some degree of synaptic recovery. It is known that once the above-described sequence of axonal swelling and disconnection occurs, the distal axonal segment, now disconnected from the sustaining soma, undergoes Wallerian change, which results in the demise of the distal axonal segment and its terminal/synaptic field. In animals, traumatically induced axonal injury results in relatively rapid ultrastructural change in the terminal projections of the damaged axons, and these can be identified due to their increased electron density and/or neurofilamentous content within 24 h of traumatic episode (Erb and Povlishock, 1991). Typically, these damaged axons are shed from their target sites within 72 h and engulfed by reactive glia. Although we have argued that the traumatically induced axonal damage and its attendant synaptic loss and target deafferentation contribute to the morbidity associated
with traumatically induced axonal injury, such a linkage may represent an oversimplification of those processes ongoing in the injured brain. The linkage of traumatically induced axonal injury with the morbidity seen in the acute period posttrauma is by no means fully validated. In both the clinical and experimental literature, the occurrence of diffuse axonal injury has long been postulated to have a link with the ensuing morbidity, and, in fact, in some primate studies, Gennarelli and colleagues (1982) observed that with increasing severity of injury and increased morbidity, there were increased numbers of damaged axons throughout the neuraxis. Unfortunately, in all studies conducted, to date, there has been no attempt to actually explain precisely how such axonal damage could translate into morbidity reflected in unconsciousness and enduring behavioral dysfunction. In this context, it is well recognized that the traumatically induced axonal damage detected by morphological endpoints involves only a relatively modest proportion of the total axons coursing in the anatomical field under observation. Therefore, based upon this observation, it is difficult to explain how such limited axonal damage could translate into such widespread global neurological abnormalities. Perhaps the axonal damage detected by morphological means represents the most severe end of the spectrum of an axonal pathology, which may be ongoing in all of the fibers in the field, yet undetectable by routine morphological methods. Although there is no direct literature to support this conclusion, it is noteworthy that studies conducted in stretched optic nerve axons suggest that while pathological change can be identified in only a small proportion of the optic nerve fibers, there occurs a generalized change in electrophysiological properties of the nerve. This suggests the existence of a population of traumatically perturbed axons that exhibits no morphologically detectable abnormality but are nonetheless functionally impaired (Tomei et al., 1990).

In terms of the chronic or more enduring consequences of traumatically induced axonal injury, a general picture is beginning to emerge. In the case of mild to moderate injury, the more limited and dispersed axonal damage translates into a diffuse deafferentation of the target sites with retention of many related intact fiber systems (Steward, 1989, 1994; Erb and Povlishock, 1991). In the case of mild to moderate brain injury, this typically results, over a several month period, in the return of normal synaptic input, suggesting that an adaptive neuroplasticity has occurred. This adaptive neuroplastic response is consistent with the more adaptive recovery seen with mild to moderate traumatic brain injury. In the case of severe traumatic brain injury, virtually no information exists on potential synaptic rearrangements that occur postinjury. Perhaps, with the larger number of fibers damaged as in the case of severe traumatic brain injury, the potential for adaptive plasticity becomes more remote as the potential for the ingrowth of maladaptive or nonhomologous fiber systems becomes more prevalent (Steward, 1989). Further, in the case of severe traumatic brain injury, other traumatically induced events such as neuroexcitation and/or secondary insults could superimpose their damaging effects upon the brain. Credibility is given to this argument by some more recent studies utilizing neuroexcitation in combination with experimentally controlled deafferentation (Gordon et al., 1994; Phillips et al., 1994). Through this approach, it was recognized that the combination of neuroexcitation and deafferentation exacerbated the morbidity associated with traumatic brain injury, and, interestingly, triggered an unanticipated maladaptive synaptic rearrangement (Phillips et al., 1994).

**AXONAL INJURY IN TRAUMATICALLY BRAIN-INJURED HUMANS**

As noted in the outset of this review, traumatic brain injury in humans has long been associated with the occurrence of damaged axons. Typically, it is noted that these axons are found widely scattered throughout the neuraxis, leading to their characterization as diffuse (Strich, 1956; Adams et al., 1982; Gennarelli et al., 1982). As noted previously, few animal models replicate the full spectrum of diffuse axonal injury as seen in traumatically injured humans; however, it is noteworthy that data from these and other models collectively indicate that the pathogenesis of traumatically induced axonal injury appears comparable in both humans and animals. In other words, while not fully validated, it appears that delayed or secondary axotomy occurs in traumatically brain-injured humans and the pathogenesis of this delayed axotomy mimics that previously described in experimental animals. In brain-injured humans, focal intraaxonal alterations in the neurofilamentous/cytoskeletal network have been described, and these have been associated with focal impairment of axoplasmic transport, leading to axoplasmic swelling and detachment over a 12- to 24-h posttraumatic period (Grady et al., 1993; Christman et al., 1994). It has not been determined by the rigorous measurement procedures used in experimental animals (Pettus et al., 1994) whether sidearm loss and neurofilamentous compaction occur in humans. However, we are continuing to address this issue in an experimental setting using antibodies targeted to sidearms and related rod domains of the 150-kDa neurofilament subunit (vida supra).

Perhaps one of the most exciting findings in the study
of traumatically induced human axonal injury has been the observation, by several laboratories, that antibodies targeted to \( \beta \)-amyloid precursor protein, a neuronal glycoprotein conveyed by rapid anterograde transport (Shigematsu and McGeer, 1992), are useful in the early detection of traumatically induced axonal damage. The benefits associated with use of \( \beta \)-amyloid precursor protein antibodies include the fact that the antibodies apparently target only injured axons, and, therefore, are not compromised by immunoreactivity in uninjured neural fibers coursing in the field. It has been posited that since traumatically induced axonal damage involves alterations in axoplasmic transport, focal accumulations in the \( \beta \)-amyloid precursor protein denote the sites of injury and impaired transport (Gentleman et al., 1993; Blumbergs et al., 1994, 1995; Sherriff et al., 1994a,b,c). In the above investigators’ hands, traumatically induced axonal damage has been recognized in human postmortem analysis after brief (in some cases, hours) posttraumatic survival. The observed changes appear to reflect many of the changes previously described in animals. Typically, with amyloid precursor protein, subtle focal axonal swelling is recognized to progress over time to more dramatic swelling, leading to axonal separation, with the formation of a mature reactive axonal swelling.

It is interesting that in one study (Sherriff et al., 1994a) analysis of sections of injured human brain marked with antibodies to the 68-kDa neurofilament subunit seemed to target fewer injured axons and a greater number of normal ones than did antibodies to \( \beta \)-amyloid precursor protein used in adjacent sections. It should be noted, however, that previous studies using the 68-kDa antibody (Yaghmai and Povlishock, 1992; Grady et al., 1993; Christman et al., 1994) also revealed such nonspecific staining, which led to the use of exclusion factors (evidence of distention, separation, etc.) in the identification of injured axons. An additional consideration is the finding that 68 kDa immunoreactivity is not necessarily confined to the site of actual swelling but may extend to varying degrees along the axis cylinder (Povlishock, 1992; Yaghmai and Povlishock, 1992; Grady et al., 1993). Thus, it is conceivable that sites of neurofilamentous abnormality need not be precisely colocalized with the actual site of swelling. Careful interpretation is essential regarding data obtained with the use of \( \beta \)-amyloid precursor protein, as well, since blocked transport of this substance may similarly result in immunoreactivity in axons of normal diameter whose reactive axonal swellings are presumed to be out of the plane of section (Sherriff et al., 1994a).

Taking into account all of the above observations, the broader view may be that each of these antibodies makes its own contribution to an understanding of the events ongoing in diffuse axonal injury. Antibodies to the 68-kDa subunit target a relatively stationary cytoskeletal element, and antibodies to \( \beta \)-amyloid precursor protein target a more mobile constituent that undergoes rapid axonal transport. Furthermore, the selection of antibodies to components of normal and of injured axons is large and, it is hoped, still expanding. In the future, perhaps, the application of a variety of such antibodies, used with the limitations and advantages of each kept in mind, will further the general understanding of DAI.

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