JOURNAL OF NEUROTRAUMA
Volume 23, Number 8, 2006
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Pp. 1261–1273

Recombinant Human TNFα Induces Concentration-Dependent and Reversible Alterations in the Electrophysiological Properties of Axons in Mammalian Spinal Cord

ANDREW L. DAVIES,[1,2] KEITH C. HAYES,[1,2] and RIYI SHI[3]

ABSTRACT

Increased expression of the proinflammatory cytokine tumor necrosis factor–alpha (TNFα) and its soluble receptors is evident within the central nervous system (CNS) following traumatic brain injury and spinal cord injury. TNFα is integral to the acute inflammatory cascade that follows neurotrauma and has been shown to have both beneficial and detrimental properties. We examined the effects of varying concentrations (1–5000 ng/mL) of recombinant human TNFα (rhTNFα) on select electrophysiological properties of excised guinea pig spinal cord tissue. Pulsed electrical stimuli (0.33 Hz) were delivered to strips of isolated ventral white matter in a double sucrose gap chamber. Recordings were made of the compound action potential (CAP) and membrane potential before, during, and after bathing the tissue with rhTNFα for 30 min. Increasing concentrations of rhTNFα yielded progressively greater reductions in amplitude of the CAP that were temporally associated with depolarization of the resting compound membrane potential. These effects were largely reversed on washout of rhTNFα and were not present when heat-denatured rhTNFα was introduced. The results provide evidence that elevated concentrations of TNFα induce reversible depolarization of the compound membrane potential and reduction in CAP amplitude, sometimes to the point of extinction of the CAP, suggestive of impaired axonal conduction. These observations point to a new mechanism of immune-mediated central conduction deficit. Cytokine-induced alterations in membrane properties and axonal conduction may contribute to neurological deficits following CNS injury by compounding trauma-induced myelinopathy and axonopathy.

Key words: axons; compound action potential; conduction; cytokine; electrophysiology; guinea pig; recombinant human TNFα

INTRODUCTION

The proinflammatory cytokine tumor necrosis factor–alpha (TNFα) and its two principal receptors (TNF-R1 and TNF-R2) are constitutively expressed by neurons, astrocytes, and oligodendrocytes within the spinal cord and brain (Benveniste, 1998, 1992; Sredni-Kenigsbuch, 2002; Woodroofe, 1995). TNFα secreted by circulating immune cells also passes through the blood–brain barrier (BBB) via active transport mechanisms (Pan and Kastin, 2002) to influence central nervous system (CNS) function (Banks et al., 1995). Indeed,

[1]Neuroscience Program, University of Western Ontario, London, Ontario, Canada.
[2]Lawson Health Research Institute, London, Ontario, Canada.
[3]Department of Basic Medical Sciences, Institute for Applied Neurology, Purdue University, West Lafayette, Indiana.

one of the properties of proinflammatory cytokines that pass through the BBB is to upregulate their own expression within the CNS (Hopkins and Rothwell, 1995). TNFα is clearly involved in immune-CNS signaling and is known to contribute to normal physiological functions such as sleep (Krueger et al., 1998) and appetite regulation (Dantzer, 2001; Vitkovic et al., 2000).

Increased neuronal expression of TNFα and its receptors has been reported following traumatic brain injury (TBI) (Ahn et al., 2004; Fan et al., 1996; Goodman et al., 1990; Knoblach et al., 1999; Vitarbo et al., 2004) and spinal cord injury (SCI) (Reece et al., 2004; Yune et al., 2003). Although most evident in the acute stage following vascular accidents (Gruber et al., 2000; Zaremba et al., 2001) or trauma (Knoblach et al., 1999), persistently elevated levels of TNFα in CNS tissue have also been reported (Holmin et al., 1997; Holmin and Mathiesen, 1999). Increased neuronal expression of TNFα has been definitively established in animal models (Knoblach et al., 1999; Vitarbo et al., 2004; Wang et al., 2002; Yin et al., 2003), and comparable observations have been made from studies of human CNS tissue following TBI (Holmin and Hojeberg, 2004) and SCI (Yang et al., 2004).

The functional consequences of increased neuronal expression of TNFα following trauma are multifaceted. TNFα is instrumental in the acute inflammatory cascade that follows neurotrauma (Feuerstein et al., 1994) and has been shown to have both proinflammatory and anti-inflammatory properties (Schmidt et al., 2004). TNFα also has neuroprotective (Liu et al., 1998, 1999; Shinpo et al., 1999), neurotoxic (MacEwan, 2002; Viviani et al., 2004), and neuroplastic properties (Beattie et al., 2002; Covey et al., 2000). Enduring expression of neuronal TNFα has been thought to reflect a chronic neuroinflammatory process (Holtmann and Neurath, 2004; Kollias et al., 1999; Kollias, 2005).

At physiological levels, TNFα has well-documented neuromodulatory properties that include modifying ion channel kinetics (Hribar et al., 1999; Kagan et al., 1992; Koller et al., 1998; van der Goot et al., 1999) and enhancing synaptic transmission (Beattie et al., 2002; Pickering et al., 2005; Tancredi et al., 1992). At high concentrations, and with prolonged exposure, TNFα can be cytotoxic, for example by inducing axonopathy (Sipe et al., 1996; Talley et al., 1995) or oligodendrogliopathy (Hisahara et al., 1997; Selmaj and Raine, 1988a,b) through apoptotic pathways (Lee et al., 2000; Talley et al., 1995; Yune et al., 2003). TNFα-induced cytotoxicity is considered to contribute to the pathogenesis of certain autoimmune neuroinflammatory diseases in which demyelination is a prominent feature (Glabinski et al., 1995; Matusevicius et al., 1996; Sun et al., 2004; Taupin et al., 1997). High cerebrospinal fluid (CSF) TNFα concentrations also correlate with magnetic resonance imaging-defined human white matter injury (Ellison et al., 2005).

While appreciable evidence exists as to the physiological effects of low or very high extracellular concentrations of TNFα (Blatteis, 1990; Hermann et al., 2005; Leem and Bove, 2002; Pickering et al., 2005; Rothwell and Hopkins, 1995; Soliven and Albert, 1992; Sorkin et al., 1997; Vitkovic et al., 2000), less is known about the effects of intermediate concentrations ie. elevated but not cytotoxic. We have previously introduced the possibility that elevated concentrations of TNFα in serum or CSF following CNS trauma may be surrogate markers of an immune-mediated "channelopathy" (ie altered axonal ion channel conductance) leading to conduction failure that compounds the neurologic deficits associated with axonopathy and myelinopathy (Hayes et al., 2002). This proposition of cytokine-mediated conduction deficits within the CNS following SCI or TBI has not previously been tested.

The present study thus examined the effects of a range (1–5000 ng/mL) of concentrations of recombinant human TNFα (rhTNFα) on membrane and action potential properties in isolated strips of ventral spinal cord tissue from adult guinea pigs. In this initial, proof of principle study, uninjured spinal cord tissue was used to avoid the added complication of trauma-induced disruption of axonal membrane (Borgens, 2001) or myelin sheath. In particular we tested the hypothesis that elevated extracellular concentrations of rhTNFα would modify axonal membrane and conduction properties during short-term exposure as would be predicted by observations that TNFα increases membrane permeability to Na^+ in a pH- and voltage-dependent fashion independent of receptor binding (Hribar et al., 1999; Kagan et al., 1992; van der Goot et al., 1999).

METHODS

Isolation of Spinal Cord Tissue

The surgical procedure for isolating the spinal cord tissue has been described previously (Shi et al., 2002; Shi and Blight, 1996, 1997; Shi and Borgens, 1999). Adult female guinea pigs of 350–500 g in body weight (n = 17, Harlan, USA) were anesthetized prior to surgery (80 mg/kg ketamine hydrochloride, 0.8 mg/kg acepromazine maleate, and 12 mg/kg xylazine, i.m.). Following anesthesia, animals were perfused transcardially with 500 mL of cold (15°C), oxygenated Krebs' solution (NaCl 124 mM, KCl 2 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.3 mM, $CaCl_2$ 1.2 mM, dextrose 10 mM, $NaHCO_3$ 26 mM,

sodium ascorbate 10 mM, equilibrated with 95% O_2, 5% CO_2 to a pH of 7.2–7.4), and the vertebral column was excised rapidly. The spinal cord was carefully removed from the vertebrae and placed in cold Krebs' solution. The cord was initially separated into two halves by midline sagittal division, and the ventral white matter was then isolated (Fig. 1A). White matter strips were maintained in continuously oxygenated Krebs' solution for at least 1 h prior to mounting in the recording chamber to ensure recovery from dissection before experimentation. The experimental protocols were reviewed and approved by the Purdue University Animal Care and Use Committee (PACUC). All efforts were made to minimize the number of animals used and their distress.

Electrophysiological Recording and Analysis

The design and construction of the double sucrose gap chamber used for the electrophysiological recordings has been described previously (Shi et al., 2002; Shi and Blight, 1996, 1997; Shi and Borgens, 1999) and is illustrated in Figure 1B. The central compartment, 20 mm in diameter, was continuously superfused with oxygenated Krebs' solution at 2 mL/min into which the rhTNFα was introduced. The temperature of the central compartment was maintained at 36.0–37.0°C with an in-line solution warmer (Warner Instruments, Hamden, CT), and monitored using a thermocouple probe. The two end compartments contained isotonic potassium chloride (120 mM) and were electrically isolated from the central compartment by narrow channels through which sucrose solution (320 mM) continuously flowed.

The strip of tissue was stimulated through silver-silver chloride pin electrodes positioned within a side compartment and the central bath (Fig. 1B). Constant current supramaximal stimuli were generated by a digital stimulator (Cygnus Technology, PA) and delivered via a stimulus isolation unit (WP Instruments, Sarasota, FL) in the form of 0.1-msec constant current rectangular pulses at a rate of 0.33 Hz. Compound action potentials (CAP) and the compound membrane potential were recorded. The electrophysiological data were digitized and stored with a Neurodata Instruments Neurocorder for subsequent analysis. All the on-line recording and subsequent analysis was performed using LabView software (National Instruments, Austin, TX).

rhTNFα Treatments

rhTNFa (eBioscience, San Diego, CA) derived from *Escherichia coli*, with >98% purity by SDS-PAGE and maintained in a phosphate buffer with pH 7.2 (150 mM NaCl, 1% BSA), was added to oxygenated Krebs' solution in concentrations of 1, 10, 100, 1000, and 5000 ng/mL. The duration of tissue perfusion with rhTNFα was 30 min. After 30 min, the rhTNFα was washed out by perfusion with Krebs solution alone and the electrophysiological properties were monitored for an additional

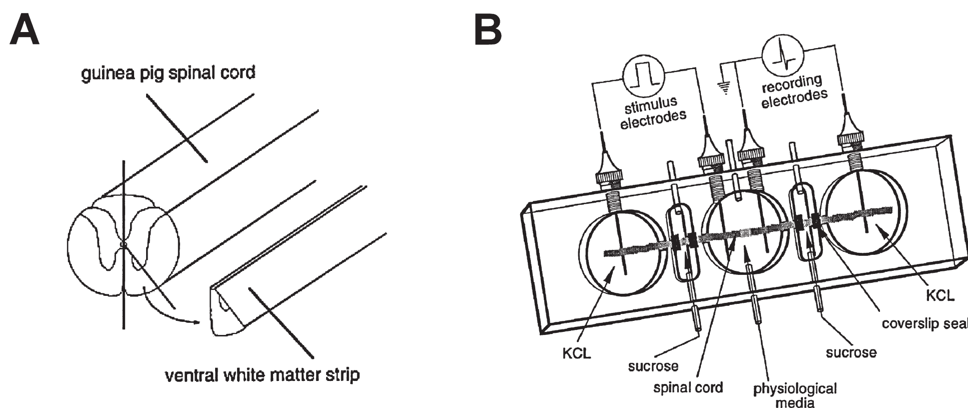

FIG. 1. Isolation of ventral white matter and double sucrose gap chamber for *ex vivo* recording of axonal conduction properties of excised guinea pig ventral white matter. (**A**) Separation of ventral white matter strips for mounting in the double sucrose gap recording chamber. (**B**) Double sucrose gap recording chamber viewed from above showing the two outside compartments filled with isotonic KCl, a central compartment filled with oxygenated Krebs' solution, and two sucrose gap compartments maintaining electrical isolation between the KCl and Krebs. rhTNFα is added to the Krebs' solution to bathe the cord in the central compartment. The guinea pig cord is positioned in the trough that traverses the various compartments and is covered by the fluids. Stimulation electrodes (silver-silver chloride) with anode and cathode positioned in the KCL and Krebs' compartments, and bipolar recording (pin) electrodes in the opposite KCl compartment and the common (central) Krebs' compartment are also shown. The central recording electrode is referenced to ground.

60 min. Fresh strips of tissue were used for each preparation and each condition was replicated 5 times ($n = 5$) to establish reproducibility of the observations.

Control Condition

In order to dissociate the effects of rhTNFα from the effects of high concentrations of protein in solution, heat-denatured 5000 ng/mL rhTNFα in Krebs' solution was perfused through the central compartment for 30 min. The cytokine was denatured by immersion in a $>$95°C water bath for 10 min. A second control condition tested whether changing the perfusion solution altered the conduction properties of the spinal cord preparation. To achieve this, the Krebs' solution used prior to rhTNFα treatment was replaced with a new Krebs' solution while all other aspects of the protocol remained identical.

Statistical Analysis

Linear regression analysis was used to characterize the association between rhTNFα concentration (natural log-ln transformation) and CAP amplitude. The CAP amplitude was expressed as a percentage of the baseline mean CAP amplitude for each preparation.

RESULTS

The compound membrane potential stabilized over a mean 53.0 $\pm$ 11.1 (SD) min in the 30 preparations studied. Once stabilized, the membrane potential varied minimally (0.003–0.062 mV; SEM) prior to introduction of TNFα. Monophasic CAPs were recorded from each preparation. Stable recordings of the peak amplitude of the CAP were obtained prior to the introduction of rhTNFα treatment (SEM = 0.004–0.012 mV, or 0.17–1.32% of the mean).

1 ng/mL rhTNFα

Application of rhTNFα at a concentration of 1 ng/mL ($n = 5$) had no detectable effect on the CAP or membrane potential throughout the duration of the exposure phase (30 min) and the subsequent washout period (60 min).

10 ng/mL rhTNFα

rhTNFα at a concentration of 10 ng/mL ($n = 5$) resulted in a slow and persistent partial depolarization of the membrane potential in all preparations. After 20 min of exposure to rhTNFα, the mean CAP amplitude was reduced to 88.96 $\pm$ 10.26% of pre-treatment CAP amplitude and remained slightly depressed (92.62 $\pm$ 10.31%) at 30 min of rhTNFα exposure. Following 30 min of washout with oxygenated Krebs' solution, mean CAP amplitude returned to 96.16 $\pm$ 17.81% of pre-treatment levels and the membrane potential returned to baseline level.

100 ng/mL rhTNFα

Application of rhTNFα at a concentration of 100 ng/mL ($n = 5$) again resulted in a small and prolonged depolarization of the membrane potential in the majority of preparations. After 20 min of exposure to rhTNFα, the mean CAP amplitude was reduced to 87.64 $\pm$ 31.68% of pre-treatment CAP amplitude and continued to decrease (79.07 $\pm$ 42.04%) at 30 min of rhTNFα exposure. Following 30 minutes of washout with oxygenated Krebs' solution, mean CAP amplitude had recovered to 89.70 $\pm$ 28.86% of pre-treatment levels. The membrane potential returned to its baseline level. In Figure 2A, the upper panel illustrates CAPs recorded prior to, during, and following bathing of the tissue in 100 ng/mL rhTNFα in a representative preparation. The inverse relationship between CAP peak amplitude and the membrane potential is shown in the lower panel.

1000 ng/mL rhTNFα

rhTNFα at a concentration of 1000 ng/mL ($n = 5$) also resulted in a gradual and prolonged partial depolarization of the membrane potential, again persisting throughout the entire duration of the exposure phase. After 20 min of rhTNFα exposure, mean CAP amplitude was reduced to 72.71 $\pm$ 23.08% of pre-treatment amplitude. This decrease was maintained throughout rhTNFα application, and after an additional 10 min of exposure, CAP amplitude remained (73.81 $\pm$ 17.70%) below the pre-treatment baseline. Thirty minutes of washout with oxygenated Krebs' solution partially reversed the decrease, but the CAP amplitude remained depressed at 81.55 $\pm$ 16.42% of pre-treatment. The membrane potential also remained partially depolarized at this time. Figure 2B illustrates data for the CAP and membrane potential obtained from a preparation treated with 1000 ng/mL rhTNFα.

5000 ng/mL rhTNFα

Application of rhTNFα at a concentration of 5000 ng/mL ($n = 5$) resulted in an immediate partial depolarization of the membrane potential in all five preparations that persisted throughout the duration of the exposure phase. On washout the change in membrane potential was partially reversed in three preparations; the membrane remained in its depolarized state in the other two preparations. Almost immediately following introduction of 5000 ng/mL rhTNFα the mean CAP amplitude was re-

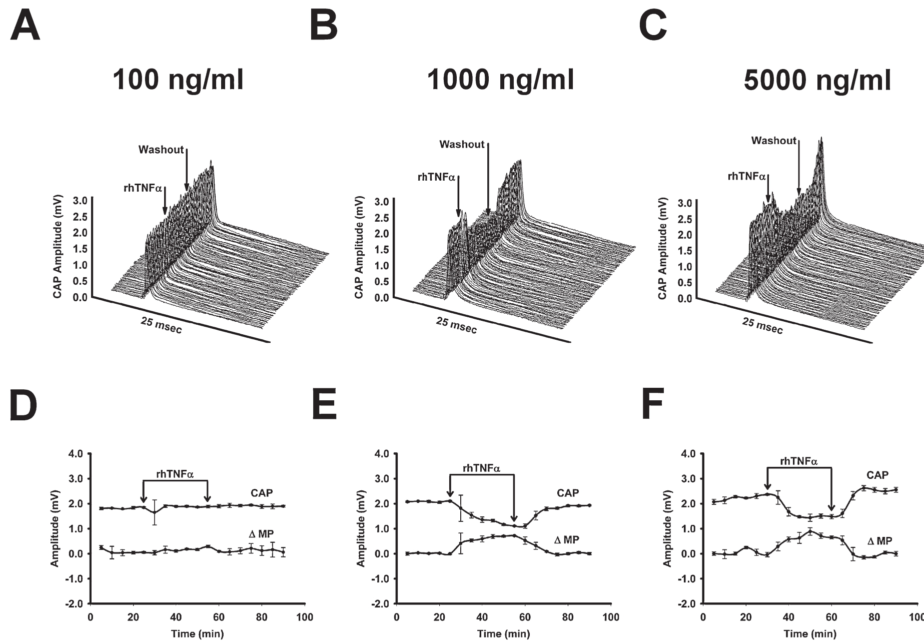

FIG. 2. Compound action potential (CAP) waveforms, mean CAP amplitude, and mean change in membrane potential before (30 min), during (30 min), and after (30 min) treatment with 100, 1000, and 5000 ng/mL rhTNFα. (**A**) CAP waveforms recorded before, during, and after treatment with 100 ng/mL rhTNFα. The cytokine had little effect on CAP waveform or amplitude. The duration of each trace is 20 msec. (**B**) CAP waveforms recorded before, during, and after treatment with 1000 ng/mL rhTNFα. The cytokine reversibly blocked conduction and this effect was partially reversed on washout. (**C**) CAP waveforms recorded before, during, and after treatment with 5000 ng/mL rhTNFα. (**D**) Mean CAP amplitude and change in membrane potential before, during, and after treatment with 100 ng/mL rhTNFα. (**E**) Mean CAP amplitude and change in membrane potential before, during, and after treatment with 1000 ng/mL rhTNFα. (**F**) Mean CAP amplitude and change in membrane potential before, during, and after treatment with 5000 ng/mL rhTNFα.

duced to 43.98 $\pm$ 40.34% of the pre-treatment value. Mean CAP amplitude was depressed throughout rhTNFα application and remained at 59.82 $\pm$ 53.77% of the pre-treatment baseline after 30 min of exposure. Two of the preparations exhibited complete extinction of the CAP indicative of conduction failure and the CAP was not restored during washout. Thirty minutes of washout with oxygenated Krebs' solution partially reversed the decrease in those preparations where reduction in CAP amplitude was incomplete, and the overall mean CAP amplitude remained depressed at 63.19 $\pm$ 57.44% of the pre-treatment value. Figure 2C shows CAP and membrane potential data obtained from a preparation treated with 5000 ng/mL rhTNFα and showing attenuation of the amplitude but not complete loss of CAP.

Concentration-Dependent Conduction Failure

The concentration-dependent nature of the rhTNFα-induced conduction deficits across all preparations is illustrated in Figure 3. The normalized peak CAP amplitude (y) was found to reduce linearly with the ln-transformed rhTNFα treatment concentration ($y = 1.06 - 0.06 \cdot \ln[\text{rhTNF}\alpha]$, $R^2 = 0.95$, $p < 0.05$). As treatment concentration increased, reductions in CAP amplitude and depolarization of the membrane potential occurred at a faster rate.

Control Conditions

Replication of the treatment protocol using heat-denatured rhTNFα at the highest concentration tested (5000 ng/mL) yielded no reduction in peak CAP amplitude or change in membrane potential. Illustrative data are shown in Figure 4. Similar observations were made when a replacement Krebs solution containing no cytokine alone was used instead of the rhTNFα.

DISCUSSION

Neuronal and glial expression of TNFα and its soluble receptors is increased following trauma to the brain (Holmin and Mathiesen, 1999; Knoblach et al., 1999; Vitarbo et al., 2004) or spinal cord (Lee et al., 2000; Wang et al., 2002; Yune et al., 2003), and a variety of poten-

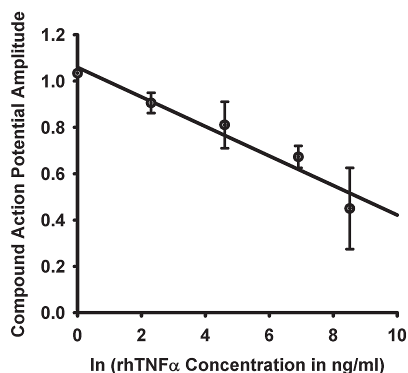

FIG. 3. Concentration-dependency of rhTNFα-induced conduction block. A linear relationship existed between normalized mean CAP amplitude and the ln-transformed rhTNFα concentration (mean [normalized] CAP amplitude $= 1.06 - 0.06 \cdot \ln[\text{rhTNF}\alpha]$, $R^2 = 0.95$, $p < 0.05$).

tially beneficial and detrimental consequences have been identified (Barnhart and Peter, 2003; Blatteis, 1990; Lenzlinger et al., 2001; Pan et al., 1997b). TNFα is also synthesized by circulating immune cells and is selectively transported across the blood–brain barrier and blood–spinal cord barrier (Banks et al., 2001; Gutierrez et al., 1993). This selective permeability is increased following brain injury (Ott et al., 1994) and SCI (Pan et al., 1997a, 1999, 2003; Pan and Kastin, 2001a, b), and varies with time since injury, as well as the location and type of lesion (Pan et al., 1996, 2002; Pan and Kastin, 2001a). Elevated concentrations of TNFα and its receptors are evident in the CSF of patients with TBI shortly after trauma (Goodman et al., 1990; Ross et al., 1994). The physiological consequences of increased neuronal expression and concentrations of TNFα within the extracellular fluid and CSF compartments of the CNS are gradually being elucidated (Campbell et al., 1997; Shohami et al., 1999; Tonelli and Postolache, 2005; Wang and Shuaib, 2002).

In the present study, we examined the effects of elevated extracellular concentrations of rhTNFα on axonal membrane and action potential properties in the CNS utilizing an electrophysiological recording protocol that has been previously shown to yield sensitive yet stable indices of axonal conduction in excised guinea pig spinal cord tissue (Shi et al., 2002; Shi and Blight, 1996, 1997; Shi and Borgens, 1999). In this initial set of studies, we examined the immediate effects of relatively brief (30 min) exposure of the tissue to rhTNFα and employed a wide range of concentrations that were considered to be relevant to the levels present in pathologic states. Our intent was to further explore the possibility that an immune-mediated "channelopathy" contributes to the neurologic deficits seen following CNS trauma (Hayes et al., 2002).

The main result of this study was that 30 min of exposure to rhTNFα induced a dose-dependent reduction in CAP amplitude and depolarization of the compound membrane potential in axons of guinea pig spinal cord. These electrophysiological changes occurred faster at higher concentrations; the reduction in CAP amplitude was linearly proportional to the natural log of the rhT-

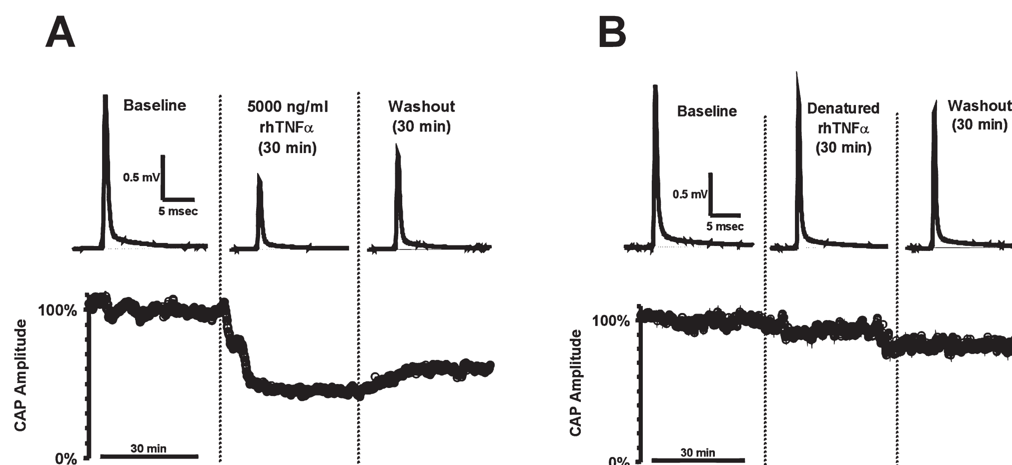

FIG. 4. Heat-denatured control condition. Effects of denatured (control) 5000 ng/mL rhTNFα on CAP amplitudes. (**A**) Effects of 5000 ng/mL rhTNFα on CAP amplitudes recorded during baseline, treatment, and washout periods. Upper trace shows average CAPs. Lower trace shows peak amplitudes recorded throughout each period. (**B**) Effects of denatured (5000 ng/mL) rhTNFα on CAP amplitudes. Upper trace shows average CAPs. Lower trace shows peak amplitudes recorded throughout each period. Note the preservation of large amplitude CAPs (>80% of pretreatment condition) throughout the application and washout of denatured (control) rhTNFα solution.

NFα concentration and was largely reversed during 60 min of washout with Krebs' solution. The reduction in amplitude of the CAP was, in the vast majority of cases, temporally associated with depolarization of the resting compound membrane potential. Heat-denatured rhTNFα (5000 ng/mL) did not lead to changes in CAP amplitude or membrane potential, thereby implying that it was the molecular structure of the rhTNFα per se rather than the presence of constitutive proteins that caused the altered electrophysiological properties. Collectively, these observations provide support for the notion that immune-mediated conduction deficits may contribute to neurologic dysfunction following trauma.

The mechanisms underlying this form of cytokine-mediated alteration of membrane and CAP properties remain to be determined. At the concentrations employed in the present study, and with the limited duration of exposure, the reduction in CAP amplitude and depolarization of the membrane potential were largely reversible. This implies that cytotoxicity was not a factor (i.e., there was unlikely any appreciable TNFα-induced axonopathy or oligodendrogliopathy) leading to demyelination-based conduction failure. Moreover, the rapid onset of membrane and CAP changes would likely be more consistent with changes in ion channel kinetics rather than in slower acting receptor-mediated second messenger signaling.

TNFα has been shown previously to increase membrane permeability to Na^+ through direct interaction with endogenous ion channels or membrane proteins coupled with ion channels (van der Goot et al., 1999). Altered channel conductance resulting from exposure to extracellular TNFα is pH- and voltage-dependent, and receptor-independent (Hribar et al., 1999). Accumulation of axoplasmic Na^+ per se can induce membrane depolarization sufficient to cause conduction failure. Moreover, Na^+ accumulation is functionally coupled to extra-axonal Ca^{2+} import. Increases in intracellular [Na^+] cause reverse operation of the Na^+-Ca^{2+} exchanger, which admits potentially disruptive quantities of Ca^{2+} into the cell (Li et al., 2000; Stys et al., 1992). Calcium accumulation, in turn, results in increased phospholipase and protease activity, production of reactive oxygen species such as nitric oxide (Kristian and Siesjo, 1998; Paschen, 1999, 2000, 2003), which can also block conduction (Kapoor et al., 1999; Redford et al., 1997; Shrager et al., 1998).

TNFα also enhances (Houzen et al., 1997; Ilschner et al., 1995; Koller et al., 1998; McLarnon et al., 1993, 2001; Nietsch et al., 2000) or impairs (Diem et al., 2001; Sawada et al., 1990, 1991a,b; Soliven et al., 1991) K^+ currents in excitable membranes. In rat cortical neuron cultures, prolonged (12–48 h) application of 10–100 ng/mL TNFα enhanced outward K^+ currents (Houzen et al., 1997). TNFα also modulates Ca^{2+} and K^+ channel activity in human microglia (McLarnon et al., 1993, 2001). Changes in either Na^+, Ca^{2+}, or K^+ conductances in axons or glia might therefore underlie the membrane and CAP changes observed in the present study.

Another candidate mechanism stems from the observation that elevated concentrations of TNFα directly increase expression of inducible nitric oxide synthase (iNOS) in macrophages and astrocytes within the CNS with release of greater amounts of nitric oxide (NO) (Yune et al., 2003). NO causes a dose-dependent and reversible axonal conduction failure that is activity-dependent; demyelinated axons are especially susceptible (Kapoor et al., 1999). Disruption of mitochondrial respiration and insufficient production of adenosine triphosphate (ATP) to support the membrane potential is thought to underlie this form of conduction deficit.

Central to the plausibility of immune-mediated conduction deficits *in vivo* is the question of how closely the concentrations of TNFα used in the present study approximate those found in the clinical state. Where there exists local neuronal expression of TNFα in the CNS, as in the case of a localized inflammatory response, the extracellular concentration (gradient) falls off rapidly as a function of both time and space; drainage into the larger volume CSF fluid compartment then results in a dilution effect, and CSF concentrations underestimate local extracellular concentrations (de Lange and Danhof, 2002; Shen et al., 2004). The problem of CSF as a surrogate marker of extracellular concentration is further complicated by the fact that circulating (serum) TNFα-secreting cells and the cytokine itself can pass through both the blood–brain barrier and the blood–CSF barrier and into the extracellular space at rates that depend on disease state; thus neuronal and glial expression, serum and CSF sources all influence extracellular TNFα concentrations. Acknowledging these caveats, it follows that experimental (extracellular) concentrations needed to induce electrophysiological changes of the type reported here would need to be many times higher than the concentrations detectable in CSF. Abnormally elevated TNFα CSF concentrations of $\sim$20 pg/mL have been reported following human TBI (Hayakata et al., 2004), and we have detected levels up to 37 pg/mL following human SCI (unpublished studies).

Also relevant to the concentration question is the cross-species protocol employed herein. Cross-reactivity between rhTNFα and rodent tissue, at various concentrations, has been demonstrated previously (Catanzaro et al., 1991; Fischer et al., 1999; Hocking et al., 1990; Mallick et al., 1989; Pennings et al., 1998; Shibata and Blatteis, 1991a,b; Stephens et al., 1988) and the amino acid alignment of TNFα is remarkably similar for human and guinea pig, particularly at signature sequences (Goetz et

al., 2004). Nevertheless, it has been shown previously that higher (×1000) concentrations of human TNFα than rodent TNFα are required to induce equivalent changes in firing rates of rat neurons. This suggests that the *in vivo* extracellular concentrations of TNFα that will induce electrophysiological changes may be appreciably less than the concentrations employed in our cross-species *ex vivo* model.

The extracellular concentration of TNFα necessary to induce conduction deficits *in vivo* may well be dependent on the physiological status of the axons, glia, and surrounding milieu. For reasons noted previously we used uninjured tissue in the present study. Trauma to cord parenchyma introduces additional considerations most notably with respect to the astroglial scar. Astrocytes are a major immunoresponsive source of TNFα within the brain and spinal cord (Aschner, 1998; DeLeo et al., 2000), and a pathological increase in their density at a lesion site (astrocytosis or astrogliosis) may render injured tissue exquisitely sensitive to immune-mediated alterations in neurological function. The astrocyte marker glial fibrillary acidic protein (GFAP) (Du et al., 1999; Eng et al., 2000; Menet et al., 2003) which is upregulated following neurotrauma (Haghighi et al., 2004), and is elevated in the CSF of patients with TBI (Regner et al., 2001) and SCI (Guez et al., 2003), has been associated with acidification (Oh et al., 1995). TNFα-induced modulation of Na^+ channel activity occurs in a pH-dependent manner (Kagan et al., 1992; van der Goot et al., 1999), with increased activity at lower pH. A focal, astrocyte-induced, acidification of the axonal environment at a lesion site may therefore result in altered axonal conduction at relatively lower concentrations of TNFα following CNS trauma. The implication here is that injured CNS tissue, where reactive gliosis is present, may be even more sensitive to cytokine-mediated conduction deficits than the uninjured tissue used in the present study.

The potential clinical implications of the present findings are numerous. In the acute stage of CNS trauma when neuronal expression of TNFα is increased (Vitarbo et al., 2004) and CSF levels of TNFα are high (Schmidt et al., 2004), there exists the possibility that TNFα-mediated impairment of axonal conduction contributes to the observed neurologic deficits. Immune-mediated deficits in conduction, of the type described here, would be distinguishable from those attributable to axonopathy or myelinopathy by virtue of their reversibility and their resolution may contribute to spontaneous neurologic recovery. Since elevated CNS levels of TNFα may endure during chronic neuroinflammatory processes (Holtmann and Neurath, 2004; Kollias et al., 1999; Kollias, 2005), because of infection, autoimmune disease, or elevated levels of TNFα passing through the blood-spinal cord barrier, there also exists the potential for a sustained impairment of axonal conduction that would only reverse on resolution of the infection or autoimmune response. Reversal of neurologic deficit of this type might contribute to elements of late-onset recovery. If this is the case, anti-inflammatory, anti-cytokine or anti-TNFα therapy may be expected to foster accelerated recovery or resolution of longstanding deficits. This recovery of function would occur earlier than could be reasonably attributed to remyelination.

Reversible, cytokine-mediated impairment of axonal conduction is thus a potentially new mechanism of central neurological deficit following CNS trauma. If attributable to altered membrane permeability to various ions, this form of neuroimmunomodulation would fit into the recently identified category of deficits termed "acquired channelopathy" (Waxman, 2001).

In summary, the results of the present study demonstrated concentration-dependent effects of rhTNFα on membrane and CAP properties in guinea pig spinal cord tissue. Increasing concentrations of rhTNFα yielded progressively greater reductions in amplitude of the CAP accompanied by depolarization of the membrane potential. These effects were reversed on washout of rhTNFα and were not present when denatured rhTNFα was introduced. TNFα-mediated changes in the compound membrane potential and axonal conduction may underlie some of the neurologic deficits observed following various forms of neurotrauma and provide a target for future neuro-restorative therapies.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance and support provided by P. Zickmund and R. Borgens, Ph.D. This work was funded by grants from Parkwood Hospital Foundation and The Cooperators, and a studentship award (to A.L.D.) from the Ontario Neurotrauma Foundation.

REFERENCES

AHN, M.J., SHERWOOD, E.R., PROUGH, D.S., LIN, C.Y., and DEWITT, D.S. (2004). The effects of traumatic brain injury on cerebral blood flow and brain tissue nitric oxide levels and cytokine expression. J. Neurotrauma **21,** 1431–1442.

ASCHNER, M. (1998). Astrocytes as mediators of immune and inflammatory responses in the CNS. Neurotoxicology **19,** 269–281.

BANKS, W.A., KASTIN, A.J., and BROADWELL, R.D. (1995). Passage of cytokines across the blood–brain barrier. Neuroimmunomodulation **2,** 241–248.

BANKS, W.A., MOINUDDIN, A., and MORLEY, J.E. (2001). Regional transport of TNF-alpha across the blood–brain barrier in young ICR and young and aged SAMP8 mice. Neurobiol. Aging **22,** 671–676.

BARNHART, B.C., and PETER, M.E. (2003). The TNF receptor 1: a split personality complex. Cell **114,** 148–150.

BEATTIE, E.C., STELLWAGEN, D., MORISHITA, W., et al. (2002). Control of synaptic strength by glial TNFalpha. Science **295,** 2282–2285.

BENVENISTE, E.N. (1992). Inflammatory cytokines within the central nervous system: sources, function, and mechanism of action. Am. J. Physiol. **263,** C1–C16.

BENVENISTE, E.N. (1998). Cytokine actions in the central nervous system. Cytokine Growth Factor Rev. **9,** 259–275.

BLATTEIS, C.M. (1990). Neuromodulative actions of cytokines. Yale J. Biol. Med. **63,** 133–146.

BORGENS, R.B. (2001). Cellular engineering: molecular repair of membranes to rescue cells of the damaged nervous system. Neurosurgery **49,** 370–378.

CAMPBELL, I.L., STALDER, A.K., CHIANG, C.S., et al. (1997). Transgenic models to assess the pathogenic actions of cytokines in the central nervous system. Mol. Psychiatry **2,** 125–129.

CATANZARO, A., RYAN, A., BATCHER, S., and WASSERMAN, S.I. (1991). The response to human rIL-1, rIL-2, and rTNF in the middle ear of guinea pigs. Laryngoscope **101,** 271–275.

COVEY, W.C., IGNATOWSKI, T.A., KNIGHT, P.R., and SPENGLER, R.N. (2000). Brain-derived TNFalpha: involvement in neuroplastic changes implicated in the conscious perception of persistent pain. Brain Res. **859,** 113–122.

DANTZER, R. (2001). Cytokine-induced sickness behavior: mechanisms and implications. Ann. N. Y. Acad. Sci. **933,** 222–234.

DE LANGE, E.C., and DANHOF, M. (2002). Considerations in the use of cerebrospinal fluid pharmacokinetics to predict brain target concentrations in the clinical setting: implications of the barriers between blood and brain. Clin. Pharmacokinet. **41,** 691–703.

DELEO, J.A., RUTKOWSKI, M.D., STALDER, A.K., and CAMPBELL, I.L. (2000). Transgenic expression of TNF by astrocytes increases mechanical allodynia in a mouse neuropathy model. Neuroreport **11,** 599–602.

DIEM, R., MEYER, R., WEISHAUPT, J.H., and BAHR, M. (2001). Reduction of potassium currents and phosphatidylinositol 3-kinase-dependent AKT phosphorylation by tumor necrosis factor–α rescues axotomized retinal ganglion cells from retrograde cell death *in vivo*. J. Neurosci. **21,** 2058–2066.

DU, S., RUBIN, A., KLEPPER, S., et al. (1999). Calcium influx and activation of calpain I mediate acute reactive gliosis in injured spinal cord. Exp. Neurol. **157,** 96–105.

ELLISON, V.J., MOCATTA, T.J., WINTERBOURN, C.C., DARLOW, B.A., VOLPE, J.J., and INDER, T.E. (2005). The relationship of CSF and plasma cytokine levels to cerebral white matter injury in the premature newborn. Pediatr. Res. **57,** 282–286.

ENG, L.F., GHIRNIKAR, R.S., and LEE, Y.L. (2000). Glial fibrillary acidic protein (GFAP)—thirty-one years (1969–2000). Neurochem. Res. **25,** 1439–1451.

FAN, L., YOUNG, P.R., BARONE, F.C., FEUERSTEIN, G.Z., SMITH, D.H., and McINTOSH, T.K. (1996). Experimental brain injury induces differential expression of tumor necrosis factor–alpha mRNA in the CNS. Brain Res. Mol. Brain Res. **36,** 287–291.

FEUERSTEIN, G.Z., LIU, T., and BARONE, F.C. (1994). Cytokines, inflammation, and brain injury: role of tumor necrosis factor–alpha. Cerebrovasc. Brain Metab Rev. **6,** 341–360.

FISCHER, B.M., ROCHELLE, L.G., VOYNOW, J.A., AKLEY, N.J., and ADLER, K.B. (1999). Tumor necrosis factor–alpha stimulates mucin secretion and cyclic GMP production by guinea pig tracheal epithelial cells *in vitro*. Am. J. Respir. Cell Mol. Biol. **20,** 413–422.

GLABINSKI, A., MIRECKA, M., and POKOCA, L. (1995). Tumor necrosis factor–alpha but not lymphotoxin is overproduced by blood mononuclear cells in multiple sclerosis. Acta Neurol. Scand. **91,** 276–279.

GOETZ, F.W., PLANAS, J.V., and MACKENZIE, S. (2004). Tumor necrosis factors. Dev. Comp. Immunol. **28,** 487–497.

GOODMAN, J.C., ROBERTSON, C.S., GROSSMAN, R.G., and NARAYAN, R.K. (1990). Elevation of tumor necrosis factor in head injury. J. Neuroimmunol. **30,** 213–217.

GRUBER, A., ROSSLER, K., GRANINGER, W., DONNER, A., ILLIEVICH, M.U., and CZECH, T. (2000). Ventricular cerebrospinal fluid and serum concentrations of sTNFR-I, IL-1ra, and IL-6 after aneurysmal subarachnoid hemorrhage. J. Neurosurg. Anesthesiol. **12,** 297–306.

GUEZ, M., HILDINGSSON, C., ROSENGREN, L., KARLSSON, K., and TOOLANEN, G. (2003). Nervous tissue damage markers in cerebrospinal fluid after cervical spine injuries and whiplash trauma. J. Neurotrauma **20,** 853–858.

GUTIERREZ, E.G., BANKS, W.A., and KASTIN, A.J. (1993). Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. J. Neuroimmunol. **47,** 169–176.

HAGHIGHI, S., ANDERSEN, O., ODEN, A., and ROSENGREN, L. (2004). Cerebrospinal fluid markers in MS patients and their healthy siblings. Acta Neurol. Scand. **109,** 97–99.

HAYAKATA, T., SHIOZAKI, T., TASAKI, O., et al. (2004). Changes in CSF S100B and cytokine concentrations in early-phase severe traumatic brain injury. Shock **22,** 102–107.

HAYES, K.C., HULL, T.C., DELANEY, G.A., et al. (2002). Elevated serum titers of proinflammatory cytokines and CNS

autoantibodies in patients with chronic spinal cord injury. J. Neurotrauma **19,** 753–761.

HERMANN, G.E., HOLMES, G.M., and ROGERS, R.C. (2005). TNF(alpha) modulation of visceral and spinal sensory processing. Curr. Pharm. Des **11,** 1391–1409.

HISAHARA, S., SHOJI, S., OKANO, H., and MIURA, M. (1997). ICE/CED-3 family executes oligodendrocyte apoptosis by tumor necrosis factor. J. Neurochem. **69,** 10–20.

HOCKING, D.C., PHILLIPS, P.G., FERRO, T.J., and JOHNSON, A. (1990). Mechanisms of pulmonary edema induced by tumor necrosis factor-alpha. Circ. Res. **67,** 68–77.

HOLMIN, S., and HOJEBERG, B. (2004). *In situ* detection of intracerebral cytokine expression after human brain contusion. Neurosci. Lett. **369,** 108–114.

HOLMIN, S., and MATHIESEN, T. (1999). Long-term intracerebral inflammatory response after experimental focal brain injury in rat. Neuroreport **10,** 1889–1891.

HOLMIN, S., SCHALLING, M., HOJEBERG, B., NORDQVIST, A.C., SKEFTRUNA, A.K., and MATHIESEN, T. (1997). Delayed cytokine expression in rat brain following experimental contusion. J. Neurosurg. **86,** 493–504.

HOLTMANN, M.H., and NEURATH, M.F. (2004). Differential TNF-signaling in chronic inflammatory disorders. Curr. Mol. Med. **4,** 439–444.

HOPKINS, S.J., and ROTHWELL, N.J. (1995). Cytokines and the nervous system. I: Expression and recognition. Trends Neurosci. **18,** 83–88.

HOUZEN, H., KIKUCHI, S., KANNO, M., SHINPO, K., and TASHIRO, K. (1997). Tumor necrosis factor enhancement of transient outward potassium currents in cultured rat cortical neurons. J. Neurosci. Res. **50,** 990–999.

HRIBAR, M., BLOC, A., VAN DER GOOT, F.G., et al. (1999). The lectin-like domain of tumor necrosis factor-alpha increases membrane conductance in microvascular endothelial cells and peritoneal macrophages. Eur. J. Immunol. **29,** 3105–3111.

ILSCHNER, S., OHLEMEYER, C., GIMPL, G., and KETTENMANN, H. (1995). Modulation of potassium currents in cultured murine microglial cells by receptor activation and intracellular pathways. Neuroscience **66,** 983–1000.

KAGAN, B.L., BALDWIN, R.L., MUNOZ, D., and WISNIESKI, B.J. (1992). Formation of ion-permeable channels by tumor necrosis factor-alpha. Science **255,** 1427–1430.

KAPOOR, R., DAVIES, M., and SMITH, K.J. (1999). Temporary axonal conduction block and axonal loss in inflammatory neurological disease. A potential role for nitric oxide? Ann. N. Y. Acad. Sci. **893,** 304–308.

KNOBLACH, S.M., FAN, L., and FADEN, A.I. (1999). Early neuronal expression of tumor necrosis factor-alpha after experimental brain injury contributes to neurological impairment. J. Neuroimmunol. **95,** 115–125.

KOLLER, H., ALLERT, N., OEL, D., STOLL, G., and SIEBLER, M. (1998). TNF alpha induces a protein kinase C-dependent reduction in astroglial K+ conductance. Neuroreport **9,** 1375–1378.

KOLLIAS, G. (2005). TNF pathophysiology in murine models of chronic inflammation and autoimmunity. Semin. Arthritis Rheum. **34,** 3–6.

KOLLIAS, G., DOUNI, E., KASSIOTIS, G., and KONTOYIANNIS, D. (1999). The function of tumour necrosis factor and receptors in models of multi-organ inflammation, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease. Ann. Rheum. Dis. **58,** I32–I39.

KRISTIAN, T., and SIESJO, B.K. (1998). Calcium in ischemic cell death. Stroke **29,** 705–718.

KRUEGER, J.M., FANG, J., TAISHI, P., CHEN, Z., KUSHIKATA, T., and GARDI, J. (1998). Sleep. A physiologic role for IL-1 beta and TNF-alpha. Ann. N. Y. Acad. Sci. **856,** 148–159.

LEE, Y.B., YUNE, T.Y., BAIK, S.Y., et al. (2000). Role of tumor necrosis factor-alpha in neuronal and glial apoptosis after spinal cord injury. Exp. Neurol. **166,** 190–195.

LEEM, J.G., and BOVE, G.M. (2002). Mid-axonal tumor necrosis factor-alpha induces ectopic activity in a subset of slowly conducting cutaneous and deep afferent neurons. J. Pain **3,** 45–49.

LENZLINGER, P.M., MORGANTI-KOSSMANN, M.C., LAURER, H.L., and McINTOSH, T.K. (2001). The duality of the inflammatory response to traumatic brain injury. Mol. Neurobiol. **24,** 169–181.

LI, S., JIANG, Q., and STYS, P. K. (2000). Important role of reverse Na^{+}-Ca^{2+} exchange in spinal cord white matter injury at physiological temperature. J. Neurophysiol. **84,** 1116–1119.

LIU, J., MARINO, M.W., WONG, G., et al. (1998). TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination. Nat. Med. **4,** 78–83.

LIU, X.H., XU, H., and BARKS, J.D. (1999). Tumor necrosis factor-a attenuates N-methyl-D-aspartate-mediated neurotoxicity in neonatal rat hippocampus. Brain Res. **851,** 94–104.

MACEWAN, D.J. (2002). TNF receptor subtype signalling: differences and cellular consequences. Cell Signal. **14,** 477–492.

MALLICK, A.A., ISHIZAKA, A., STEPHENS, K.E., HATHERILL, J.R., TAZELAAR, H.D., and RAFFIN, T.A. (1989). Multiple organ damage caused by tumor necrosis factor and prevented by prior neutrophil depletion. Chest **95,** 1114–1120.

MATUSEVICIUS, D., NAVIKAS, V., SODERSTROM, M., et al. (1996). Multiple sclerosis: the proinflammatory cytokines lymphotoxin-alpha and tumour necrosis factor-alpha are upregulated in cerebrospinal fluid mononuclear cells. J. Neuroimmunol. **66,** 115–123.

McLARNON, J.G., FRANCIOSI, S., WANG, X., BAE, J.H., CHOI, H.B., and KIM, S.U. (2001). Acute actions of tumor necrosis factor-alpha on intracellular Ca(2+) and K(+) currents in human microglia. Neuroscience **104,** 1175–1184.

McLARNON, J.G., MICHIKAWA, M., and KIM, S.U. (1993). Effects of tumor necrosis factor on inward potassium current and cell morphology in cultured human oligodendrocytes. Glia **9,** 120–126.

MENET, V., PRIETO, M., PRIVAT, A., and RIBOTTA, M. (2003). Axonal plasticity and functional recovery after spinal cord injury in mice deficient in both glial fibrillary acidic protein and vimentin genes. Proc. Natl. Acad. Sci. U.S.A. **100,** 8999–9004.

NIETSCH, H.H., ROE, M.W., FIEKERS, J.F., MOORE, A.L., and LIDOFSKY, S.D. (2000). Activation of potassium and chloride channels by tumor necrosis factor alpha. Role in liver cell death. J. Biol. Chem. **275,** 20556–20561.

OH, T.H., MARKELONIS, G.J., VON VISGER, J.R., BAIK, B., and SHIPLEY, M.T. (1995). Acidic pH rapidly increases immunoreactivity of glial fibrillary acidic protein in cultured astrocytes. Glia **13,** 319–322.

OTT, L., McCLAIN, C.J., GILLESPIE, M., and YOUNG, B. (1994). Cytokines and metabolic dysfunction after severe head injury. J. Neurotrauma **11,** 447–472.

PAN, W., BANKS, W.A., and KASTIN, A.J. (1997a). Blood–brain barrier permeability to ebiratide and TNF in acute spinal cord injury. Exp. Neurol. **146,** 367–373.

PAN, W., BANKS, W.A., KENNEDY, M.K., GUTIERREZ, E.G., and KASTIN, A.J. (1996). Differential permeability of the BBB in acute EAE: enhanced transport of TNT-alpha. Am. J. Physiol **271,** E636–E642.

PAN, W., CORNELISSEN, G., HALBERG, F., and KASTIN, A.J. (2002). Selected contribution: circadian rhythm of tumor necrosis factor-alpha uptake into mouse spinal cord. J. Appl. Physiol **92,** 1357–1362.

PAN, W., and KASTIN, A.J. (2001a). Increase in TNFalpha transport after SCI is specific for time, region, and type of lesion. Exp. Neurol. **170,** 357–363.

PAN, W., and KASTIN, A.J. (2001b). Upregulation of the transport system for TNFalpha at the blood-brain barrier. Arch. Physiol Biochem. **109,** 350–353.

PAN, W., and KASTIN, A.J. (2002). TNFalpha transport across the blood-brain barrier is abolished in receptor knockout mice. Exp. Neurol. **174,** 193–200.

PAN, W., KASTIN, A.J., BELL, R.L., and OLSON, R.D. (1999). Upregulation of tumor necrosis factor alpha transport across the blood-brain barrier after acute compressive spinal cord injury. J. Neurosci. **19,** 3649–3655.

PAN, W., ZADINA, J.E., HARLAN, R.E., WEBER, J.T., BANKS, W.A., and KASTIN, A.J. (1997b). Tumor necrosis factor-alpha: a neuromodulator in the CNS. Neurosci. Biobehav. Rev. **21,** 603–613.

PAN, W., ZHANG, L., LIAO, J., CSERNUS, B., and KASTIN, A.J. (2003). Selective increase in TNF-alpha permeation across the blood-spinal cord barrier after SCI. J. Neuroimmunol. **134,** 111–117.

PASCHEN, W. (1999). Calcium neurotoxicity. J. Neurochem. **72,** 2625–2626.

PASCHEN, W. (2000). Role of calcium in neuronal cell injury: which subcellular compartment is involved? Brain Res. Bull. **53,** 409–413.

PASCHEN, W. (2003). Mechanisms of neuronal cell death: diverse roles of calcium in the various subcellular compartments. Cell Calcium **34,** 305–310.

PENNINGS, H.J., KRAMER, K., BAST, A., BUURMAN, W.A., and WOUTERS, E.F. (1998). Tumour necrosis factor–alpha induces hyperreactivity in tracheal smooth muscle of the guinea-pig *in vitro*. Eur. Respir. J. **12,** 45–49.

PICKERING, M., CUMISKEY, D., and O'CONNOR, J.J. (2005). Actions of TNF-α on glutamatergic synaptic transmission in the central nervous sysyem. Exp. Physiol. **90,** 663–670.

REDFORD, E.J., KAPOOR, R., and SMITH, K.J. (1997). Nitric oxide donors reversibly block axonal conduction: demyelinated axons are especially susceptible. Brain **120,** 2149–2157.

REECE, T.B., OKONKWO, D.O., ELLMAN, P.I., et al. (2004). The evolution of ischemic spinal cord injury in function, cytoarchitecture, and inflammation and the effects of adenosine A2A receptor activation. J. Thorac. Cardiovasc. Surg. **128,** 925–932.

REGNER, A., ALVES, L.B., CHEMALE, I., et al. (2001). Neurochemical characterization of traumatic brain injury in humans. J. Neurotrauma **18,** 783–792.

ROSS, S.A., HALLIDAY, M.I., CAMPBELL, G.C., BYRNES, D.P., and ROWLANDS, B.J. (1994). The presence of tumour necrosis factor in CSF and plasma after severe head injury. Br. J. Neurosurg. **8,** 419–425.

ROTHWELL, N.J., and HOPKINS, S.J. (1995). Cytokines and the nervous system II: Actions and mechanisms of action. Trends Neurosci. **18,** 130–136.

SAWADA, M., HARA, N., and MAENO, T. (1990). Extracellular tumor necrosis factor induces a decreased K^+ conductance in an identified neuron of Aplysia kurodai. Neurosci. Lett. **115,** 219–225.

SAWADA, M., HARA, N., and MAENO, T. (1991a). Analysis of a decreased Na+ conductance by tumor necrosis factor in identified neurons of Aplysia kurodai. J. Neurosci. Res. **28,** 466–473.

SAWADA, M., HARA, N., and MAENO, T. (1991b). Tumor necrosis factor reduces the ACh-induced outward current in identified Aplysia neurons. Neurosci. Lett. **131,** 217–220.

SCHMIDT, O.I., MORGANTI-KOSSMANN, M.C., HEYDE, C.E., et al. (2004). Tumor necrosis factor-mediated inhibi-

tion of interleukin-18 in the brain: a clinical and experimental study in head-injured patients and in a murine model of closed head injury. J. Neuroinflammation **1,** 13.

SELMAJ, K., and RAINE, C.S. (1988a). Tumor necrosis factor mediates myelin damage in organotypic cultures of nervous tissue. Ann. N. Y. Acad. Sci. **540,** 568–570.

SELMAJ, K.W., and RAINE, C.S. (1988b). Tumor necrosis factor mediates myelin and oligodendrocyte damage *in vitro*. Ann. Neurol. **23,** 339–346.

SHEN, D.D., ARTRU, A.A., and ADKISON, K.K. (2004). Principles and applicability of CSF sampling for the assessment of CNS drug delivery and pharmacodynamics. Adv. Drug Deliv. Rev. **56,** 1825–1857.

SHI, R., and BLIGHT, A.R. (1996). Compression injury of mammalian spinal cord *in vitro* and the dynamics of action potential conduction failure. J. Neurophysiol. **76,** 1572–1580.

SHI, R., and BLIGHT, A.R. (1997). Differential effects of low and high concentrations of 4-aminopyridine on axonal conduction in normal and injured spinal cord. Neuroscience **77,** 553–562.

SHI, R., and BORGENS, R.B. (1999). Acute repair of crushed guinea pig spinal cord by polyethylene glycol. J. Neurophysiol. **81,** 2406–2414.

SHI, R., LUO, J., and PEASLEY, M. (2002). Acrolein inflicts axonal membrane disruption and conduction loss in isolated guinea-pig spinal cord. Neuroscience **115,** 337–340.

SHIBATA, M., and BLATTEIS, C.M. (1991a). Differential effects of cytokines on thermosensitive neurons in guinea pig preoptic area slices. Am. J. Physiol **261,** R1096–R1103.

SHIBATA, M., and BLATTEIS, C.M. (1991b). Human recombinant tumor necrosis factor and interferon affect the activity of neurons in the organum vasculosum laminae terminalis. Brain Res. **562,** 323–326.

SHINPO, K., KIKUCHI, S., MORIWAKA, F., and TASHIRO, K. (1999). Protective effects of the TNF-ceramide pathway against glutamate neurotoxicity on cultured mesencephalic neurons. Brain Res. **819,** 170–173.

SHOHAMI, E., GINIS, I., and HALLENBECK, J.M. (1999). Dual role of tumor necrosis factor alpha in brain injury. Cytokine Growth Factor Rev. **10,** 119–130.

SHRAGER, P., CUSTER, A.W., KAZARINOVA, K., RASBAND, M.N., and MATTSON, D. (1998). Nerve conduction block by nitric oxide that is mediated by the axonal environment. J. Neurophysiol. **79,** 529–536.

SIPE, K.J., SRISAWASDI, D., DANTZER, R., KELLEY, K.W., and WEYHENMEYER, J.A. (1996). An endogenous 55-kDa TNF receptor mediates cell death in a neural cell line. Brain Res. Mol. Brain Res. **38,** 222–232.

SOLIVEN, B., and ALBERT, J. (1992). Tumor necrosis factor modulates Ca2+ currents in cultured sympathetic neurons. J. Neurosci. **12,** 2665–2671.

SOLIVEN, B., SZUCHET, S., and NELSON, D.J. (1991). Tumor necrosis factor inhibits K+ current expression in cultured oligodendrocytes. J. Membr. Biol. **124,** 127–137.

SORKIN, L.S., XIAO, W.H., WAGNER, R., and MYERS, R.R. (1997). Tumour necrosis factor–alpha induces ectopic activity in nociceptive primary afferent fibres. Neuroscience **81,** 255–262.

SREDNI-KENIGSBUCH, D. (2002). TH1/TH2 cytokines in the central nervous system. Int. J. Neurosci. **112,** 665–703.

STEPHENS, K.E., ISHIZAKA, A., LARRICK, J.W., and RAFFIN, T.A. (1988). Tumor necrosis factor causes increased pulmonary permeability and edema. Comparison to septic acute lung injury. Am. Rev. Respir. Dis. **137,** 1364–1370.

STYS, P.K., WAXMAN, S.G., and RANSOM, B.R. (1992). Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na+ channels and Na^{+}-Ca^{2+} exchanger. J. Neurosci. **12,** 430–439.

SUN, D., NEWMAN, T.A., PERRY, V.H., and WELLER, R.O. (2004). Cytokine-induced enhancement of autoimmune inflammation in the brain and spinal cord: implications for multiple sclerosis. Neuropathol. Appl. Neurobiol. **30,** 374–384.

TALLEY, A.K., DEWHURST, S., PERRY, S.W., et al. (1995). Tumor necrosis factor alpha-induced apoptosis in human neuronal cells: protection by the antioxidant N-acetylcysteine and the genes bcl-2 and crmA. Mol. Cell Biol. **15,** 2359–2366.

TANCREDI, V., D'ARCANGELO, G., GRASSI, F., et al. (1992). Tumor necrosis factor alters synaptic transmission in rat hippocampal slices. Neurosci. Lett. **146,** 176–178.

TAUPIN, V., RENNO, T., BOURBONNIERE, L., PETERSON, A.C., RODRIGUEZ, M., and OWENS, T. (1997). Increased severity of experimental autoimmune encephalomyelitis, chronic macrophage/microglial reactivity, and demyelination in transgenic mice producing tumor necrosis factor-alpha in the central nervous system. Eur. J. Immunol. **27,** 905–913.

TONELLI, L.H., and POSTOLACHE, T.T. (2005). Tumor necrosis factor alpha, interleukin-1 beta, interleukin-6 and major histocompatibility complex molecules in the normal brain and after peripheral immune challenge. Neurol. Res. **27,** 679–684.

VAN DER GOOT, F.G., PUGIN, J., HRIBAR, M., et al. (1999). Membrane interaction of TNF is not sufficient to trigger increase in membrane conductance in mammalian cells. FEBS Lett. **460,** 107–111.

VITARBO, E.A., CHATZIPANTELI, K., KINOSHITA, K., TRUETTNER, J.S., ALONSO, O.F., and DIETRICH, W.D. (2004). Tumor necrosis factor alpha expression and protein levels after fluid percussion injury in rats: the effect of injury severity and brain temperature. Neurosurgery **55,** 416–424.

VITKOVIC, L., BOCKAERT, J., and JACQUE, C. (2000). "Inflammatory" cytokines: neuromodulators in normal brain? J. Neurochem. **74,** 457–471.

VIVIANI, B., BARTESAGHI, S., CORSINI, E., GALLI, C.L., and MARINOVICH, M. (2004). Cytokines role in neurodegenerative events. Toxicol. Lett. **149,** 85–89.

WANG, C.X., REECE, C., WRATHALL, J.R., SHUAIB, A., OLSCHOWKA, J.A., and HAO, C. (2002). Expression of tumor necrosis factor–alpha and its mRNA in the spinal cord following a weight-drop injury. Neuroreport **13,** 1391–1393.

WANG, C.X., and SHUAIB, A. (2002). Involvement of inflammatory cytokines in central nervous system injury. Prog. Neurobiol. **67,** 161–172.

WAXMAN, S.G. (2001). Acquired channelopathies in nerve injury and MS. Neurology **56,** 1621–1627.

WOODROOFE, M.N. (1995). Cytokine production in the central nervous system. Neurology **45,** S6–10.

YANG, L., BLUMBERGS, P.C., JONES, N.R., MANAVIS, J., SARVESTANI, G.T., and GHABRIEL, M.N. (2004). Early expression and cellular localization of proinflammatory cytokines interleukin-1_{beta}, interleukin-6, and tumor necrosis factor–alpha in human traumatic spinal cord injury. Spine **29,** 966–971.

YIN, L., OHTAKI, H., NAKAMACHI, T., et al. (2003). Expression of tumor necrosis factor–alpha (TNFα) following transient cerebral ischemia. Acta Neurochir. Suppl. **86,** 93–96.

YUNE, T.Y., CHANG, M.J., KIM, S.J., et al. (2003). Increased production of tumor necrosis factor-alpha induces apoptosis after traumatic spinal cord injury in rats. J. Neurotrauma **20,** 207–219.

ZAREMBA, J., SKROBANSKI, P., and LOSY, J. (2001). Tumour necrosis factor–alpha is increased in the cerebrospinal fluid and serum of ischaemic stroke patients and correlates with the volume of evolving brain infarct. Biomed. Pharmacother. **55,** 258–263.

Address reprint requests to:
Keith C. Hayes, Ph.D.
Lawson Health Research Institute
801 Commissioners Road East
London, Ontario, Canada, N6C 5J1

E-mail: kchayes@uwo.ca