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In Vitro Central Nervous System Models of Mechanically Induced Trauma: A Review

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ABSTRACT

Injury is one of the leading causes of death among all people below the age of 45 years. In the United States, traumatic brain injury (TBI) and spinal cord injury (SCI) together are responsible for an estimated 90,000 disabled persons annually. To improve treatment of the patient and thereby decrease the associated mortality, morbidity, and cost, several *in vivo* models of central nervous system (CNS) injury have been developed and characterized over the past two decades. To complement the ability of these *in vivo* models to reproduce the sequelae of human CNS injury, *in vitro* models of neuronal injury have also been developed. Despite the inherent simplifications of these *in vitro* systems, many aspects of the posttraumatic sequelae are faithfully reproduced in cultured cells, including ultrastructural changes, ionic derangements, alterations in electrophysiology, and free radical generation. This review presents a number of these *in vitro* systems, detailing the mechanical stimuli, the types of tissue injured, and the *in vivo* injury conditions most closely reproduced by the models. The data generated with these systems is then compared and contrasted with data from *in vivo* models of CNS injury. We believe that *in vitro* models of mechanical injury will continue to be a valuable tool to study the cellular consequences and evaluate the potential therapeutic strategies for the treatment of traumatic injury of the CNS.

Key words: cell culture; glia; injury mechanisms; intervention; mechanical stimulus; neurons

INTRODUCTION

INJURY IS ONE OF THE LEADING CAUSES OF death among all people under the age of 45 years in the United States, with an incidence of death in the entire population of 17–57 per 100,000 (Kraus and McArthur, 1996; Rice and MacKenzie, 1989; Sosin et al., 1989). The occurrence of traumatic brain injury (TBI) requiring hospitalization is 200–500 per 100,000 (Coburn, 1992; Kraus et al., 1994; Walleck, 1992). If all types of head injuries are included, the incidence jumps to 790–5,000 per 100,000 or 2–12.5 million head injuries per year (Coburn, 1992; Kraus et al., 1994; Kraus and Sorenson, 1994; Pope and Tarlov, 1991). The estimated cost of hospitalization for these injuries is $1–$25 billion per year, with an estimated cost in lost wages of $3 billion per year based only on the duration of hospital stay (Coburn, 1992; Kraus et al., 1994). As estimated by Kraus and Sorenson (1994), these injuries result in ~82,800 new disabled persons per year. The occurrence of spinal cord injury (SCI), although not as prevalent as TBI, has been estimated to be ~3 per 100,000, resulting in 10,000 new cases per year (Runge, 1993). Given the high incidence of injury in the younger population, its morbidity and cost to society are staggering. The lifetime cost of all injuries has been estimated at $200 billion, of which TBI and SCI ac-

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count for a significant proportion because of their profound, lifelong impact on the patient (Runge, 1993). The high incidence, mortality, and cost demand a more complete understanding of central nervous system (CNS) trauma so that the patient may be treated more effectively and efficiently.

In response to this need, a growing number of animal models have been introduced, characterized, and utilized over the past two decades to study the mechanisms of mechanical CNS trauma. These models are important because they permit the measurement of two broad categories of parameters that are directly comparable to the clinical situation: (a) the physiologic/pathophysiologic response of the whole animal and (b) the neurobehavioral response to trauma. *In vivo* models, however, are not without their disadvantages. For example, the presence of the circulatory system can confound mechanistic interpretation of the brain's response to the mechanical trauma. Changes in brain temperature, if not carefully monitored and controlled, can lead to conflicting results when conducting preclinical pharmacological studies (Buchan and Pulsinelli, 1990). The complexity of the *in vivo* situation may result in a limited accessibility to the tissue of interest, preventing real-time and spatial measurement of biological or mechanical parameters. The cost of these animal models as well as support facilities may be prohibitively expensive. Conversely, *in vitro* models of CNS injury allow for the precise control of the extracellular environment, easy and perhaps repeated access to the cells, and lower associated costs.

Tissue damage associated with CNS injury is hypothesized to be a consequence of an extended neurochemical or cellular cascade set in motion by an initial, primary mechanical event (Hovda et al., 1992; McIntosh et al., 1996; Siesjo, 1993). This event, a rapid deformation of CNS tissue, is the primary cause of traumatic injury and is ultimately responsible for any resultant damage. If the sequelae of traumatic injury are to be faithfully reproduced *in vitro,* the injury cascade should be initiated by a mechanical stimulus similar to that which occurs in the human body. To aid in understanding the pathology of CNS injury, the primary initiator, i.e., the mechanical stress and strain experienced by the tissue, must also be understood.

Finite element method (FEM) models are computational models that can predict the state of stress and strain in an object subjected to a prescribed force. As these models have become more complex, they have been able to predict reasonably well the state of stress and strain in an object as complicated as the brain, when subjected to a realistic, traumatic load (King et al., 1995; Ruan et al., 1994; Ueno and Melvin, 1995). For a given macroscopic loading condition, FEM models can predict the cellular loading within the brain. However, interpretation of the consequences of this deformation remain difficult because of a lack of knowledge concerning the functional response of living brain tissue to different levels of strain at different strain rates. Through the use of *in vitro* models of CNS trauma, a microscopic load consistent with that assocaited with human CNS trauma can be applied to a cell so that the cellular response to injury can be studied. However, the biological responses to and mechanisms of injury are likely to be dependent upon the exact nature of the pathologic stimuli. Therefore, to provide a more complete understanding of the mechanisms of CNS injury, *in vitro* systems should not only provide for the measurement of various biochemical or physiological parameters, but also allow mechanical parameters such as strain and strain rate to be assessed accurately. Data from the *in vitro* systems could then be integrated into FEM models to create a computational model that could predict functional damage as well as mechanical damage, so that, for example, the response of the whole brain could be predicted on a cellular level, to a given macroscopic load.

This review will focus on *in vitro* models of mammalian CNS trauma (both brain and spinal cord) that induce injury with a primary mechanical stimulus. Many of these devices are flexible enough so that a combination of secondary injury cascades, such as hypoxia or excitotoxicity, may be superimposed over the primary mechanical injury. These models differ, primarily, in how the mechanical stimulus is applied to the cultured tissue and, secondarily, by what type of tissue is cultured and what cellular parameters are measured. Other models have been developed to study the response of peripheral nervous system tissue to trauma (e.g., Edbladh et al., 1994; Ekstrom, 1995a) as well as the response of non-mammalian nervous sytems to traumatic injury (e.g., Ekstrom, 1995b; Krause et al., 1994; Yawo and Kuno, 1985). Pioneering work by several investigators performed with cultures of chick neurons is not addressed in this review; however, the interested reader is directed to the following references (Bird, 1978; Levi and Meyer, 1945; Shaw and Bray, 1977; Sole, 1980). This literature is scientifically valuable since many of the techniques and data associated with these fields are applicable to the study of mechanical trauma of the mammalian CNS; however, a review of that literature is beyond the scope of this article.

DESCRIPTION OF INJURY MODELS

The design of *in vitro* models of TBI has been constrained by the contemporary understanding of the injury process. Consequently, one of the earliest *in vitro* mod-

els of CNS injury, in which a 1-mm^3 piece of rat cortex was placed in a nutrient medium and subjected to trauma induced by a rotating stir-bar, did not address the mechanics of the induced trauma (Epstein, 1971). The severity of the insult was controlled by the duration of tissue injury, lasting between 3 min and 48 h. By culturing the explants after injury, neurons were found to be the most susceptible element of the parenchyma followed by astrocytes and then fibroblasts. Although the loading conditions used in this and other early models of *in vitro* tissue injury were not intended to reproduce directly any clinically relevant traumatic insult, increased knowledge of injury mechanisms in recent years has led to the development of models exploring the effects of transection, compression, acceleration, stretch, and shear, all of which are believed to be relevant in SCI and TBI in humans. A schematic representation of the models described herein is presented in Fig. 1 and summarized in Table 1.

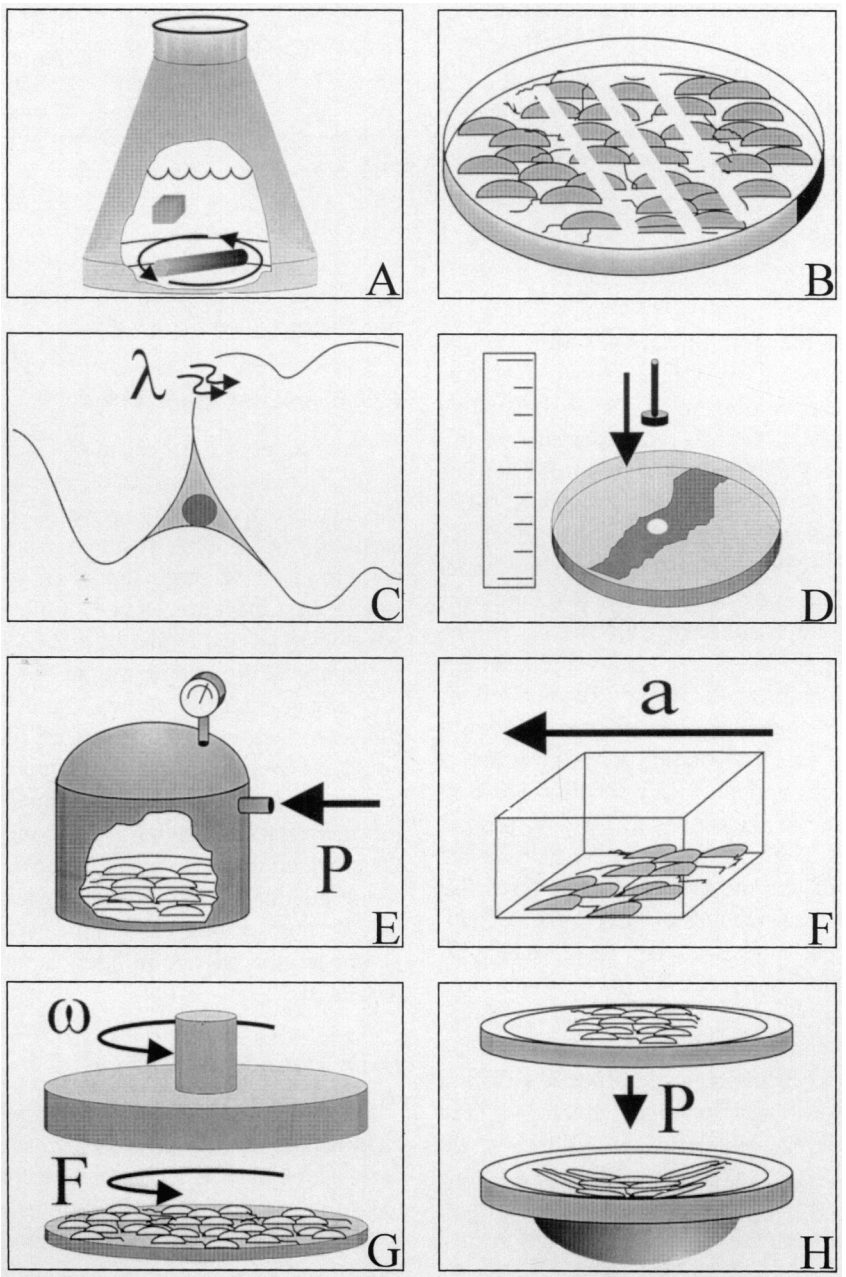

FIG. 1. *In vitro* mechanical injury models (for full descriptions, see text). (A) Rotating stir-bar. (B) Stylet transection. (C) Laser transection. (D) Weight drop. (E) Hydrostatic pressure. (F) Acceleration. (G) Hydrodynamic shear. (H) Substrate strain. λ, laser light; **P,** pressure; **a,** acceleration, ω, rotational velocity; **F,** force.

TABLE 1. SUMMARY OF *IN VITRO* INJURY MODELS AND CELL CULTURE SYSTEMS

Injury model	*Culture system*	*Schematic*[a]	*Reference*
Rotating stir-bar	1-mm^3 rat cortex	A	Epstein, 1971
Transection (stylet)	Neurons and glia	B	Tecoma et al., 1989
Transection (rotating scribe)	Neurons and glia	B	Muhkin et al., 1997
Transection (laser)	Neurons	C	Lucas et al., 1985
Weight drop	Organotypic spinal cord	D	Balentine et al., 1988
Barotrauma (quasistatic)	Cell lines	E	Murphy and Horrocks, 1993
Barotrauma (fluid percussion)	Glia	E	Shepard et al., 1991
Barotrauma (weight drop)	Organotypic brain (acute)	E	Wallis and Panizzon, 1995
Acceleration	Neurons and glia	F	Lucas and Wolf, 1991
Hydrodynamic shear	Differentiated cell line	G	LaPlaca and Thibault, 1997
Substrate strain	Neurons and glia	H	Ellis et al., 1995
Substrate strain	Differentiated cell line	H	Cargill and Thibault, 1996
Substrate strain	Organotypic brain	H	Morrison et al., 1998

[a]The schematic representation of the injury model, as shown in Fig. 1.

Transection Models

Historically, since the work of Ramon y Cajal (1928) as well as others, axotomy has been suggested to play an important role in the development of CNS-associated injury pathology, although the relative importance of primary versus secondary axotomy continues to be debated (Povlishock, 1993; Povlishock and Christman, 1995; Povlishock et al., 1997; Rand and Courville, 1934). To study primary axotomy *in vitro* further, two types of models have been designed and evaluated. The first employed a plastic stylet to scrape adherent cells from a culture dish, thereby tearing processes and soma while leaving a significant proportion of cells intact (Tecoma et al., 1989). Recently, this model has been adapted to increase throughput with the use of a rotating scribe (Muhkin et al., 1997). In this later variant, injury severity was controlled by adding one to six scribes to vary the proportion of cells injured. These systems offer certain advantages such as ease of use and low cost, advantages that may be well suited for screening of novel pharmaceutical compounds. These models attempt to reproduce aspects of the secondary injury cascade such as neurotoxicity and simulate injuries such as stab wounds, penetrating skull frcatures, gunshots, or other injuries involving transection of cell bodies and processes.

The second type of transection model employs a laser to perform microsurgery on single cells (Gross et al., 1983; Lucas et al., 1985). With this system, a single process from a single cell could be transected at a predetermined distance from the cell body. This last system offers exquisite control over injury parameters such as distance of lesion from cell body and has been put forth as a model of spinal cord transection injury. A potential drawback of these transection models is that mechanical data (force, strain, strain rate) concerning the process of transection cannot be obtained.

Compression Trauma Model

One proposed mechanism of CNS injury, particularly in the spinal cord, is that of compression. To simulate this compressive trauma *in vitro* (and to eliminate potentially confounding factors such as ischemia due to compromised autoregulation), an *in vitro* compression model has been developed. Ballentine et al. (1988) adapted an *in vivo* model of SCI (Allen, 1914), simulating injury by dropping a weight from a prescribed height onto organotypically cultured spinal tissue. By varying the prescribed height and weight, the severity of injury could be changed. Despite the technical challenges inherent in working with organotypic explant cultures, this model produced many of the pathophysiological changes documented after *in vivo* SCI. However, the system offered little insight into the biomechanics of the tissue injury because neither the strain field produced at the site of impact nor the rate of application of this strain were measured.

Hydrostatic Pressure Models

Several models have employed specially constructed chambers to subject cells to increased hydrostatic pressure, which may be a potential mechanism of cell injury (Murphy and Horrocks, 1993; Shepard et al., 1991; Wallis and Panizzon, 1995). In one model, cells were subjected to pressures as high as 15 atm for as long as 10 min (Murphy and Horrocks, 1993). The long duration and very high hydrostatic pressures necessary to generate injury with this device are not readily translatable to typi-

cal, clinical SCI scenarios. Other pressure models have employed modified fluid percussion devices to subject cells to a transient pressure pulse. Sheppard et al. (1991) applied an ~20-ms pressure pulse as high as 6 atm to cultured cells, while Wallis and Panizzon (1995) dropped a 1-kg weight from a height of 61 cm onto a piston that sealed a fluid-filled chamber. Although the pressure transient in the weight-drop variant was not measured, the magnitude and duration of the forces generated in these latter two models appear to mimic the conditions of a clinical injury more closely than those of the prolonged hydrostatic pressure model, although hydrostatic pressure rarely exceeds 2 atm in physical or FEM models of TBI (Chu et al., 1994; Ruan et al., 1993; Zhou et al., 1995). However, CNS tissue is not generally deformed significantly under hydrostatic pressure because of its incompressible material property (Sahay et al., 1992), but compression *in vivo* may give rise to pressure gradients, which in turn generate strain fields within the tissue, thereby causing primary injury. Therefore, the most likely mechanical injury mechanism associated with *in vivo* compression trauma may not be reproduced by hydrostatic pressure.

Acceleration Model

One important mechanism underlying neuronal death and damage following human head injury appears to be the acceleration/deceleration of the head, which in turn leads to tissue strain throughout the brain (inertial loading). Features of clinical, closed-head injuries such as diffuse axonal injury (DAI), contusion, or subdural hematoma may result from shear strain of brain matter due to inertial loading (Adams et al., 1981; Chu et al, 1994; Gennarelli et al., 1982; Graham et al, 1993; Povlishock, 1993; Povlishock et al., 1997; Voigt et al, 1977). In an effort to mimic the mechanics of such an injury, Lucas and Wolf (1991) designed an *in vitro* system that applied an acceleration as high as 200*g* to a flask of cultured cells via an impacting pendulum. The system produced cellular damage after a minimum of three successive accelerations provided that the acceleration was tangential rather than normal to the layer of cells. This system has the advantages of increased speed and ease of use, although it is constrained to modeling injuries of multiple impacts (Lucas and Wolf, 1991). One potential drawback of this system is that cellular deformation in response to acceleration cannot be measured.

The manner in which *in vitro* tissue deformation is produced need not mimic the *in vivo* scenario as long as the magnitude and rate of the applied strain are closely reproduced. Strain guidelines, which could be used as design criteria for *in vitro* models, were generated for TBI with the aid of physical model simulations, which duplicated animal studies that produced clinically relevant, postinjury outcomes (Margulies et al., 1990; Meaney and Thibault, 1990). Physical models were developed using the skulls of nonhuman primates that were filled with a material mimicking the mechanical properties of brain and were then subjected to the same inertial loads known to produce coma and DAI in animal experiments. The pattern of strain within the models was captured on high-speed film, and it was determined that strains of 0.10–0.50 applied at strain rates of 10–50 s^{-1} produced clinically relevant injuries (Margulies et al., 1990; Meaney and Thibault, 1990). This information led to the design of *in vitro* injury models that stretch CNS tissue without the use of an acceleration/deceleration pulse.

Hydrodynamic Model

Based on earlier studies described above, an *in vitro* model was recently developed to study injuries caused by inertial loading of the head that utilized hydrodynamic forces to shear or stretch cultured cells (LaPlaca and Thibault, 1997). This system consists of a parallel plate viscometer with cells grown on one of its plates. The hydrodynamic force applied to the cells is controlled by the speed of the rotating plate and the gap distance between the two plates. Two novel strengths of this system are that the cells could be visualized under a microscope while the injury occurred, and, through the use of adherent microbeads, individual cell strain could be calculated. The system is capable of generating cell strain up to 0.53 at strain rates from <1 s^{-1} to as high as 10 s^{-1}. A potential limitaion of this device, however, is that the temporal resolution of the strain measurements is limited to the video frame rate of 30 Hz or one calculation every 33 ms. Moreover, like the hydrostatic pressure models, the mechanistic correlate to the *in vivo* condition during trauma is not clear.

Cell Stretch Models

If it is assumed that tissue or cells are well adhered to the substrate on which they are cultured, then they can be deformed by stretching that substrate. Cell or tissue strain can then be indirectly measured by measuring substrate strain. A number of injury models have been recently developed that exploit this principle (Cargill and Thibault, 1996; Ellis et al.,1995; Morrison et al., 1998). Ellis et al. (1995) utilized the commercially available Flex Plate® which consists of a six-well cell culture plate, with the bottom of the plastic wells replaced with a 2-mm-thick silicone plate. With a predetermined pressure pulse, the silicone plate in individual wells is deformed, thereby deforming the adherent cells. Substrate strain of up to

0.72 could be generated, although strain rate was not reported (Ellis et al., 1995). An advantage of this systm is that it utilizes a commercially available, standard format culture plate offering the potential of widespread acceptance and use.

A second substrate strain model, in which cells were cultured on a thin silicone membrane incorporated into a custom-built well, was described by Cargill and Thibault (1996). The membrane is deformed with a vacuum pulse generating strains as large as 0.45 at strain rates between 10 s^{-1} and <1 s^{-1}. After each experiment, the centerline deflection of the membrane is calibrated to vacuum pressure, which is measured during the experiment. Pressure data is then used to calculate strain at the center of the membrane. The major advantages of this system are that strain rate can be calculated and that cells can be stretched at both low and high strain rates.

The latest variant of the substrate strain models, recently reported by Morrison et al. (1998), can achieve strains up to 0.65 at strain rates as high as 15 s^{-1} and as low as 0.04 s^{-1}. Two major improvements integrated into the device include a laser displacement transducer to measure directly the dynamic displacement of the center of the membrane as it deforms, and needle valves and computer-controlled solenoid valves that control the duration and shape of the injury pulse. Because brain tissue is viscoelastic, this level of control may be useful in comparing cellular responses to physiologic and injurious deformations and may be important in elucidating mechanisms of TBI.

CELL CULTURE SYSTEMS

The various cell culture systems that have been utilized with the *in vitro* injury models described above fit into three main categories: (a) immortal cell lines, (b) primary cultures, and (c) explant or organotypic cultures. Each culture system has specific advantages and disadvantages, so the particular culture system employed may need to be chosen carefully depending on the ultimate goal of the proposed experiments. Although not all cell culture systems have been used with each injury system, each device appears flexible enough to be adapted for use with multiple culture systems.

Immortalized Cell Lines

The use of immortalized cell lines offer certain advantages over other types of cultured cells. They are available from commercial sources, can be frozen, and can be passaged many times to provide a continuous supply of cells. Cell lines are also usually more robust and easier to maintain in culture than are primary cultures. Certain cell lines can also be differentiated from their normal growth state to a more quiescent state in which they take on characteristics of neurons, although it remains unclear as to whether these cells respond to mechanical stimuli as normal neurons would.

Several researchers have utilized neuroblastoma (Kirkpatrick et al., 1985; Murphy and Horrocks, 1993) or neuroblastoma cross glioma (Cargill and Thibault, 1996) cell lines for their experiments. The latter experiments were performed on cells (NG108-15) that had been terminally differentiated by serum withdrawal and cAMP administration (Cargill and Thibault, 1996). Other lines such as human teratocarcinoma (NT2) cells have also been used (LaPlaca and Thibault, 1997). This cell line can be terminally differentiated with retinoic acid treatment to generate a >95% pure population of postmitotic cells that closely resemble neurons in many respects (Pleasure and Lee, 1993).

The use of cell lines in *in vitro* injury models present one significant disadvantage. Although some are derived from neuronal cells, these lines consist of immortalized or cancerous cells with the ability to divide uncontrollably, suggesting that their pattern of gene and/or protein expression may be significantly different from the terminally differentiated, functioning neuron. The atypical traits of cell lines must be considered when interpreting the response of such cells to mechanical injury.

Primary Cultures

One alternative to using cell lines is to isolate primary cells of chioce. Several techniques have been developed to culture both astrocytes and neurons, although maintaining oligodendrocytes *in vitro* remains challenging. Primary cultures afford the opportunity to study either a single cell population or a heterogeneous cell population, allowing interactions between cell types to be explored. These cultures require no treatments for differentiation and retain biochemical and molecular fidelity to similar cell types *in vivo*.

Astrocytes can readily be cultured as homogenous populations for the study of *in vitro* mechanical injury (Ellis et al., 1995; Shepard et al., 1991). Although neurons may be more susceptible to mechanical damage than are astrocytes, neurons, to date, have not been injured in isolation. Due to technical limitations, neuronal injury studies are often performed on neurons cultured on a monolayer of nonneuronal cells (Kirkpatrick et al., 1985; Lucas and Wolf, 1991; Tecoma et al., 1989). The formulation of neuron-specific culture media may permit future use of isolated neurons in *in vitro* models of CNS injury (Brewer et al., 1993).

The use of primary cell culture can be complicated by several factors. Harvest of target tissue may be complex

since it is often necessary to culture embryonic cells for best results. Additionally, interpretation of experimental results could be limited to the immature or developing CNS. During the harvest procedure, cells are thoroughly dissociated through mechanical and perhaps enzymatic means, potentially leading to severe cellular injury as well as a loss of the intricate connectivity and cytoarchitecture found *in vivo*. While injury to a homogenous population of cells may facilitate mechanistic studies about the role of a single cell type, the simplicity of the system may provide incomplete or artificial answers. Moreover, the role of a cell population could change dramatically when cocultured with another cell type (Charles, 1994).

Organotypic Cultures

Organotypic cultures are slices or chunks of tissue cultured whole without dissociation of the individual cells. The heterogeneous populations of cells found *in vivo* are maintained *in vitro* with these preparations, as are the complex, three-dimensional connections between these cells.

Organotypic cultures of both spinal cord and brain have been injured with a variety of devices (Balentine et al., 1988; Epstein, 1971; Morrison et al., 1997). Ballentine et al. (1988) injured cultures consisting of pieces of mouse spinal cord cut into longitudinal segments 2.0 × 0.5 mm that were allowed to mature in culture for at least 21 days. These cultures contained astrocytes and mature neurons with myelinated axons as demonstrated by Nissl staining and electron microscopy (Balentine et al., 1988). One of the early models of brain trauma cultured small chunks of the cortex in spinner bottles (Epstein, 1971), whereas other recent studies have cultured coronal slices of 4-day-old rat brain maintained for 18 days *in vitro* before injury (Morrison et al., 1997).

In electrophysiology studies, acute organotypic slice preparations have been maintained for a maximum of ~8 h *in vitro* after injury in a barotrauma device (Wallis and Panizzon, 1995). Although the duration of experiments must be kept short (≤8 h) when using acute organotypic slices, these slices may be generated from immature or adult animals. In contrast, long-term organotypic slice cultures have been generated only from newborn animals, and the slices mature *in vitro*. However, the long-term culture technique allows the slice to recover from the trauma of slice preparation, which could confound interpretation of the effects of an applied injury.

Organotypic cultures can be technically challenging and difficult to prepare. Cultures must be very thin (350–475 μm) slices or very small (1 mm^3) tissue sections to avoid hypoxia and necrosis of the central tissue; consequently, they are difficult to manipulate. The visualization of cellular changes in real time, as well as measurement of cellular strain is impeded by the thickness of the slice, although tissue strain can be measured. However, these cultures maintain the heterogeneous cell populations found *in vivo*, allowing for the interaction between cell types to be studied. The spatial orientation of and complex electrophysiological pathways between these cell types are maintained in three dimensions, with greater fidelity to the *in vivo* state than is possible with other culture systems. Furthermore, within organotypic cultures, the distribution of extracellular signaling molecules may be replicated more faithfully since their local concentration may be elevated in the intercellular spaces within the tissue, whereas in dissociated cultures these molecules may diffuse away freely, once released. Although they cannot exactly duplicate the *in vivo* situation, organotypic cultures may offer a useful compromise between dissociated cultures and *in vivo* models.

BIOLOGICAL RESPONSE TO *IN VITRO* INJURY

A wealth of useful information has been obtained through the experimental use of the above described *in vitro* models of traumatic injury in conjunction with different cell culture systems. Because it is possible to eliminate confounding factors found *in vivo, in vitro* models may help to elucidate mechanisms underlying a "pure" mechanically induced trauma. No single model of *in vitro* mechanical trauma will reproduce the *in vivo* situation with exact fidelity; therefore, it is imperative that multiple models are utilized to fully elucidate mechanisms and evaluate therapeutic interventions of mechanical trauma of the CNS. The wealth of data generated from all *in vitro* models must be interpreted, compared, and contrasted as a whole.

Cell Membrane Damage After Mechanical Stimuli

Cell death or damage following *in vitro* CNS injury has been assessed in a number of ways, including cell counting with a vital dye such as trypan blue or erythrosine B, which stains only those cells with compromised plasma membranes. This method is fast, requires no specialized equipment except for a microscope, and is relatively accurate and reproducible. However, interpretation of staining with these dyes can be complicated by the use of serum or a high concentration of protein in the medium, as noncellular debris may stain as well (Murphy and Horrocks, 1994). Vital dyes in another class such as propidium iodide and ethidium homodimer increase their fluorescence once bound to DNA. Staining of a cell with these dyes indicates that the plasma membrane has

been compromised and that the dye has bound to DNA. Interpretation of results with these DNA binding dyes is more straightforward than with trypan blue or erythrosine B since there is almost no background straining.

Another method to assess membrane damage is to measure the medium concentration of a normally intracellular protein. The activity of lactate dehydrogenase (LDH), a large intracellular protein that is not transported out of the cell, is a widely used measure of plasma membrane damage. The LDH assay itself uses a small volume of culture medium so that multiple samples at different time points can be assayed from the same culture. However, because different cell types contain different amounts of LDH, these levels may not correlate directly with the number of damaged cells in mixed cultures (Murphy and Horrocks, 1994).

Utilizing either LDH assays or vital dyes, several studies have shown that the plasma membranes of various cell types may be immediately damaged by a primary mechanical event (Ellis et al., 1995; LaPlaca et al., 1997; LaPlaca and Thibault, 1997; Lucas and Wolf, 1991; McKinney et al., 1996; Muhkin et al., 1997; Murphy and Horrocks, 1993; Regan and Choi, 1994; Shepard et al., 1991; Tecoma et al., 1989). As the injury level increased, as indicated by an increased stretch or increased pressure, the plasma membranes of more cells were damaged. This observation is rather critical, particularly in light of the varied injury models, in demonstrating that an increased membrane permeability is a direct consequence of the primary mechanical input to the system. It also demonstrates that these models can generate injuries of graded severity that correlate with specific parameters of the mechanical input.

Histological and Ultrastructural Response to In Vitro *Injury*

Although various models have produced cell injury via different means, the morphological, histological, and ultrastructural manifestations of *in vitro* injury reveal many similarities. After transection injury, neurite retraction is observed and has been hypothesized to help seal the damaged process, thereby preventing an influx of ions and promoting cell survival (Lucas, 1987; Povlishock, 1993; Povlishock and Christman, 1995). A gradient of damage from the severed process to the nucleus is observed, revealing the progression of damage over time (Emery et al., 1987; Gross and Higgins, 1987; Lucas et al., 1990a). The Golgi and smooth endoplasmic reticulum swell first, followed by swelling of the mitochondria and, later, dissolution of the microtubules (Emery et al., 1987; Gross and Higgins, 1987). Near the site of transection and, eventually, at points more remote along the severed process, the density of neurofilaments and microtubules is decreased (Gross and Higgins, 1987; Gross et al., 1983; Lucas et al., 1985). Schlaepfer and Bunge in 1973 determined that, after transection injury, the loss of microtubules and neurofilaments was dependent on $[Ca^{2+}]_e$. While organelles progressively swell over time and become electron-lucent and indistinguishable, indicating osmotic swelling and ionic derangements, the plasma membrane appears to remain continuous (Gross and Higgins, 1987; Lucas et al., 1985; Mire et al., 1970).

In other models of *in vitro* mechanical injury which employ either stretch, weight-drop, or acceleration techniques, cells demonstrated posttraumatic pathologies such as swollen and granular cytoplasm and prominent nuclei (Balentine et al., 1988; Ellis et al., 1995; Lucas and Wolf, 1991). As injury levels increased, these structural changes became more prominent as mitochondria swelled, Golgi and smooth endoplasmic reticulum dilated, and vacuoles formed (Ellis et al., 1995; Emery et al., 1987; Gross and Higgins, 1987).

Similar morphological, histological, and ultrastructural changes have been reported following *in vivo* experimental TBI and SCI as well as clinical injuries (Christman et al., 1994; Foda and Marmarou, 1994; Povlishock and Christman, 1995; Tator, 1995). For instance, after experimental TBI in the rat, neurons undergo extensive vacuolization as mitochondria swell, and, at early stages (2 h) of degeneration, neurofilaments initially collapse, to be degraded at later time points (24 h) (Povlishock et al., 1997). Similar changes were found in human postmortem tissue, although the time course was somewhat more protracted (Christman et al, 1994). Comparable changes have been described in animal models of SCI as well as in humans (Tator, 1995). Therefore, many *in vitro* models of mechanical cellular injury have been able to reproduce morphological features found in both *in vivo* models of CNS injury and the clinical situation following human head and spinal cord injuries.

Disruption of Ionic Homeostasis After In Vitro *Injury*

Neuronal loss after traumatic CNS injury is often hypothesized to be due to a disruption of ionic homeostasis as a consequence of a massive release of K^+ from neurons (Chen et al., 1997; Choi, 1994; Olney, 1994; Rothman and Olney, 1995). As the $[K^+]_e$ rises, neurons indiscriminately depolarize, releasing quantities of glutamate, which activate ionophore-linked glutamate receptors resulting in an increase of $[Ca^{2+}]_i$, $[Na^+]_i$, and $[K^+]_e$. Influx of Na^+ has been hypothesized to cause an initial swelling and acidification of various cellular organelles (Choi, 1994; Lucas et al., 1997; Mire et al.,

1970). In pioneering work performed by Mire et al. in 1970, it was hypothesized that swelling after transection injury was caused by a failure of active ion-pumping mechanisms. Influx of Na^+ may also promote release of Ca^{2+} from mitochondria and Ca^{2+} influx via reversal of the Na^+/Ca^{2+} transporter (Young, 1992). Elevated $[Ca^{2+}]_i$ may activate Ca^{2+}-dependent enzymes such as phospholipase A_2 (PLA_2) or proteases such as calpain, contributing to Ca^{2+}-dependent neuronal degeneration (LoPachin and Lehning, 1997). It is hypothesized that, in an attempt to restore ionic homeostasis after these derangements, a cell becomes depleted of energy reserves and dies (Lucas et al., 1997).

Alterations of ion concentrations have been observed *in vivo* in various CNS trauma models. Total tissue Na^+ accumulation has been documented as early as 10 min following fluid-permission injury (FPI) and 5 min following spinal cord weight-drop injury in the rat as measured by atomic absorption spectroscopy and Na^+-selective electrodes (Lemke et al., 1987; Leybaert and De Ley, 1994; Soares et al., 1992). Similarly, in weight-drop models of head injury and spinal cord injury in the rat, total tissue Ca^{2+} was increased after injury as measured by atomic absorption spectroscopy and Ca^{2+}-sensitive electrodes (Leybaert and De Ley, 1994: Moriya et al., 1994; Shapira et al., 1989). *In vivo* $^{45}Ca^{2+}$ studies have also demonstrated increased Ca^{2+} flux for up to 4 days in rats after FPI (Fineman et al., 1993; Nilsson et al., 1996).

Although intracellular Na^+ has not been measured *in vitro*, both Ca^{2+} flux and intracellular free Ca^{2+} have been studied in response to *in vitro* mechanical CNS trauma. Pyroantimonate was used to measure Ca^{2+} accumulation in injured spinal cord explants, demonstrating that Ca^{2+} did accumulate, but did not precipitate (Balentine et al., 1988). The fluorescent indicator Fura-2, has been used for real-time, continuous monitoring of $[Ca^{2+}]_i$ and has shown that $[Ca^{2+}]_i$ increased after injury in a similar organotypic spinal cord injury model (Leybaert and de Hemptinne, 1996). In other experiments, intracellular $^{45}Ca^{2+}$ was shown to accumulate in injured astrocytes two- to threefold above uninjured controls for at least 90 min postinjury as measured by scintillation counting (Rzigalinski et al., 1997). Increased Ca^{2+} accumulation appears to be a robust consequence of mechanical injury, since many of the *in vitro* findings have duplicated results obtained with animal experiments.

In two different *in vitro* injury models utilizing different neuronal-like cell lines, $[Ca^{2+}]_i$ increased in response to high strain rate ($>1\ s^{-1}$) loading, but remained unaltered following deformations of similar magnitude applied at lower strain rates ($<1\ s^{-1}$) as measured by Fura-2 fluorescence (Cargill and Thibault, 1996; LaPlaca et al., 1997; LaPlaca and Thibault, 1997). These experiments exemplify one of the strengths of *in vitro* models to control precisely and measure the cellular loading conditions to differentiate a subthreshold response from a traumatic response. These data suggest that the biological response of neurons to mechanical stimuli may be strain rate dependent, just as the physical response of the brain has been shown to be (Engin and Wang, 1970; Shuck and Advani, 1972; Wang and Wineman, 1972).

In vitro models of mechanical CNS trauma provide a level of control with which to test precisely various hypotheses of the posttraumatic sequelae. It has been hypothesized that both extracellular Ca^{2+} and Na^+ play a role in cell damage after injury. By precisely controlling the levels of these ions in *in vitro* models, it has been concluded that both extracellular Ca^{2+} and Na^+ contribute significantly to the development of damage after injury (Emery et al., 1991; Lucas et al., 1990a; Schlaepfer and Bunge, 1973). By reducing the $[NaCl]_e$ to 50% of controls, cell death caused by a laser transection injury was reduced by ~50% (Lucas et al., 1997; Rosenberg and Lucas, 1996). Similarly, by decreasing the $[Ca^{2+}]_e$, ultrastructural damage was decreased after transection, although not eliminated completely (Lucas et al., 1990a; Schlaepfer and Bunge, 1973). However, low $[Ca^{2+}]_e$ combined with low $[Na^+]_e$ decreased the damage even further (Emery et al., 1991).

Another strength of *in vitro* models is the ability to reveal cell type–specific responses to similar experimental conditions. In contrast with data indicating the neuroprotective effect of low $[Ca^{2+}]_e$ after injury, a decrease in $[Ca^{2+}]_e$ has been found to be associated with increased plasma membrane damage in astrocytes subjected to stretch injury as measured by propidium iodide uptake (Rzigalinski et al., 1997). Similarly, pharmacologically increasing $[Ca^{2+}]_i$ concentration with thapsigargin or the ionophore A23187 protected astrocytes in the same study. It is a reasonable assumption that different cell types will respond to similar mechanical stimuli differently, and *in vitro* models of mechanical injury will be useful in determining those differences.

Derangement of Electrophysiological Function After In Vitro *Injury*

Immediately following TBI, a massive increase of $[K^+]_e$ and subsequent spreading depression have been documented (Katayama et al., 1990; Takahashi et al., 1981). It is hypothesized that this efflux of K^+ leads to a subsequent pathological release of glutamate, initiating a cascade that results in excitotoxicity (Katayama et al., 1990). *In vitro* models of CNS trauma have been used to study these phenomena in greater detail. Using the laser transection model of injury, neuronal cell bodies were

found to be depolarized immediately by the transection of a process (Gross et al., 1983; Lucas et al., 1985). The degree of depolarization was dependent upon the distance of the transection from the soma, with transections closer to the soma producing a larger depolariation and transections $\geq$300 μm from the soma producing no depolarization. The precise time course varied from one cell to another, but, interestingly, even after 24 h, injured neurons were depolarized by ~8% relative to uninjured neurons (Lucas et al., 1985).

Similarly, cortical neurons depolarize after stretch-induced injury (Tavalin et al., 1995). Depolarization of ~10 mV required incubation at 37°C for approximately 1 h postinjury to develop and was not due to an increase of leak currents. Depolarization in these studies also required neuronal firing, Ca^{2+} entry, and *N*-methyl-D-aspartate (NMDA) receptor activation (Tavalin et al., 1995). In a series of follow-up experiments, injury-induced depolarization was shown to be due to an inhibition of the electrogenic Na^+/K^+-ATPase pump, since the inhibitor ouabain mimicked the postinjury depolarization, and exogenous ATP restored the resting potential (Tavalin et al., 1997).

Stretch-induced injury can also affect other integral membrane proteins such as the NMDA receptor. After stretch-induced injury, the voltage-dependent Mg^{2+} block of the NMDA receptor–associated ionophore was reduced such that activation of the NMDA receptor generated larger ionic currents and increases of $[Ca^{2+}]_i$ (Zhang et al., 1996). This inhibition could be partially restored by increasing $[Mg^{2+}]_e$ to 50 mM, although the inhibition was only 50% of that seen in control cells. It was further suggested that protein kinase C (PKC) activation may also lead to altered NMDA channel kinetics, since administration of calphostin C, a PKC inhibitor, partially restored the Mg^{2+} block of the NMDA channel (Zhang et al., 1996).

Although data obtained from primary cultures have established electrophysiological derangements in single cells, these models provide little information on intercellular electrophysiology or synaptic transmission. Historically, the acute organotypic brain slice preparation has been utilized to obtain critical data on neuronal pathways. Wallis and Panizzon (1995) have injured hippocampal slices in a barotrauma, fluid-percussion model of TBI and found a complete suppression of the orthodromic and antidromic responses of the CA1 region of the hippocampus 1 min after injury. The authors also found that both initiation and maintenance of long-term potentiation (LTP) were permanently depressed postinjury and remained depressed for the duration of the experiment (8 h) (Wallis and Panizzon, 1995).

In the FPI model in the rat, *in vivo* orthodromic CA1 electrophysiological responses showed similar, although not as dramatic, changes after injury (Miyazaki et al., 1992). Between 2 and 3 h after injury, there was a 50% reduction in the CA1 population spike, while LTP was reported to be suppressed postinjury (Miyazaki et al., 1992; Reeves et al., 1995). Other studies in the same animal model have reported a decrease in spontaneous electrical activity of the brain, which was correlated with injury severity (McIntosh et al., 1989). The *in vitro* model of mechanical injury of organotypic brain slice cultures appears to reproduce the general phenomenon of reduced electrical activity at early time points after injury, and offers the added benefits of increased accessibility of the tissue preparation and better control over extracellular parameters. However, *in vivo* data suggests that, at 2 days postinjury, the electrophysiology of the brain changes dramatically as cellular excitability increases in CA1 of the hippocampus and inhibitor cells in the dentate hilus die (Lowenstein et al., 1992; Reeves et al., 1995, 1997). Due to the limited duration of experiments feasible with acute organotypic slices, this model does not seem suited to study the electrophysiological changes at later times postinjury.

Altered Lipid and Cytokine Metabolism After In Vitro *Injury*

Plasma membrane components, such as phospholipids, are sensitive to attack by oxygen radicals, and *in vivo* studies of TBI have suggested that reactive oxygen species such as the superoxide anion, nitric oxide (NO), and the hydroxyl radical play a role in the development of posttraumatic injury (Cheeseman, 1993; Kontos, 1989). In a weight-drop model of head injury and in FPI in the rat, production of prostaglandins E_2 and 6-keto-PGF1-α, and thromboxane was elevated and activity of PLA_2 was increased after injury (DeWitt et al., 1988; Shohami et al., 1987, 1989). These data suggest that increases in arachidonic acid metabolism are a generalized response to mechanical trauma (DeWitt et al., 1988; Shohami et al., 1987). To gain a better understanding of the possible effects of oxygen radicals in TBI, lipid metabolism after mechanical injury has been investigated in several *in vitro* systems.

Subjecting a neuroblastoma cell line to several atmospheres of hydrostatic pressure caused the release of various fatty acids (Murphy and Horrocks, 1993). The authors speculated that this release could have been due to activation of acylhydrolase activity such as PLA_2, which was initiated by physical rearrangements of the lipid membrane due to the pressure (Murphy and Horrocks, 1993). Similarly, in a fluid-percussion barotrauma model of astrocytes, production of leukotriene C_4, a product of

arachidonic acid metabolism, was shown to increase after injury (Shepard et al., 1991). Interestingly, in these studies, leukotriene C_4 production was biphasically regulated by injury severity, increasing dramatically up to a certain injury level and then falling back sharply at higher levels.

Stretch-injured astrocytes have been shown to increase phosphatidylcholine (PC) synthesis as measured by incorporation of ^{3}H-choline (Lamb et al., 1997). The activity of enzymes associated with lipid metabolism such as PLA_2, phospholipase C (PLC), and phosphocoline cytidylyltransferase (PCT) were also increased after stretch. The stretch-induced increase in PC metabolism could be reduced by iron chelators, reactive oxygen scavengers, and antioxidants leading the authors to suggest that mitochondrial disruption induced by traumatic injury generated oxygen-free radicals, which in turn generated hydroxyl radicals in an iron-dependent reaction (Lamb et al., 1997).

Modulation of cytokines may also play a role in the postinjury sequelae (for review, see Rothwell et al., 1997; Rothwell and Relton, 1993; Rothwell and Strijbos, 1995). In the FPI model in the rat, mRNA for both interleukin-1β (IL-1β) and tumor necrosis factor–α (TNF-α) were acutely elevated after injury (Fan et al., 1995, 1996), while levels of IL-1β, IL-6, and TNF-α protein were increased immediately after FPI, weight-drop, and penetrating injuries (Shohami et al., 1994; Taupin et al., 1993; Woodroofe et al., 1991). Using an *in vitro* percussion injury model, adult human astroctyes increased production of IL-6 after injury (Hariri et al, 1994). Given the dual nature of many cytokines, the significance of IL-6 production after mechanical trauma *in vitro* is difficult to interpret. Production of IL-6 may be a pathological response, which is neurotoxic, or may be a beneficial response, which is neuroprotective. Resolving the importance of cytokines after injury requires further investigation.

Using In Vitro *Models to Study Neuroprotective Strategies*

An important use of *in vitro* models is rapid and cost-effective screening of potentially therapeutic interventions. Due to the accessibility of cultured cells, the effects of potentially therapeutic compounds on cell viability can be readily assessed. *In vitro* models may also prove useful in elucidating the mechanisms of neuroprotective compounds.

In *in vitro* transection and acceleration injury models, inhibition of the NMDA receptor and voltage-sensitive Ca^{2+} channels (VSCC) has been shown to be protective. Cell death after injury has been shown to be reduced by application of both competitive and noncompetitive NMDA antagonists including ketamine (Lucas and Wolf, 1991), detrorphan, and D-2-amino-5-phosphonovalerate (D-APV) (Tecoma et al., 1989). Furthermore, MK-801 given in conjunction with dihydropyridine VSCC antagonists such as nimodipine and nifedipine increased the number of surviving neurons after injury more than either class of compounds alone (Regan and Choi, 1994). Although the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was also found to be neuroprotective in these studies, its effects were likely mediated through the NMDA receptor since the more specific AMPA/kainate receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline (NBQX) did not reduce cell death after injury (Regan and Choi, 1994). In an *in vitro* model of trauma that employed an acute organotypic hippocampal slice subjected to barotrauma, felbamate (an NMDA receptor–associated glycine site antagonist) was shown to preserve CA1 orthodromic and antidromic responses as well as LTP after injury (Wallis and Panizzon, 1995).

It is interesting to note that in a model of laser transection injury, D-APV was not neuroprotective (Lucas et al., 1994). This difference in response highlights the advantages of utilizing multiple injury models to study neuroprotection. The laser transection model injures very few neurons in a particular culture, whereas the other models in which glutamate antagonists demonstrated neuroprotection injure a large portion of cells in a given culture. These data would suggest that transection of a cell process is not sufficient to cause cell death by excitotoxic mechanisms, which may require damage to a large population of cells with the subsequent release of intracellular components (including excitatory amino acids). Taken together, these results suggest that the *in vitro* models of mechanical injury faithfully duplicate excitatory, amino acid–related, injury mechanisms, as well as other mechanisms, and can be useful in predicting which compounds may warrant further study *in vivo*.

Another avenue of intervention is the prevention of free radical generation and subsequent lipid membrane oxidation. Inhibitors of lipid peroxidation such as methylprednisolone (Rosenberg et al., 1996; Rosenberg-Schaffer and Lucas, 1993) and 21-aminosteroids (lazaroids) (Regan and Panter, 1995) have been shown to limit neuronal death after *in vitro* mechanical injury. When used in conjunction with MK-801, the 21-aminosteroids (U74500A and U74389F) increased the number of surviving neurons after transection more than if each compound had been used alone. These data suggest that the neuroprotective mechanisms of the 21-aminosteroids do not involve the same mechanisms as MK-801, but rather involve membrane stabilization and free radical scav-

enging (Regan and Panter, 1995). Both methylprednisolone and the 21-aminosteroids have been used to treat experimental and clinical SCI with success, indicating the utility of *in vitro* models of CNS trauma (Clark et al., 1995; Hall, 1992; Rossignol and Barbeau, 1993; Villa and Gorini, 1997). *In vitro* studies also demonstrated that the effective dose of methylprednisolone was within a narrow range (Rosenberg et al., 1996; Rosenberg-Schaffer and Lucas, 1993).

Metalloporphyrins are inhibitors of heme oxygenase and are believed to be neuroprotective by preventing the release from heme oxygenase of carbon monoxide and free iron, which can catalyze the production of free radicals. Metalloporphyrins have been shown to be neuroprotective as determined by electrophysiological measures in a barotrauma injury model in the acute organotypic hippocampal slice (Panizzon et al., 1996). Although polyethylene glycol–conjugated superoxide dismutase did not show protective effects as measured by propidium iodide staining in a model of mechanical injury of astrocyte and neuronal/glial cultures (McKinney et al., 1996), iron chelators and antioxidants were shown to be protective when measuring lipid membrane damage with more sensitive assays in the same model (Lamb et al., 1997).

In an acute organotypic hippocampal slice barotrauma model, the inhibitor of nitric oxide synthetase, methyl-L-arginine, was shown to be protective as measured electrophysiologically (Wallis et al., 1996). A potential consequence of NO production is ADP-ribosylation, which can quickly alter the activity of enzymes, potentially destabilize microtubules, and deplete the energy reserves of a cell (Brune et al., 1994; Dimmeler et al., 1993; Zhang et al., 1994). In the same injury model, inhibitors of both mono- and poly-ADP-ribosylation such as *meta*-iodobenzylguanidine, novobiocin, 3-aminobenzamide, and 3-methoxybenzamide also confered neuroprotection after injury (Wallis et al., 1996).

Several inhibitors of lipid metabolism, specifically inhibitors of leukotriene metabolism, have been shown to be protective after fluid-percussion barotrauma of an organotypic hippocampal slice *in vitro* (Girard et al., 1996). Azelastine, an inhibitor of leukotriene C_4 synthesis and release, demonstrated neuroprotection after injury as measured by the recovery of orthodromic and antidromic responses of the CA1 region of the hippocampus. Interestingly, MK-571, an antagonist of the leukotriene D_4 receptor, and MK-886, an inhibitor of 5-lipoxygenase activating protein, produced similar protection.

The potentially beneficial effects of hypothermia have been investigated *in vitro* as well. After a transection injury, cultures of neurons were cooled for 2–6 h at 27°C, 17°C, and 7°C and then allowed to re-warm to 37°C (Lucas et al., 1994, 1990b). It was found that cooling to 17°C for 2 h increased survival of neurons after transection injury; however, neuronal protection was lost if the 17°C hypothermia was extended to 6 h. It was also observed that cooling below 17°C caused an NMDA receptor–linked lethal cold stress to both injured and control neurons in the same culture (Lucas et al., 1994, 1990b). Lucas et al. (1994) sugested that the neuroprotective effects of hypothermia were mediated by a reduction in cellular energy demands, since barbiturate-induced, neuronal electrical silence was found to be neuroprotective as well (Lucas et al., 1994). *In vivo*, 3 h of 30°C hypothermia after FPI in the rat has been shown to reduce histological damage and attenuate enlargement of the lateral ventricles (Bramlett et al., 1997; Dietrich et al., 1994). *In vitro* injury systems may therefore be ideal for studying the mechanisms of hypothermic neuroprotection because of the ease with which hypothermia can be initiated, maintained, and verified during the course of an experiment.

The therapeutic strategy of gene therapy has also been explored in an *in vitro* transection injury model of cocultures (Lefrancois et al., 1997). It was hypothesized that neuronal outgrowth into the injured regions of the culture dish was inhibited by the reactive nature of the underlying astrocytes. Prior to injury, astrocytes were transfected with an antisense glial fibrillary–associated protein (GFAP) riboprobe, which decreased the cells' transcription capacity of GFAP by 46%. In antisense GFAP transfected cultures, the number and length of neurites entering the lesioned area was increased significantly at 72 h postinjury over cultures transfected with a control riboprobe. Given the complexity of the *in vivo* situation, *in vitro* models may prove to be an invaluable screening tool for novel gene therapy strategies. Moreover, *in vitro* transfection is more efficient than *in vivo*, without associated complications of inflammation and necrosis. These *in vitro* models can be used to search for gene therapies that are beneficial, so that, in the future, these cDNAs can be inserted into improved vectors and evaluated *in vivo*.

CONCLUSIONS

The mechanisms by which a mechanical stimulus causes CNS injury are extremely complex and involve multiple pathways that end in cell dysfunction or death. These pathways include, but are not limited to, ionic derangements, excitotoxicity, free radical generation, lipid damage, and cytoskeleton disruption. From the data presented herein, *in vitro* models of mechanical injury to the

CNS appear to reproduce much of this posttraumatic sequelae and should continue to be a useful complement to *in vivo* models of TBI and SCI. *In vitro* models possess advantages over *in vivo* models, including precise control of the extracellular environment, elimination of confounding factors such as hypoxia or ischemia, and the promise of lower costs and faster discovery. *In vitro* models also offer the opportunity to study the biomechanics of trauma in the most basic and universally applicable engineering terms: strain and strain rate. With these models, new tolerance criteria on a tissue level could be determined and used to give new interpretations to complex and realistic FEM models, interpretations based on functional instead of mechanical failure criteria. These models could be further refined to include simulations of the direct molecular transduction mechanisms, thereby providing insight into their relationship with more macroscopic measurements of injury response such as ionic derangements or gene expression.

There are a number of *in vitro* models of CNS injury that have been described in the literature and presented in this review. It is hoped that this review has highlighted each model's strengths and weaknesses in an attempt to facilitate the choice of a specific model for a particular set of experiments. *In vitro* models of CNS injury will not likely replace *in vivo* models, but, if applied carefully, *in vitro* systems may provide insights that *in vivo* models cannot. It is hoped that these insights will ultimately lead to novel therapeutic interventions and, eventually, the improved management of the traumatically injured patient, so that the mortality, morbidity, and cost associated with CNS injury can be minimized.

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