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# IMMUNOLOGICALLY INDUCED ELECTROPHYSIOLOGICAL DYSFUNCTION: IMPLICATIONS FOR INFLAMMATORY DISEASES OF THE CNS AND PNS

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**Abstract**—During inflammation of the central or peripheral nervous system, a high number of immunologically active molecules, including bacterial or viral products as well as host-derived cytokines, are released. Patients suffering from inflammatory CNS or PNS diseases often develop transient symptoms with a rapid recovery, which obviously cannot be accounted for by immunologically induced tissue damage. These observations led to the hypothesis that immunologically active molecules can affect directly the electrophysiological functions of neurons and glial cells. Evidence for this hypothesis came from *in vitro* studies showing that cytokines, such as interleukins or tumor necrosis factors, arachidonic acid and its metabolites, interfere with electrophysiological properties of neurons or glial cells. These molecules affect ion currents, intracellular $Ca^{2+}$ homeostasis, membrane potentials, and suppress or enhance the induction and maintenance of long-term potentiation. Similarly, virus proteins from human immunodeficiency virus type 1 were found to alter intracellular $Ca^{2+}$ concentrations of neurons and astrocytes by modulating either transmitter receptors and channels or membrane transporters. Cerebrospinal fluid from MS patients contains factors which increase $Na^+$ current inactivation and thereby reduce neuronal excitability. Immunoglobulins in sera of patients suffering from multifocal motor neuropathy and from acquired neuromyotonia interfere with nerve fibers, inducing alterations of conduction. Increased knowledge of these mechanisms will help to explain the pathogenesis of neurological symptoms and may provide a rationale for new therapeutic strategies. 

## CONTENTS



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## ABBREVIATIONS

| | | | |
|---|---|---|---|
| AIDS | Acquired immunodeficiency syndrome | IFN | Interferon |
| ALS | Amyotrophic lateral sclerosis | IL | Interleukin |
| 4-AP | 4-Aminopyridine | IPSP | Inhibitory postsynaptic potential |
| APV | D-2-Amino-5-phosphonovalerate | LPS | Lipopolysaccharides |
| CNS | Central nervous system | LT | Leukotriene |
| CNQX | 6-Cyano-7-nitroquinoxaline-2,3-dione | LTP | Long-term potentiation |
| CSF | Cerebrospinal fluid | M-CSF | Macrophage colony-stimulating factor |
| CTRL | Control | MFS | Miller-Fisher syndrome |
| DNQX | 6,7-Dinitroquinoxaline-,2,3-dione | MEPP | Miniature endplate potentials |
| EAE | Experimental autoimmune encephalomyelitis | MHC | Major histocompatibility complex |
| EEG | Electroencephalography | MMN | Multifocal motor neuropathy |
| EPSP | Excitatory postsynaptic potential | MRI | Magnetic resonance imaging |
| FCS | Fetal calf serum | MS | Multiple sclerosis |
| GABA | $\gamma$-Amino-butyric acid | NDGA | Nordihydroguaiaretic acid |
| GBS | Guillain-Barré syndrome | NMDA | *N*-methyl-D-aspartate |
| GFAP | Glial fibrillary acid protein | PAF | Platelet activating factor |
| Glu | Glutamate | PG | Prostaglandin |
| GluR | Glutamate receptor | PNS | Peripheral nervous system |
| GM-CSF | Granulocyte–macrophage colony-stimulating factor | TGF | Transforming growth factor |
| gp | Glycoprotein | TEA | Tetraethylammonium |
| HIV | Human immunodeficiency virus | TNF | Tumor necrosis factor |
| HIVE | HIV-associated encephalopathy | TTX | Tetrodotoxin |
| $I_{Ca}$ | $Ca^{2+}$ current | VEP | Visual evoked potential |
| $I_K$ | $K^+$ current | VRR | Ventral root response |
| $I_{Na}$ | $Na^+$ current | WM | Waldenström's macroglobulinemia |

## 1. INTRODUCTION

In inflammatory diseases of the central or peripheral nervous system (CNS, PNS), immunologically active substances are released into the cerebrospinal fluid (CSF) or in the surroundings of inflammatory lesions. These substances include bacterial- or virus-derived molecules like lipopolysaccharides (Raetz, 1990) or viral glycoproteins (Dubois-Dalcq *et al.*, 1995). In response to infection, or due to autoimmunological processes, immunocompetent cells of the host also release immunologically active molecules, cytokines including interleukins, chemokines, tumor necrosis factors, interferons, immunoglobulins and others (Hopkins and Rothwell, 1995). These immune mediators either are secreted within the CNS by cells invading from the blood stream, like blood monocytes or lymphocytes, or are secreted by local immunocompetent cells, like microglia and astrocytes. Additionally, some cytokines are transported across the blood–brain barrier by saturable active transport processes (Banks *et al.*, 1991; Gutierrez *et al.*, 1993; Luheshi *et al.*, 1994).

The relevance of these immune mediators has been elucidated in the last few years and has been summarized under various aspects in recently published reviews:

- Immune mediators are part of the immunological cascade coordinating host defence mechanisms, activating immunocompetent cells and contribute to demyelination (Compston *et al.*, 1991; Martin *et al.*, 1992; Hartung *et al.*, 1992; Hartung, 1993, 1995; Rothwell and Hopkins, 1995).
- Cytokines induce fever (LeMay *et al.*, 1990; Kluger, 1991), are putatively involved in sleep regulation [tumor necrosis factor$\alpha$ (TNF$\alpha$), Takahashi *et al.*, 1995], and have somnogenic effects (Krueger *et al.*, 1984; Shoham *et al.*, 1987; Opp *et al.*, 1992; Kapás *et al.*, 1994).

- Cytokines induce alterations in the neuroendocrine system, e.g. by activating the hypothalamic–pituitary–adrenal axis (Plata-Salamán, 1991; Rothwell and Hopkins, 1995) and by modulation of neuronal activity in limbic areas (Haas and Schauenstein, 1997).
- Cytokines affect the behaviour of animals, especially food intake and social exploration (Solomon, 1987; De Sarro *et al.*, 1990; Plata-Salamán, 1991; Kent *et al.*, 1992).
- During development and during repair mechanisms after injury, cytokines regulate neuronal gene expression and cell proliferation and are supposed to influence synaptic plasticity (Mehler *et al.*, 1993; Patterson and Nawa, 1993).
- In disease states, immune mediators also contribute to acute and chronic neurodegeneration (Rothwell and Relton, 1993; Mrak *et al.*, 1995).

The aim of the present review is to describe the current concepts, how bacterial toxins, virus proteins and host-derived immune mediators affect the electrophysiological function of CNS cells: neurons, astrocytes, microglia, and oligodendrocytes. These immune mediators include cytokines, like interleukins and tumor necrosis factors, as well as arachidonic acid and its metabolites, leukotrienes and prostaglandins. The rationale for these investigations arose from clinical observations, which showed that patients suffering from inflammatory diseases of the CNS often develop neurological symptoms, like psychomotor slowing, impairment of consciousness or seizures. In many cases, these symptoms (i) may only persist for a short period and diminish rapidly upon antimicrobial or anti-inflammatory therapy; (ii) occur in patients without detectable intracerebral lesions on magnetic resonance imaging (MRI), which could explain the dysfunction; but (iii) may be correlated to titers of bacterial endotoxins or cytokines in the cerebrospinal fluid (Dwelle *et al.*, 1987; Arditi *et al.*, 1989, 1990). Functional investigations, such as electroencephalography (EEG) or evoked potentials, may be effective in revealing cerebral dysfunction (Chequer *et al.*, 1992; Anderson, 1993). Therefore, the hypothesis was established that diffusible factors may be released, which interfere with the function of the CNS or PNS. We describe the present state of experimental studies investigating the interaction between immune mediators and the electrophysiological function of single cells or neuronal circuits.

In the second part of this review, we describe the present knowledge and hypotheses on how injurious molecules of bacterial or viral origin or those derived from host defence mechanisms (cytokines, immunoglobulins) may impair the function of CNS cells or nerve fibers in various CNS and PNS diseases, such as multiple sclerosis (MS), AIDS-associated encephalopathy, meningitis and encephalitis, and inflammatory diseases of peripheral nerves. We do not discuss the autoimmunological diseases of the neuromuscular junction, myasthenia gravis (Engel *et al.*, 1993; Drachman, 1994) and Lambert Eaton myasthenic syndrome (Sher *et al.*, 1993), which are well-known examples for immunologically induced dysfunction in diseases, excellently reviewed elsewhere (Vincent *et al.*, 1995).

## 2. IMMUNOLOGICALLY INDUCED ALTERATIONS OF ELECTROPHYSIOLOGICAL FUNCTIONS OF SINGLE CNS CELLS

### 2.1. Effects on Voltage-dependent and Transmitter Receptor-operated Ion Currents of Neurons

#### 2.1.1. *Effects of Identified Immune Mediators: Cytokines*

Only a few reports have been published to date which describe direct effects of immune mediators on ion currents of single neurons.

In acutely isolated hippocampal neurons from guinea pig Plata-Salamán and ffrench-Mullen (1992) observed an IL-1-induced decrease of $Ca^{2+}$ currents ($I_{Ca}$). Within 30 sec after application, a dose-dependent reduction of $I_{Ca}$ appeared, which could be blocked by an IL-1 receptor antagonist (rhIL-Ira). The depression of $Ca^{2+}$ channel current was prevented by pertussis toxin and by inhibitors of protein kinase C, suggesting that the IL-1$\beta$-induced reduction of $Ca^{2+}$ current is mediated by an activation of protein kinase C through a G-protein-coupled IL-1 receptor (Plata-Salamán and ffrench-Mullen, 1994).

IL-2 also reduced $Ca^{2+}$ currents in hippocampal neurons from guinea pig. Plata-Salamán and ffrench-Mullen (1993) described that IL-2 induced a decrease of the peak and late voltage-dependent $Ca^{2+}$ currents.

The effects of IL-1 and IL-2 on the $Na^+$ current of a neuroblastoma × glioma hybrid cell line (NH15-CA2) were tested (Hamm *et al.*, 1996). IL-2 induced a reduction of the $Na^+$ current which was evoked by a leftward shift of the $Na^+$ current inactivation curve by 7.7 mV. IL-2 was effective in about 30% of cells tested and led to a partial block of the $Na^+$ conductance without changing the activation or inactivation kinetics.

Sawada and coworkers reported that TNF$\alpha$ reduced the sodium conductance of aplysia neurons (Sawada *et al.*, 1991a). Immediately after local application of TNF$\alpha$, the membrane potential of identified single aplysia neurons hyperpolarized and the firing rate of the neurons decreased. From ion substitution and pharmacological experiments, these authors concluded that the hyperpolarization of the membrane was caused by a reduction of the $Na^+$ current ($I_{Na}$). The same group reported that TNF$\alpha$ decreased the $K^+$ conductance in another population of aplysia neurons (Sawada *et al.*, 1990). Extracellularly applied IL-1 also immediately induced a hyperpolarization of aplysia neurons, which was associated with a reduced $Na^+$ conductance (Sawada *et al.*, 1991c). The IL-1-induced hyperpolarization was independent of changes of $K^+$, $Ca^{2+}$ and $Cl^-$ concentrations and could not be blocked by the $K^+$ channel blockers tetraethylammoniumchloride (TEA) or 4-aminopyridine (4-AP).

Szucs *et al.* (1992) reported that IL-1 induced a decrease of voltage-activated $Ca^{2+}$ inward currents

and an increase of transient and delayed $K^+$ outward currents in neurons from *Helix pomatia*. In identified neurons in the mollusc *Onchidium*, Mimura *et al.* (1994) observed an inhibitory, hyperpolarizing effect of IL-1α and TNFα. In voltage clamp experiments, an outward current was elicited by IL-1α and TNFα, which was independent of changes in extracellular concentration of $K^+$, $CL^-$ or $Ca^{2+}$. The outward current, however, was sensitive to an alteration of the external $Na^+$ concentration and could be blocked by ouabain, an inhibitor of $Na^+$ transporters. The authors concluded that decreases of $Na^+$ conductance in addition to activation of a $Na^+$-dependent transporter are putative underlying mechanisms.

However, to our knowledge, these results have not been confirmed for mammalian neurons. In our system of cultured cortical neurons from embryonic rats, TNFα neither reduced $Na^+$ or $K^+$ conductances nor induced changes of neuronal membrane potential (Köller *et al.*, 1996a, 1996b).

TNFα and TNFβ also were found to interfere with intracellular $Ca^{2+}$ concentrations. Incubation with TNFα for 12–24 hr increased the peak $Ca^{2+}$ current in cultured rat sympathetic neurons, while TNFα did not affect $Ca^{2+}$ current amplitudes immediately after application (Soliven and Albert, 1992).

Treatment with TNFα and TNFβ led to an increased expression of calbindin in hippocampal, septal and cortical neurons from embryonic rat (Cheng *et al.*, 1994). Pretreatment with both TNFα and TNFβ resulted in an attenuated glucose deprivation-induced increase of intracellular $Ca^{2+}$ and it was concluded that TNFα and TNFβ may have a protective function on neurons against metabolic–excitotoxic insults. TNFα and IFNγ did not affect the membrane potential and the spontaneous action potential firing in rat hippocampal neurons (Neumann *et al.*, 1997). Cultures were incubated with IFNγ or TNFα and electrophysiological properties, assessed by whole cell patch clamp recordings, were unchanged.

The effect of some cytokines on neurotransmitter receptor function and transmitter-induced ion currents have been investigated. Miller *et al.* (1991) reported that IL-1 enhanced the GABA-mediated increase of $Cl^-$ permeability in cultured cortical neurons from mice. Yu and Shinnick-Gallagher (1994) observed an IL-1β-induced hyperpolarization accompanied by a decrease of input resistance in amygdala neurons in slice preparations. The IL-1β-induced hyperpolarization had a reversal potential near the equilibrium potential of $Cl^-$ and could be blocked by the GABA-A receptor antagonist bicuculline, suggesting that it was mediated by enhancing the action of endogenous GABA. While responses to direct application of GABA, glutamate or glutamate agonists were not altered, IL-1β reduced inhibitory and excitatory synaptic potentials. The authors concluded that IL-1β acts at presynaptic sites, depressing synaptic transmission, and hyperpolarizes the neurons.

In cultured cerebellar granule cells, chronically applied IL-6 was found to increase intracellular $Ca^{2+}$ levels (Qiu *et al.*, 1995) and to increase the $Ca^{2+}$ responses to extracellularly applied glutamate (Holliday *et al.*, 1995) and NMDA (Qiu *et al.*, 1995).

In aplysia neurons, IL-2 reduces the GABA-activated $Cl^-$ current (Sawada *et al.*, 1992) and TNFα was reported to diminish the acetylcholine-induced outward current (Sawada *et al.*, 1991b). To our knowledge, these observations on invertebrates have not yet been reproduced on mammalian neurons.

### 2.1.2. *Arachidonic Acid and Its Metabolites*

Arachidonic acid and its metabolites, prostaglandins and leukotrienes, which can be synthesized locally within the CNS, have been suggested to function as second messengers (Piomelli *et al.*, 1987). In aplysia sensory neurons, arachidonic acid induced a slow hyperpolarization of the membrane associated with an increase of membrane conductance. In single channel experiments, Piomelli *et al.* (1987) showed that arachidonic acid increased the probability of $K^+$ channels to open. While an inhibitor of the cyclooxygenase pathway (indomethacin) failed to affect arachidonic acid-induced hyperpolarization, an inhibitor of the lipoxygenase pathway (nordihydroguaiaretic acid, NDGA) suppressed the hyperpolarizing response, indicating that lipoxygenase products may be involved. In cultured rat neocortical neurons, arachidonic acid also augmented $K^+$ currents, recorded in the whole cell mode of the patch clamp technique (Zona *et al.*, 1993). In contrast to the findings in aplysia neurons, the arachidonic acid-induced increase of $K^+$ current was blunted by indomethacin in cortical neurons. Zona and coworkers concluded that cyclooxygenase products may participate in this effect.

Arachidonic acid induced suppression of the L-type $Ca^{2+}$ current in acutely dissociated hippocampal neurons from guinea pigs and in cultured hippocampal neurons from fetal rat (Keyser and Alger, 1990). This effect was blocked by specific inhibitors of protein kinase C [pseudosubstrate 19–36 (PKCI) and H-7], indicating an activation of protein kinase C as underlying mechanism. Free radicals also seemed to contribute to this effect, since superoxide free radical scavengers also blocked the $I_{Ca}$ depression. Lipoxygenase (NDGA) and cyclooxygenase inhibitors (indomethacin) were ineffective. Prostaglandin $E_2$ was noted to increase $Ca^{2+}$ currents, presumably the L-type one, in cultured avian dorsal root ganglia neurons (Nicol *et al.*, 1992). Arachidonic acid itself (in contrast to the findings on hippocampal neurons), as well as other metabolites, prostaglandin $F_{2\alpha}$ and leukotriene $B_4$, had no effect on the amplitude of the $Ca^{2+}$ current.

In cultured cortical neurons from embryonic rat, we found that leukotriene $B_4$ induced a decrease of voltage-dependent $K^+$ outward currents in a subpopulation (about one-third) of neurons (Köller and Siebler, 1993). In contrast to our findings in astrocytes (see below), the membrane potential of neurons was not affected.

Miller *et al.* (1992) reported an arachidonic acid-induced potentiation of NMDA evoked currents in isolated cerebellar granule cells and in acutely isolated hippocampal neurons. This potentiation was

due to an increase in channel open probability. A conversion of arachidonic acid to its lipoxygenase or cyclooxygenase derivatives was not necessary for the potentiation of the NMDA-evoked currents and inhibition of protein kinase C activity was without effect.

The effects of immune mediators on voltage-gated and ligand-operated ion currents are summarized in Fig. 1.

### 2.1.3. *Effects of Virus Proteins*

Human immunodeficiency virus (HIV) type 1 envelope protein gp 120 was documented as elevating intracellular $Ca^{2+}$ concentrations and subsequently inducing neurotoxicity (Dreyer *et al.*, 1990; Lipton, 1991). The intracellular $Ca^{2+}$ increase was mediated by a $Ca^{2+}$ influx into the cell, as well as by a release from internal stores. Neuronal $Ca^{2+}$ overloading, as well as neurotoxicity, could be prevented by $Ca^{2+}$ channel antagonists of the dihydropyridine type (nimodipine and nifedipine, Dreyer *et al.*, 1990), by a diphenylalkylamine piperazine derivative (flunarizine) and by a phenylalkylamine (verapamil, Lipton, 1991). Lipton concluded that gp 120 activates neuronal L-type $Ca^{2+}$ channels, thereby raising intracellular $Ca^{2+}$ and inducing neuronal cell death. Lo *et al.* (1992) observed $Ca^{2+}$ oscillations in cultured hippocampal neurons after exposure to gp 120. The $Ca^{2+}$ oscillations were blocked by a $Ca^{2+}$ channel blocker (nitrendipine), a NMDA antagonist and tetrodotoxin (TTX), which blocks sodium channels. The authors surmised that gp 120 acted as an excitotoxin by augmenting synaptic activity.

Another protein from HIV type 1, tat, also interferes with electrophysiological properties of neurons. Sabatier *et al.* (1991) reported that tat caused depolarization of cockroach interneurons, accompanied by a decrease of membrane resistance. The depolarization could not be blocked by blockers of voltage-dependent $Na^+$ (TTX) or $Ca^{2+}$ channels ($Cd^{2+}$), thereby indicating an opening of unselective ion channels by tat. Intracerebroventricular application of tat in mice resulted in apathy, convulsions and death. In adult human neurons in slices and embryonic human neurons in culture (Magnuson *et al.*, 1995), tat also induced a depolarization, which was insensitive to TTX but could be blocked by the glutamate antagonist kynureate. The specific NMDA receptor antagonist D-2-amino-5-phosphonovalerate (APV) was ineffective, suggesting that the tat-induced depolarization did not mainly involve NMDA receptors. However, APV, as well as MK 801, protected against tat-induced neurotoxicity.

The effects of virus proteins on NMDA receptor function and on neuronal toxicity (Brenneman *et al.*, 1988; Dawson *et al.*, 1993; Savio and Levi, 1993) were examined in several studies. Lipton and coworkers (Lipton *et al.*, 1991; Lipton, 1992a) found that HIV-1 coat protein 120 (gp 120) increased intracellular $Ca^{2+}$

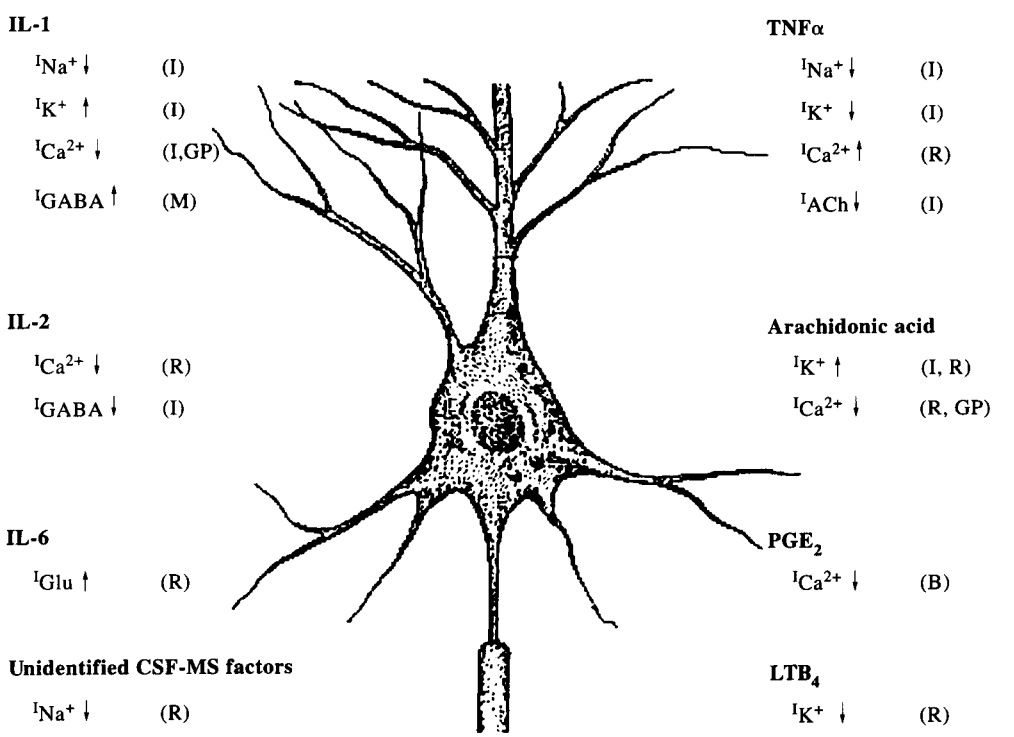


Fig. 1. Effects of immune mediators on voltage-gated and neurotransmitter-operated ion currents of neurons. This diagram summarizes the effects of identified cytokines, arachidonic acid and its metabolites and unidentified factors in the CSF of MS patients on voltage-activated $Na^+$ ($I_{Na^+}$), $K^+$ ($I_{K^+}$) and $Ca^{2+}$ ($I_{Ca^+}$) currents as well as on GABA ($I_{GABA}$) or glutamate ($I_{Glu}$) receptor coupled ion currents. Increases of currents after application of the immune mediators are indicated by ↑, decreases by ↓. The sources of neuronal preparation are given in parentheses: I, invertebrates; GP, guinea pig; M, mouse; R, rat; B, bird. For details of preparation, experimental design and for references, see text.

and acted synergistically with NMDA receptor activation to inflict damage on rat retinal ganglion cells. The gp 120-induced rise in intracellular $Ca^{2+}$, as well as neuronal damage, were prevented by NMDA receptor antagonists and by incubation with glutamate–pyruvate transaminase, which breaks down endogenous glutamate. Under their culture conditions, neither glutamate nor gp 120 on its own were noxious to neurons. The gp 120 did not alter NMDA-evoked ionic responses in voltage clamp recordings and did not induce a similar ion current by itself (Lipton *et al.*, 1991). These results have been confirmed for cultured embryonic human cortical neurons. Lannuzel *et al.* (1995) reported that gp 120 and its precursor, gp 160, did not affect voltage-activated $Na^+$ or $Ca^{2+}$ currents nor NMDA-induced currents, but potentiated NMDA-induced rises of intracellular $Ca^{2+}$. The increase of the NMDA-induced $Ca^{2+}$ response depended on the presence of extracellular $Ca^{2+}$ and was blocked by the NMDA receptor antagonist APV and by the inhibitor of voltage-gated $Ca^{2+}$ channels, nifedipine. In accordance with the conclusions of Lipton *et al.* (1991) and Lannuzel *et al.* (1995) also suggested synergistic effects of NMDA-operated and voltage-dependent $Ca^{2+}$ channels and an additional release of $Ca^{2+}$ from internal stores. They suggested also that neuronal damage was the consequence of the $Ca^{2+}$ increase. Further support came from data of another study. Dawson *et al.* (1993) observed gp 120-mediated neuronal toxicity in fetal rat neurons, which depended on extracellular $Ca^{2+}$ and glutamate, and was reduced by a NMDA receptor antagonist (MK 801), a non-NMDA–glutamate receptor antagonist (DNQX) and an antagonist of voltage-dependent $Ca^{2+}$ channels (nifedipine). From the blockade of neurotoxicity by dantrolene, they inferred also that $Ca^{2+}$ additionally was released from internal stores. The gp 120-induced neuronal damage was mitigated further by inhibitors of the NO synthetase, indicating that NO might contribute to the noxious effects of gp 120. In line with these results are findings that the NMDA receptor antagonist memantine blocked gp 120-induced neurotoxicity in rat cortical cell cultures (Müller *et al.*, 1992) and retinal ganglion cells (Lipton, 1992b). In cultured hippocampal neurons from embryonic rat, gp 120 elicited large rises of intracellular $Ca^{2+}$, partially oscillatory, which could be completely blocked by a NMDA receptor antagonist, a blocker of voltage-dependent $Ca^{2+}$ channels and in addition by TTX, which blocks voltage-dependent $Na^+$ currents and thereby dampens neuronal excitability. Diop *et al.* (1994) also reported that the gp 120-induced neurotoxicity in cortical cultures could be blocked by the $Na^+$ channel blocker TTX, the $Ca^{2+}$ channel blocker nifedipine and the NMDA receptor antagonist MK 801. The authors of both studies came to the conclusion that gp 120 acted via synaptically released glutamate, leading to a secondary activation of NMDA receptors. However, different observations were made in cultured cerebellar granule cells from postnatal rats (Sweetnam *et al.*, 1993). In these cells, gp 120 selectively inhibited radioligand binding to NMDA receptors without activating the receptor-coupled ion channel, but suppressed the NMDA-induced $Ca^{2+}$ current. In this study, gp 120 did not induce any neurotoxicity. In this instance, it turned out instead to be neuroprotective, reducing NMDA-induced neuronal damage.

#### 2.1.4. *Effects of Unidentified Factors in the CSF*

A number of cytokines has been identified in the CSF of patients suffering from an acute relapse of multiple sclerosis, and disease progression has been correlated with the cytokine profile in serum and CSF (Rieckmann *et al.*, 1995; Hartung *et al.*, 1995a). Therefore, attempts have been made to reveal factors in the CSF which impair the electrophysiological functions of neurons. We reported recently that neuronal excitability was reduced due to factors present in the CSF of MS patients (CSF–MS). We used cultured cortical neurons and investigated the activation and inactivation properties of voltage-dependent ion currents of cells bathed in CSF–MS compared to cells in CSF of patients with non-inflammatory CNS diseases (CSF–CTRL, Köller *et al.*, 1996a). We found that membrane potential and membrane resistance were unaffected. The $Na^+$ inward currents, as well as $K^+$ outward currents, could be elicited. In CSF–MS, however, the $Na^+$ current inactivation curve was shifted to more negative potentials compared to cells in CSF–CTRL. The $V_{1/2}$ value of the $Na^+$ current inactivation curve was about 10 mV more negative in CSF–MS (Fig. 2). The shift of the inactivation curve was reversible after wash. Neither IL-2 nor IL-1, IL-6 nor TNFα induced an alteration of $Na^+$ current inactivation (Köller *et al.*, 1996a). The underlying mechanism could be similar to the effects of local anesthetics (Ragsdale *et al.*, 1994), which bind to identified regions of the $Na^+$ channel and thereby directly modulate inactivation of the $Na^+$ current. We suggest that the CSF of MS patients contains factors which engage this binding site. These results are in accordance with observations of Brinkmeier *et al.*, 1993, 1996) on the effect of CSF–MS on the $Na^+$ current of human myoballs and neuron-like cells from a cell line. In contrast to their observation on the muscular $I_{Na}$ (Kaspar *et al.*, 1994; Proebstle *et al.*, 1995), the inactivation shift of neuronal $Na^+$ current could not be mimicked by IL-2.

### 2.2. Effects on Electrophysiological Properties of Astrocytes

#### 2.2.1. *Astrocytes are Immunocompetent Cells*

Astrocytes belong to the immunocompetent cells within the central nervous system. They are able to produce immune mediators such as interleukins (Norris *et al.*, 1994), TNFα, prostaglandins and leukotrienes (Hartung and Toyka, 1987; Hartung *et al.*, 1988). They are also target cells for a large number of injurious molecules that induce reactive astrogliosis (Eddleston and Mucke, 1993). Glial cells participate in immunological activation by immune mediators (Merrill and Benveniste, 1996), bacterial toxins and virus proteins inducing immunological

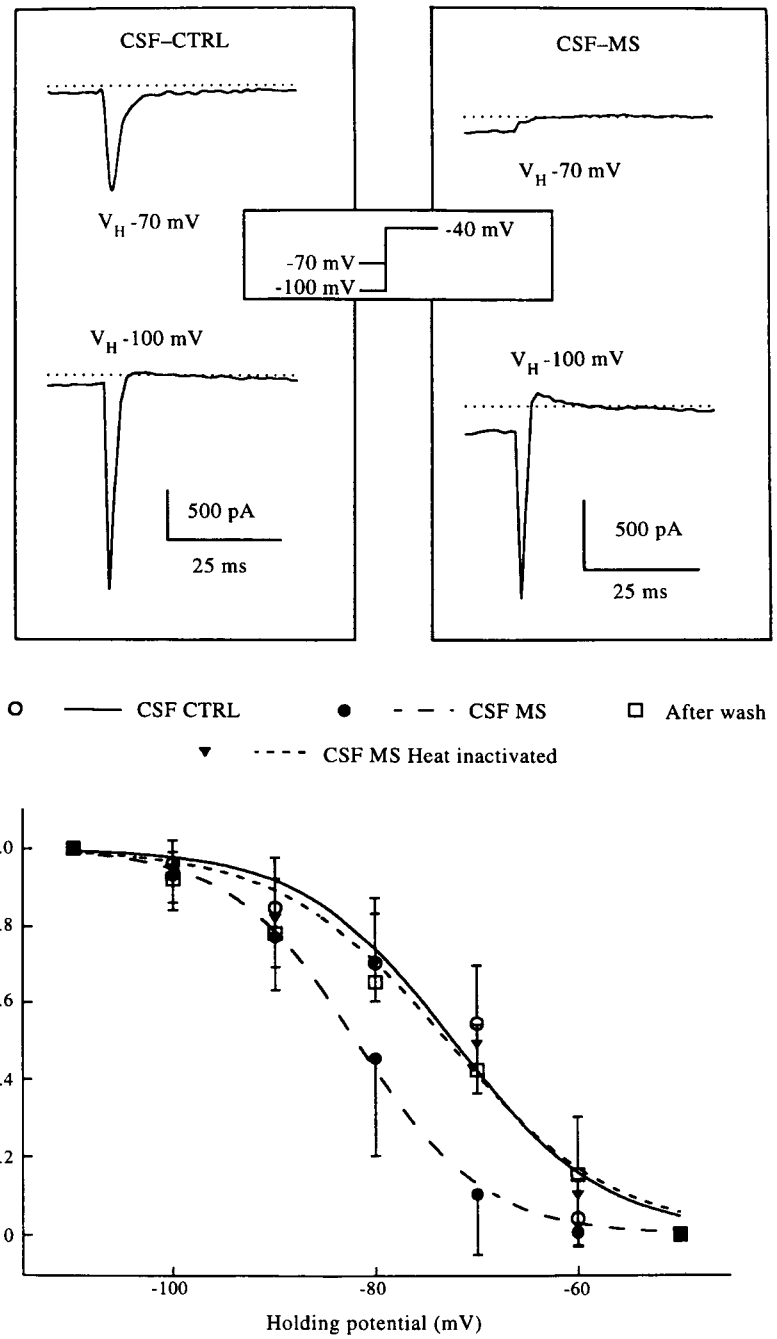


Fig. 2. The CSF from MS patients contains factors which block $Na^+$ channels. Cultured neurons from embryonic rat showed an inward current after depolarizing current pulses in CSF from patients with non-inflammatory neurological diseases (CSF–CTRL) and in CSF from MS patients during an acute relapse (CSF–MS). The amplitude of the inward current was similar in both groups, if the current pulses started from a holding potential ($V_H$) of $-100$ mV. If the cells were depolarized by a prepulse to $-70$ mV, the inward current in CSF–MS was reduced or even abolished, compared to recordings in CSF–CTRL. This reflects an increased $Na^+$ current inactivation with a shift of the inactivation curve to more hyperpolarized potentials (lower part of the figure). The shift of the inactivation curve was reversible after wash and could be prevented by CSF heat inactivation. (From Köller *et al.*, 1996a by permission of Oxford University Press.)

activation, release of immune mediators (Norris *et al.*, 1994) and proliferation (Kato *et al.*, 1991; Selmaj *et al.*, 1990). Also, they are partially competent antigen-presenting cells (Weber *et al.*, 1994; Gold *et al.*, 1996). Cytokine-induced neurotoxicity seems to be, at least partly, mediated by immunologically activated glial cells (Chao *et al.*, 1995).

### 2.2.2. *Astrocytes are Electrophysiologically Competent Cells*

Astrocytes are no longer considered to be electrophysiologically inactive cells. A number of voltage-dependent and neurotransmitter-operated ion channels have been identified (Bowman and Kimelberg, 1984; Kettenmann *et al.*, 1987; Sontheimer, 1995a; Sontheimer *et al.*, 1996). Although glial cells are not able to generate action potentials, they influence neuronal function importantly by direct and indirect mechanisms (Verkhratsky and Kettenmann, 1996). In the neuron–glia interaction, astrocytes are important in maintaining the local ion microenvironment by clearing exaggerated extracellular potassium released by neurons during firing (Walz, 1989; Barres, 1991; Sontheimer, 1995b). Potassium is transported via astrocytic syncytium or is stored locally (Barres, 1991). Astrocytes also posses neurotransmitter uptake systems, e.g. for glutamate or GABA, which clear the synaptic cleft from neuronally released transmitters (Kimelberg *et al.*, 1989; Flott and Seifert, 1991).

Direct signaling mechanisms between astrocytes and neurons have been described: Nedergaard reported that $Ca^{2+}$ waves in the astrocytic syncytium also elicited $Ca^{2+}$ elevation in neurons resting on these astrocytes. This increase in neuronal intracellular $Ca^{2+}$ was blocked by the gap junction blocker octanol, leading to the concept that gap junctions between astrocytes and neurons, which mediate the propagation of $Ca^{2+}$ waves from astrocytes to neurons, are present (Nedergaard, 1994). Parpura *et al.*, 1994, showed that glutamate, released from astrocytes upon stimulation by bradykinin, is sufficient to induce a NMDA receptor-mediated increase of intracellular $Ca^{2+}$ in co-cultured neurons. Both studies demonstrate direct signaling pathways from astrocytes to neurons, which induce changes of intracellular $Ca^{2+}$ in neurons. The electrophysiological function of astrocytes and their intracellular ion homeostasis seem to be of relevance for the function of neurons also in neuronal networks. Very recently, Herreras and coworkers (Largo *et al.*, 1996) reported on the relevance of undisturbed glial metabolism for neuronal function. After poisoning of astrocytes by the selective gliotoxin, fluorocitrate neurons were injured by recurrent spreading depression waves. The disturbed glial function also led to dysregulation of the extracellular $K^+$ concentration, pH and levels of the neurotransmitters glutamate and GABA. They concluded that undisturbed glial function is required for maintaining extracellular $K^+$ concentration and especially for recovery after pathological $K^+$ release, e.g. by spreading depression waves. Glia also regulates extracellular pH and transmitter contents.

### 2.2.3. *Effects of Identified Immune Mediators*

#### 2.2.3.1. *Cytokines*

Circumstantial evidence that ion channel function and immunological activation are coupled in astrocytes came from observations of Ohira *et al.* (1991) and Benveniste *et al.* (1991). Ohira *et al.* (1991) reported that the $K^+$ channel antagonist tetraethylammonium inhibited the interferon-$\gamma$ (IFN-$\gamma$)-induced MHC class II antigen expression and DNA synthesis in rat astrocytes. Benveniste *et al.* (1991) observed that IFN-$\gamma$ evoked an increase of intracellular $Na^+$, which could be blocked by amiloride and by the protein kinase C inhibitor H7. Both substances also diminished the MHC class II expression, so that these authors concluded that a $Na^+$ influx, mediated via $Na^+/H^+$ exchange, and activation of protein kinase C, were necessary for expression of MHC class II gene products. Interestingly, IFN-$\gamma$ activated a transmembrane $Ca^{2+}$ influx on T lymphocytes from MS patients resulting in an intracellular $Ca^{2+}$ increase which seemed to facilitate stimulus-induced proliferation (Martino *et al.*, 1995).

An activating effect of several cytokines on the astrocytic $Na^+/H^+$ exchanger also was reported by Benos *et al.* (1994a). They found that this transporter was stimulated by TNF$\alpha$, IFN-$\gamma$ and IL-1$\beta$, but not by TGF-$\beta$, IL-2 or IL-6. The IFN-$\gamma$-induced $Na^+/H^+$ exchanger activation seemed to be mediated by activation of tyrosine kinase as well as the gp 120-induced activation (see below).

TNF$\alpha$ also interferes with astrocytic $Ca^{2+}$ homeostasis and membrane potential (Köller *et al.*, 1995, 1996). After an incubation period of 30–40 min, TNF$\alpha$ induced an increase of intracellular $Ca^{2+}$ as measured by the $Ca^{2+}$ indicator dye fura-2. This rise in intracellular $Ca^{2+}$ concentration could be abrogated by a neutralizing TNF$\alpha$ antibody, indicating its specificity, by omission of extracellular $Ca^{2+}$ and by verapamil, suggesting that the TNF$\alpha$-induced $Ca^{2+}$ increase was mediated by a transmembrane $Ca^{2+}$ influx. The $Ca^{2+}$ increase was followed by a slow depolarization of the astrocytes, which could be prevented in the same way as the $Ca^{2+}$ influx. IL-1 and IL-6 did not induce a $Ca^{2+}$ influx. The $Ca^{2+}$ homeostasis and membrane potential of neurons were not affected (Köller *et al.*, 1996a, 1996b).The membrane potential of astrocytes was also reduced by TNF$\alpha$ present in the cerebrospinal fluid of patients suffering from septic or aseptic meningitis (Köller, 1997). Incubation of astrocytes in these CSF samples led to a reduction of membrane potential by nearly 40 mV compared to the membrane potential of astrocytes incubated in non-inflammatory CSF. The depolarization of meningitis – CSF was inhibited by a neutralizing anti TNF$\alpha$ antibody and it was regularly diminished in a second CSF sample from the same patients after some days of therapy.

IL-1$\beta$ also affected the intracellular $Ca^{2+}$ concentration of cultured astrocytes, especially after pretreatment with forskolin, which induced an increase of $Ca^{2+}$ channels (Holliday and Gruol, 1993). The IL-1$\beta$-induced rise of $Ca^{2+}$ appeared immediately, whereas TNF$\alpha$ did not elicit such a rapid response. After prolonged treatment with IL-1$\beta$, the quisqualate-induced $Ca^{2+}$ increase, mediated via the metabotropic glutamate receptor, was elevated. The authors suggested a role of this mechanism in disease states associated with chronic cytokine release.

2.2.3.2. *Arachidonic acid and metabolites*

Ito *et al.* (1992) investigated the effects of various prostaglandins on the intracellular $Ca^{2+}$ levels of astrocytes and found an increase of intracellular $Ca^{2+}$ after stimulation of type-1 astrocytes with the prostaglandins $PGF_2$, $PGD_2$ and some metabolites, but not after stimulation by $PGE_2$. The prostaglandin-induced $Ca^{2+}$ response could not be inhibited by an antagonist of the $PGD_2$ receptor or of the thromboxane $A_2$ receptor, leading these authors to the conclusion that the intracellular $Ca^{2+}$ increase was mediated via $PGF_{2\alpha}$ receptors coupled to phosphoinositide metabolism.

Depolarization of astrocytes was achieved by incubation of these cells with the arachidonic acid derivative leukotriene $B_4$ (Köller *et al.*, 1993), which can be released by macrophages as well as by astrocytes (Hartung *et al.*, 1988). The intracerebral levels of leukotrienes are increased in various disease states, including ischemia (Chen *et al.*, 1986; Moskowitz *et al.*, 1984) and vasogenic edema surrounding brain tumors (Black *et al.*, 1986). In contrast to $LTB_4$, other leukotrienes ($LTC_4$ or $LTD_4$) did not induce a depolarization. The $LTB_4$-induced depolarization was not affected by changing the ion concentrations of $Cl^-$ or the addition of $Co^{2+}$, which blocks $Ca^{2+}$ conductances. However, depolarization of the same degree was elicited by the unselective $K^+$ channel blocker $Ba^{2+}$, suggesting a reduced $K^+$ conductance as the origin of the depolarization. The depolarization could be blocked by cycloheximide, indicating that *de novo* synthesis of protein was required for initiating the depolarization. We concluded that $LTB_4$ induced the synthesis of a protein, which interfered with $K^+$ channels and reduced their conductance.

2.2.4. *Effects of Bacterial Toxins*

Astrocytes respond to challenge by bacterial endotoxins (lipopolysaccharides, LPS) with immunological activation (Lieberman *et al.*, 1989; Benveniste *et al.*, 1990; Chung and Benveniste, 1990). We tested whether electrophysiological functions of astrocytes are impaired by LPS. We obtained evidence for a dose-dependent depolarization of astrocytes after incubation with LPS in the range of 1.0–10.0 $\mu$g/ml (Köller *et al.*, 1994a). The depolarization could be blocked by the omission of $Na^+$ or by the addition of amiloride (250 $\mu$M). These observations suggested that the depolarization was mediated via an electrogenic $Na^+$-dependent transport mechanism and we proposed an activation of the electrogenic $Na^+/Ca^{2+}$ transporter as underlying mechanism.

Since such different immunologically active molecules as $LTB_4$ and LPS induce a depolarization of astrocytes, we examined the hypothesis that the depolarization may be a part of the immunological activation necessary or sufficient for the immunological response of astroglial cells. Using release of the cytokine IL-6 as a marker of immunological activation, we studied the effect of $LTB_4$- and LPS-induced depolarization (Köller *et al.*, 1994b). However, IL-6 was released only after incubation of astrocytes with LPS, as reported previously by others (Benveniste *et al.*, 1990), but not after incubation with $LTB_4$. The blockade of LPS-induced depolarization was insufficient to reduce or abolish IL-6 release. This indicates that depolarization and IL-6 production were differentially regulated. There were differences also in the sensitivity to immunosuppressive agents. While the $LTB_4$-induced depolarization was abolished by dexamethasone and cycloheximide, both substances did not affect depolarization-induced by LPS. In serum-free media, both immune mediators fail to stimulate proliferation (Table 1, Köller *et al.*, 1994b). In a similar vein, both substances also did not induce a change of membrane potential in neurons (Köller and Siebler, 1993; Köller *et al.*, 1994a).

2.2.5. *Effects of Virus Proteins*

GP 120 of HIV type 1 was reported to affect ion transporters and ion channels in primary rat and human astrocytes (Benos *et al.*, 1994a, 1994b; Bubien *et al.*, 1995). GP 120 induced a stimulation of the $Na^+/H^+$ antiporter in astrocytes, resulting in alkalinization of the cells. This action could be blocked by amiloride. As a consequence, an apamin-sensitive $K^+$ conductance was induced (Bubien *et al.*, 1995), which was insensitive to extracellular charybdotoxin and tetraethylammonium. It could, however, be blocked by amiloride and by omission of $Na^+$ from the extracellular bath solution. Collectively, these data indicated the involvement of the $Na^+/H^+$ exchanger. Stimulation with gp 120 also resulted in an increased efflux of glutamate from astrocytes, which is supposed to contribute to glutamate-mediated neurotoxicity.

Meldolesi and coworkers (Ciardo and Meldolesi, 1993; Codazzi *et al.*, 1995) investigated the gp 120-induced intracellular $Ca^{2+}$ responses in rat cerebellar astrocytes. A subpopulation of type-2 astrocytes, characterized by immunostaining for GFAP and A2B5, exhibited an increase of intracellular $Ca^{2+}$ upon stimulation by gp 120, which also appeared in a larger population of type-2 and most of type-1 astrocytes in $Na^+$-free solution. In

Table 1. Immuno-electrical Coupling in Astrocytes

| | LTB | LPS |
|---|---|---|
| Induction of depolarization | Yes | Yes |
| Suggested mechanism | Reduction of $K^+$ conductance | Activation of $Na^+/Ca^{2+}$ exchanger |
| Effect of amiloride | Not tested | Blockade of depolarization |
| Effect of dexamethasone | Blockade of depolarization | No effect |
| Effect of cycloheximide | Blockade of depolarization | No effect |
| Induction of IL-6 synthesis | No | Yes |
| Stimulation of proliferation | No | No |

cerebellar astrocytes, gp 120 did not induce glutamate release (Codazzi *et al.*, 1995). In cortical astrocytes, however, gp 120 impaired the glutamate uptake system (Dreyer and Lipton, 1995). In contrast to observations in neurons, gp 120 was ineffective in causing apoptosis in human cultured astrocytes (Müller *et al.*, 1992).

### 2.2.6. *Effects of Unidentified Factors in Serum or CSF*

Skaper and Varon (1987) evaluated the effects of fetal calf serum (FCS) and gangliosides including $GM_1$ on ion regulation and proliferation of astroglial cells. Astrocytes were stimulated to proliferate by both $GM_1$ and FCS, but only FCS induced a $Na^+$ influx, which was sensitive to amiloride and putatively mediated by activation of the $Na^+/H^+$ exchanger.

We tested the effect of CSF of a patient with Waldenström's macroglobulinemia (CSF–WM) on electrophysiological function of astrocytes (Köller *et al.*, 1995a). Waldenström's macroglobulinemia (WM) is a malignant disease of the immune system with an excessive production of immunoglobulins and with release of cytokines (Solary *et al.*, 1992). This patient did not reveal any morphological abnormalities on cerebral MRI, but suffered from seizures and psychomotor slowing, indicating a functional disturbance. We found that the CSF–WM contained factors capable of inducing a depolarization of astrocytes. The depolarizing activity could be blocked by heat inactivation of the CSF and by preincubation of the cells with dexamethasone. Neuronal membrane properties, including membrane potential, membrane resistance, $Na^+$ and $K^+$ currents, were unaffected (Köller *et al.*, 1995a).

### 2.2.7. *Suggested Immunological–electrophysiological Interactions in Astrocytes*

Presently, evidence is accumulating to suggest that immunological activation in astrocytes alters at least three different functional systems: (i) the regulation of the membrane potential by inducing depolarization; (ii) the maintenance of intracellular $Ca^{2+}$ homeostasis by a release of $Ca^{2+}$ from internal stores and by $Ca^{2+}$ influx; and (iii) the function of the $Na^+/H^+$ exchanger by inducing its activation with an ensuing increase of intracellular $Na^+$. As discussed above, TNFα induces a depolarization, an increase in intracellular $Ca^{2+}$ as well as an activation of the $Na^+/H^+$ exchanger; gp 120 affects intracellular $Ca^{2+}$ and activates the $Na^+/H^+$ exchanger, whereas $LTB_4$ and LPS are found to decrease the membrane potential. The depolarization is suggested to impair voltage-dependent functions of astrocytes, as discussed (see Section 2.2.2). Cytokines including TNFα were found to inhibit astrocyte glutamine synthetase, an enzyme which plays an important role in astrocytes glutamate metabolism (Chao and Hu, 1994). While glutamate uptake in astrocytes was not affected by cytokines (Piani *et al.*, 1993), the uptake of GABA was inhibited by TNFα (Hu *et al.*, 1994).

The increase of intracellular $Ca^{2+}$ may facilitate the synthesis and release of immune mediators in an autocrine or paracrine manner. Elevation of intracellular $Ca^{2+}$ by the $Ca^{2+}$ ionophore A 23187 is sufficient to induce the production of IL-6 (Benveniste *et al.*, 1990; Norris *et al.*, 1994), prostaglandins and leukotrienes (Hartung and Toyka, 1987; Hartung *et al.*, 1988, 1989).

Tumor necrosis factorα also is shown to induce the expression of calbindin in cultured hippocampal and cortical astrocytes and TNFα-treated, calbindin-expressing astrocytes were resistant to acidosis and $Ca^{2+}$ toxicity (Mattson *et al.*, 1995). Physiological stimuli, such as depolarization or moderate increases of intracellular $Ca^{2+}$ induced by low concentrations of the $Ca^{2+}$ ionophore A23187, up-regulate the calbindin expression in PC12 pheochromocytoma cells (Vyas *et al.*, 1994). Calbindin expression is believed to be a protective mechanism of cells against an increase in intracellular free $Ca^{2+}$. The TNFα-induced depolarization and increase of intracellular $Ca^{2+}$ concentration observed in our experiments (Köller *et al.*, 1996b) then could be regarded as the initial step leading to TNFα-induced calbindin expression (Mattson *et al.*, 1995), in order to prevent $Ca^{2+}$ toxicity.

Astrocytes also play an important role for the neuronal energy metabolism. In their review, Tsacopoulos and Magistretti (1996) describe the coupling of neuronal firing to glucose utilization of astrocytes: glutamate, released by activated neurons, is transported into astrocytes, together with $Na^+$ ions (a voltage-dependent process). The increase of intracellular $Na^+$ activates the glial $Na^+/K^+$ ATPase, which stimulates glycolysis by ATP consumption. The stimulated glycolysis increases the glial glucose uptake from capillaries and increases the release of lactate, which is taken up by neurons and serves as an energy substrate. Glial cells therefore mediate the coupling between neuronal firing and energy metabolism. The initial step of the glutamate/$Na^+$ uptake is voltage dependent and a depolarization of astrocytes also will impair this glial function.

Recently, Francke *et al.* (1996) reported on changes of electrophysiological properties of retinal glial cells during diseases of the eye. Human Müller cells were obtained from patients' retinas after enucleation for therapeutic reasons, e.g. after severe penetrating injury. Ion currents in these cells were compared to those of Müller cells from brain-dead organ donors without evidence for eye diseases. In Müller cells from patients, $Na^+$ current amplitude was markedly increased and membrane potential was decreased, putatively due to decreased $K^+$ inward currents (Francke *et al.*, 1996). The reasons for these changes of electrophysiological properties under pathological conditions are not clarified yet but soluble factors such as cytokines may be candidates for initiating these alterations.

Activation of the $Na^+/H^+$ exchanger with the consequence of elevated intracellular $Na^+$ is suggested to be one underlying mechanism in growth factor-induced proliferation in astrocytes (Latzkovits *et al.*, 1988, 1989; Grinstein *et al.*, 1989). The possible interactions of these mechanisms are summarized in Fig. 3.

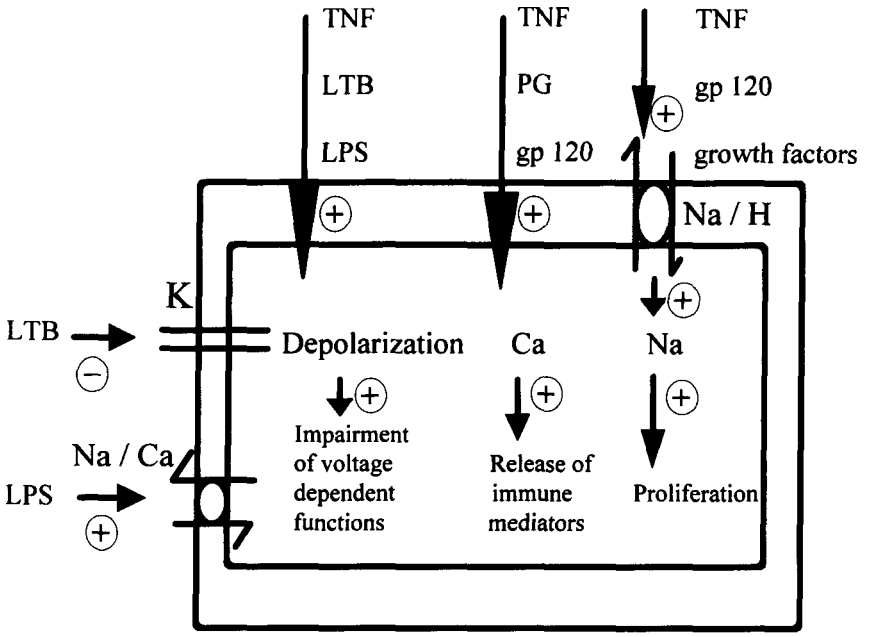


Fig. 3. Immunological–electrophysiological coupling in astrocytes. This figure summarizes the hypotheses, how immunological activation and alterations of electrophysiological properties might occur in astrocytes. TNFα, $LTB_4$ and LPS induced a depolarization. TNFα, prostaglandins (PG) and the virus glycoprotein gp 120 increase intracellular $Ca^{2+}$, which may facilitate the synthesis and release of immune mediators. TNFα, gp 120 and growth factors activate the $Na^+/H^+$ exchanger, leading to an increase of intracellular $Na^+$ and to proliferation. The $LTB_4$-induced depolarization is suggested to be mediated by a reduction of $K^+$ conductance (channel labeled with K in the figure). LPS is suggested to activate the $Na^+/H^+$ exchanger. (For further details and references, see text).

### 2.3. Microglia

#### 2.3.1. *Effects of Identified Immune Mediators*

Several reports furnished evidence that microglia, which are major local immunocompetent cells within the CNS (Gehrmann *et al.*, 1995; Kreutzberg, 1996) express a different ion channel repertoire in response to immunological stimulation. Undifferentiated microglia apparently are endowed only with inwardly rectifying $K^+$ channels (Nörenberg *et al.*, 1992, 1994). Treatment with bacterial endotoxins (LPS) and IFN-γ induced the expression of additional outward currents, which depended on an intact protein synthesis as revealed by blocking experiments using cycloheximide. Nörenberg and coworkers concluded from their pharmacological experiments (inhibition by intracellular $Cs^+$, extracellular 4-aminopyridine and tetraethylammonium) that, in microglia, immunological stimulation led to the *de novo* synthesis of $K^+$ channels.

Fischer and coworkers (Fischer *et al.*, 1995; Eder *et al.*, 1995a, 1995b) investigated the $K^+$ channel profile of microglia grown in culture media either supplemented by macrophage colony stimulating factor (M-CSF, Eder *et al.*, 1995a) or granulocyte–macrophage colony stimulating factor (GM-CSF, Eder *et al.*, 1995b). They found that the majority (about 75%) of M-CSF-grown microglia, termed resting microglia, only developed an inward rectifying $K^+$ current, whereas 75% of the GM-CSF-stimulated microglia also possessed outward $K^+$ currents. The latter type of microglia turned out to be activated in terms of their antigen-presenting capacities. IFN-γ was able to induce the development of $K^+$ outward currents in microglial cells previously grown in M-CSF. IFN-γ additionally induced a slight depolarization of these cells whereas the membrane resistance was unaffected. The authors concluded that the expression of outward $K^+$ currents in microglia is an electrophysiological activation marker, coexpressed with the antigen-presenting function. In pathological conditions like inflammation, anoxia or spreading depression with elevated $K^+$ release, the expression of outward $K^+$ currents could be helpful to maintain intracellular $K^+$ homeostasis. However, microglia, cultured on a monolayer of astrocytes, also express $K^+$ outward currents (Schmidtmayer *et al.*, 1994), which were absent in microglia cultured on fibroblasts.

Möller *et al.* (1997) investigated the intracellular $Ca^{2+}$ signals of mouse microglial cells after stimulation with complement fragments C5a and C3a. After application of these complement fragments, the intracellular $Ca^{2+}$ concentration increased due to $Ca^{2+}$ release from internal stores which were refilled by a subsequent $Ca^{2+}$ entry.

#### 2.3.2. *Effects of Virus Proteins*

Although the study was not designed to examine microglia, Bubien *et al.* (1995) reported that ion conductances of microglial cells were not affected by gp 120 in contrast to observations in astrocytes, described above (see Section 2.2.5).

### 2.4. Oligodendrocytes

#### 2.4.1. *Effects of Identified Immune Mediators*

Only very few studies investigated electrophysiological functions of oligodendrocytes (Hertz *et al.*, 1990; Barres *et al.*, 1990). After incubation of oligodendrocytes with TNFα (24–72 hr), the membrane potentials were reduced and this could be explained by a decrease of $K^+$ channel expression (Soliven *et al.*, 1991). The depolarizing effect of TNFα was not reversible within 3 days of culturing in normal media. Brief exposure (15 min) of oligodendrocytes to TNFα did not induce a depolarization (Soliven *et al.*, 1994).

Incubation with IL-2 did not affect the membrane potential of oligodendrocytes. Intracellular $Ca^{2+}$ concentrations were increased by arachidonic acid (Soliven *et al.*, 1993) and the $Ca^{2+}$ increase was mainly mediated by a transmembrane $Ca^{2+}$ influx.

Exposure of oligodendrocytes to complement resulted in a rapid increase in intracellular $Ca^{2+}$ (Scolding *et al.*, 1989), which was reduced but not abolished by the omission of extracellular $Ca^{2+}$, indicating that $Ca^{2+}$ release from internal stores, as well as influx, contribute to the intracellular $Ca^{2+}$ signal.

#### 2.4.2. *Effects of Virus Proteins*

In their study on gp 120-induced intracellular $Ca^{2+}$ increase in astrocytes, Codazzi *et al.* (1995) observed a small but progressive increase in $Ca^{2+}$ also in oligodendrocytes upon stimulation by gp 120.

## 3. IMMUNOLOGICALLY INDUCED ALTERATION OF EXCITABILITY OF NEURONAL CIRCUITS

### 3.1. Effects of Identified Immune Mediators in Experiments Using *in Vitro* and in *Vivo* Slice Preparations

In order to investigate the mechanisms of the cytokine induction of fever and reduction of food intake and anorexia, the excitability of glucose- as well as temperature-sensitive neurons, in response to cytokine application, was tested. A number of cytokines were applied iontophoretically into the brain parenchyma or intracerebroventricularly *in vivo*. Kuriyama *et al.* (1990) studied the effects of IFNα and IL-1β on neuronal excitability in slice preparations from the ventromedial hypothalamus. First, they classified the neurons with respect to their responses to changes in glucose concentration and then examined the effects of IFNα and IL-1β. They demonstrated that neurons which increase their discharge rate after a rise in glucose concentration were excited also by IFNα and IL-1β. Neurons with an increase of discharge due to a fall of glucose concentration were inhibited by IFNα and IL-1β. Glucose unresponsive neurons were not affected by these cytokines. Plata-Salamán *et al.* (1988) and Oomura (1988) noted a suppression of discharges in neurons from the lateral hypothalamic area, which has been identified as glucose sensitive (reduction of excitability in response to increase of glucose concentration), several minutes after application of TNFα and IL-1β. Glucose-insensitive neurons were affected only a little by these cytokines. Ventromedial hypothalamic glucose-responding neurons (showing an excitatory response after glucose application) displayed opposite effects after TNFα and IL-1β application: both cytokines lead to an increase in discharge rate within several minutes (Oomura, 1988).

Since IL-1 is regarded as an endogenous pyrogen, Hori and coworkers (Hori *et al.*, 1988, 1992) carried out a study of the effect of IL-1 on the discharge rate of warm- and cold-sensitive neurons in the preoptic and anterior hypothalamus. IL-1 decreased the activity of warm-sensitive neurons, while it increased the excitability of cold-sensitive neurons. This modulatory effect of IL-1 on neuronal excitability could be blocked by mepacrine, a phospholipase inhibitor, and by sodium salicylate, a cyclooxygenase inhibitor. These observations would indicate that the IL-1 effect on neuronal discharge depended on the contribution of arachidonic acid metabolites. The IL-1β-induced effects on warm- and cold-sensitive neurons could be blocked by an IL-1 receptor antagonist (Xin and Blatteis, 1992). Neurons from the bed nucleus of the stria terminalis also showed a marked increase in firing rate after local application of IL-1 (Wilkinson *et al.*, 1993).

Injection of IL-2 into the third ventricle resulted in a decrease of neuronal excitability in the ventromedial nucleus of hypothalamus and an increase in the discharge rate of neurons of the supraoptic and paraventricular nuclei (Bindoni *et al.*, 1988). IFNα also caused an increased of discharge of cortical and hippocampal neurons (Reyes-Vazquez *et al.*, 1984) while IFNγ was ineffective.

Cytokines IL-1β, IFNγ and TNFα were injected into the eye of rabbits in order to investigate the effects of these cytokines on visual evoked potentials (VEP) (Brosnan *et al.*, 1989). Within 2 hr, the peak latency of VEP was delayed by more than 2 msec after injection of IFNγ and TNFα, and the effect reversed within 24 hr. IL-1β induced a more prolonged delay in VEP latencies with a maximum notable after 24 hr and with recovery after 48 hr.

The effects of the prostaglandins $D_2$, $E_2$, and $F_{2\alpha}$ were investigated in cerebellar slice preparations. Prostaglandins induced changes of the membrane potential in Purkinje cells dendrites and a potentiation of GABAergic and glutamatergic postsynaptic potentials (Kimura *et al.*, 1985). The authors supposed alterations of intracellular cyclic nucleotide levels as underlying mechanisms. Leukotriene $C_4$, a lipoxygenase product, increased the discharge rate of cerebellar Purkinje cells, which could be blocked by a leukotriene receptor antagonist (Palmer *et al.*, 1980).

### 3.2. Effects of Identified Immune Mediators on Long-term Potentiation

Long-term potentiation (LTP) represents an increase in efficacy of synaptic transmission induced by a short train of high frequency stimulation which lasts for several hours. LTP is regarded as one

possible mechanism underlying memory and learning (Bliss and Lomo, 1973); LTP is used in many studies to investigate effects on functions of neuronal networks, which might not be assessable in the study of single cell parameters. It is found to be changed after application of growth factors such as epidermal or fibroblast growth factor (Terlau and Seifert, 1989, 1990; Abe *et al.*, 1991; Abe and Saito, 1992), but also after application of cytokines.

In rat hippocampal slices, IL-1$\beta$ increased synaptic inhibition (Zeise *et al.*, 1992). IL-1$\beta$ did not affect the resting membrane potential, membrane input resistance or amplitudes of action potentials. Electric stimulation of the Schaffer collaterals led to synaptic potentials in intracellularly recorded CA1 pyramidal neurons, an excitatory postsynaptic potential (EPSP) followed by an inhibitory postsynaptic potential (IPSP). One hour after application of IL-1$\beta$ (bath application for 10 min), the membrane conductance during the IPSP was enhanced significantly, whereas 30 min after application of IL-1$\beta$, no significant differences were found compared to controls. The increase of IPSP was abolished in recordings made after 2 hr.

IL-1$\beta$ produced an inhibition of LTP in the CA3 region of mouse hippocampus (Katsuki *et al.*, 1990), while it had no significant effect on EPSP evoked by low frequency stimulation without tetanic stimulation. Slices were perfused with IL-1$\beta$ for 25 min before tetanization and the slope of the evoked EPSP decreased. It is unlikely that the decrease of LTP is due to an increase of synaptic inhibition as reported by Zeise *et al.* (1992), since the effect on LTP was evoked at lower concentrations of IL-1$\beta$ and was established in a shorter time course. Bellinger *et al.* (1993) also found a reduction of LTP in the CA1 region of rat hippocampus. In contrast to the results of Katsuki *et al.* (1990), Bellinger *et al.* (1993) also observed a decrease of synaptic evoked EPSPs and population spikes with tetanic stimulation 10 min after IL-1$\beta$ application. LTP also was inhibited by IL-1$\beta$ in the dentate gyrus of the rat hippocampus (Cunningham *et al.*, 1996). Similar to the results previously reported by Katsuki *et al.* (1990), the low-frequency synaptic transmission was unaffected. Similar findings were obtained after incubation of the slices with TNF$\alpha$ and LPS (Cunningham *et al.*, 1996).

LTP also was affected by IL-2 (Tancredi *et al.*, 1990). Application of IL-2 inhibited the induction of short-term potentiation and LTP. It reduced also post-tetanic potentiation and LTP maintenance phase. IL-2 was added to the bath solution 10 min before conditioning tetanus. No change appeared in basal synaptic transmission. The authors suggest that IL-2 might act presynaptically, because the induction phase of LTP was disturbed.

A possible modulation of LTP by TNF$\alpha$ also was studied (Tancredi *et al.*, 1992). The induction of LTP was inhibited by TNF$\alpha$ if the conditioning tetanus was given at least 50 min after drug application. In contrast to IL-1 or IL-2, TNF$\alpha$ increased the slope of EPSPs within 30 min after application by 10–30%. The underlying mechanisms, by which TNF$\alpha$ increased basal synaptic transmission, but inhibited LTP, have not been clarified. The long time course and the obviously diverging effects on basal synaptic transmission and on LTP suggest that different mechanisms may be involved and effects on glial cells, which indirectly can affect neuronal excitability may play a role.

LTP was suppressed by interferon, which also reduced the size of short-term potentiation (D'Arcangelo *et al.*, 1991), but did not reduce the basal synaptic transmission. Interferon affected voltage-activated $Ca^{2+}$ currents in hippocampal neurons in culture (D'Arcangelo *et al.*, 1991) and it reduced NMDA-evoked inward currents.

In contrast to the cytokines IL-1, IL-2 and TNF$\alpha$, platelet-activating factor (PAF, for review of CNS effects, see Maclennan *et al.*, 1996) enhanced LTP (Wieraszko *et al.*, 1993). PAF increased excitatory postsynaptic potentials in a concentration-dependent fashion. The PAF effect was abolished by a PAF receptor antagonist and by the NMDA receptor antagonists MK 801 and APV. The induction of LTP, however, was not blocked by PAF receptor antagonists. The PAF-elicited increase in synaptic transmission was not blocked, if PAF receptor antagonists were applied after the induction of synaptic increase by PAF. From the fact that PAF-induced facilitation and LTP could not be evoked additionally to each other, the authors concluded that a common pathway may be used for PAF-mediated facilitation of synaptic transmission and LTP. The increase of intracellular $Ca^{2+}$ in neuronal cells evoked by PAF (Kornecki and Ehrlich, 1988, 1991) is supposed to contribute to the enhancement of synaptic transmission.

Arachidonic acid also enhanced synaptic transmission in hippocampal slice preparations (Williams *et al.*, 1989). Weak stimulation of the perforant path resulted in a slow-onset persistent increase in synaptic transmission associated with an increase in glutamate release. Tetanus-induced LTP and this arachidonic acid-elicited increase in excitability were not additive, indicating that the same mechanisms may be used. The lipoxygenase blocker NDGA was without effect on the arachidonic-induced increase in excitation. Drapeau *et al.* (1990) also observed an increase in synaptic transmission in the CA1 subfield of rat hippocampus after application of arachidonic acid. In contrast to the results of Williams *et al.* (1989), NDGA prevented the arachidonic acid-induced increase of synaptic transmission, suggesting involvement of lipoxygenase derivatives.

The effects of immune mediators on synaptic transmission and LTP are summarized in Table 2.

### 3.3. Effects of Unidentified Factors in Sera or CSF from MS Patients

Effects of factors present in the sera from MS patients during an acute exacerbation on neuronal excitability have been examined, using electrophysiological methods in explant cultures from cerebral cortex and spinal cord of the mouse. Sera from MS patients, as well as sera from animals with experimental allergic encephalomyelitis (EAE), produced a rapid and reversible depression of neuronal excitability (Bornstein and Crain, 1965; Crain and

Table 2. Effects of Immune Mediators on Synaptic Transmission and LTP

| Immune mediator | Effect on synaptic transmission | Effect on LTP | Reference |
|---|---|---|---|
| Il-1$\beta$ | IPSP increase | | Zeise *et al.* (1992) |
| | No effect on EPSP | Inhibition of LTP induction | Katsuki *et al.* (1990) |
| | Reduction of EPSP | Inhibition of LTP induction | Bellinger *et al.* (1993) |
| | No effect on EPSP | Inhibition of LTP induction | Cunningham *et al.* (1996) |
| IL-2 | No effect | Inhibition of LTP induction | Tancredi *et al.* (1990) |
| TNF$\alpha$ | EPSP increase | Inhibition of LTP induction | Tancredi *et al.* (1992) |
| | No effect on EPSP | Inhibition of LTP induction | Cunningham *et al.* (1996) |
| IFN (rat) | Reduction of NMDA-responses | Inhibition of LTP induction | D'Arcangelo *et al.* (1991) |
| PAF | EPSP increase | Induction of LTP by itself | Wieraszko *et al.* (1993) |
| Arachidonic acid | EPSP decrease | Facilitation of LTP induction | Williams *et al.* (1989) |

Bornstein, 1975). In these explant cultures, neurons responded to electrical stimulation by eliciting single action potentials due to pulse propagation along axons, and prolonged 'complex potentials', which are suggested to represent synaptically generated electrical activity. Sera from MS patients and from EAE animals specifically prompted a reduction of the 'complex potentials', whereas single action potentials could still be evoked, indicating that the factor(s) present in the sera could interact with synaptic transmission. This hypothesis was confirmed by the work of Schauf and colleagues, who investigated the effects of sera from MS patients and EAE animals on the excitability of spinal cord neurons from frogs (Schauf *et al.*, 1976, 1978, 1981; Stefoski *et al.*, 1982). Sera from MS patients reduced the ventral root responses (VRR) after electrical stimulation of the dorsal root by 26% (EAE sera: 36%). The VRR reduction appeared within a few minutes and the VRR recovered to prior values within 10 min. The degree of VRR reduction correlated with disease activity. The effect could be abolished by heating of serum to 56°C and was restored by the addition of complement (Schauf *et al.*, 1978). There was no effect of blocking sera on conduction of demyelinated or normal nerve fibers (Schauf *et al.*, 1981). Plasmapheresis as therapeutical intervention for MS also reduced the neuroelectric blocking activity in sera of seven patients; however, only two of seven patients also improved clinically and there was no correlation of clinical improvement and levels in blocking activity (Stefoski *et al.*, 1982). In contrast to these observations, Seil and coworkers failed to find a specific interaction of sera from MS patients (Seil *et al.*, 1976) or EAE animals (Seil *et al.*, 1975) on neuronal excitability in cerebellar or cerebral tissue cultures. They detected blocking activity in controls as well as in sera from EAE animals or MS patients. In the human sera, blocking factors were found to be thermolabile or thermostable and some depend on complement. The methodological differences between these experimental conditions and possible implications for the evaluation were discussed in a review by Seil (1977).

## 4. IMPLICATIONS FOR CNS DISEASES

### 4.1. Multiple Sclerosis

#### 4.1.1. *Soluble Cytokines in MS*

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the CNS of a presumed autoimmune origin (Hintzen *et al.*, 1992; Martin *et al.*, 1992; Hartung, 1993; Hartung *et al.*, 1995a). Soluble cytokines are believed to contribute significantly to its pathogenesis. In the MS lesion, cytokines (Hofman *et al.*, 1989; Cannella and Raine, 1995), including TNF$\alpha$, IL-1$\beta$, IL-2, IL-4, IL-10 and TGF$\beta$, have been identified. In the CSF of MS patients, IL-1$\beta$ and TNF$\alpha$ (Franciotta *et al.*, 1989; Hauser *et al.*, 1990), as well as IL-2 (Gallo *et al.*, 1988, 1991), were found to be elevated compared to controls. In longitudinal studies, the expression of TNF$\alpha$ mRNA in blood mononuclear cells correlated with disease activity (Rieckmann *et al.*, 1995). Prior to relapse, TNF$\alpha$ mRNA increased, whereas the mRNA for TGF$\beta$ and IL-10 declined. The IL-1$\beta$, IL-4, IL-6 or IFN-$\gamma$ mRNA levels did not correlate with disease activity. Levels of circulating adhesion molecules and TNF$\alpha$ receptors also were found to be elevated in MS patients, especially in those with gadolinium-enhancing lesions, reflecting acute inflammatory activity (Hartung *et al.*, 1995c).

#### 4.1.2. *Evidence for Putative Cytokine Effects on Neuronal or Glial Function in MS from Clinical and Experimental Studies*

Soluble cytokines apart from their immunomodulatory or demyelinating effects also may contribute to the pathogenesis of symptoms by direct effects on neurons or astrocytes. There is some evidence from clinical studies: Callanan *et al.* (1989) reported that the degree of cognitive impairment of MS patients in psychometric testing correlated with the degree of brain pathology in MRI but not with its localization (either periventricular, frontal or temporal) and patients with optic neuritis, lesions in the spinal cord or brainstem showed a similarly reduced performance in psychometric tests. This may indicate that soluble factors impairing neuronal function are released during the inflammation.

Additional strong evidence that cytokines induce neuronal dysfunction without demyelination, but by interaction with neuronal or glial electrophysiological function, came from clinical studies using humanized antibodies in order to produce transient lymphopenia. In parallel with a dramatic increase of circulating cytokines (mainly TNF$\alpha$, IFN-$\gamma$ and IL-6), preexisting MS symptoms

markedly exacerbated, Moreau *et al.* (1994, 1996). This worsening of the MS symptoms coincided with the raised cytokine levels and lasted for several hours only. The time course is too short for any demyelinating or remyelinating process to take place. The authors concluded that the cytokines themselves, directly or indirectly, cause neurological deterioration.

Further evidence supporting this notion may come from symptomatic therapeutical studies in MS patients using $K^+$ channel blockers such as 4-aminopyridine (4-AP, Davis *et al.*, 1990; Van Diemen *et al.*, 1992; Bever, 1994; Bever *et al.*, 1996). It was found that 4-AP led to an improvement of clinical symptoms in MS patients. The underlying mechanism is suggested to be a restoration of conduction of demyelinating fibers due to a delayed repolarization as a consequence of a blockade of the fast inactivating $K^+$ current (Gustafsson *et al.*, 1982). However, an increase in synaptic activity due to 4-AP, as suggested by Felts and Smith (1994), is another very likely mechanism. The 4-AP also has the propensity to precipitate convulsions, indicating an increase of neuronal excitability due to a delayed repolarization. Therefore, its beneficial effect in MS patients may be due to an antagonism of factors present in the CSF of MS patients that promote $Na^+$ channel inactivation (Köller *et al.*, 1996a).

Two lines of evidence, gathered from the *in vitro* studies discussed above, suggest that a direct interaction between cytokines and electrophysiological function may be relevant for symptom generation in patients:

Several studies reported the presence of factors in CSF and perhaps in serum from MS patients, which interfere with neuronal excitability. We showed that neuronal sodium current inactivation is shifted to more hyperpolarized potentials with subsequent reduction of neuronal excitability (Köller *et al.*, 1996a). These results are in line with those obtained from investigations of sodium current inactivation in myoballs (Brinkmeier *et al.*, 1993, 1996). The early results of Schauf *et al.* (1976, 1978) and Bornstein and Crain (1965) also suggest that in MS factors could be released which inhibit neuronal excitability.

As mentioned, the cytokine TNFα in particular seems to play a crucial role in the pathogenesis of MS (Franciotta *et al.*, 1989; Rieckmann *et al.*, 1995; Hartung *et al.*, 1995a; Zipp *et al.*, 1995; Spuler *et al.*, 1996; Moreau *et al.*, 1996). In invertebrates, TNFα affects neuronal $Na^+$ (Sawada *et al.*, 1991a) and $K^+$ currents *in vitro* (Sawada *et al.*, 1990), but these findings have not yet been confirmed for mammalian neurons. However, in more complex experimental situations, TNFα application was followed by electrophysiological effects: it increased basal synaptic transmission but inhibited LTP (Tancredi *et al.*, 1992) with a very long time course. After intraocular injection in the rabbit it induces an increase of cortical VEP which reversed within 24 hr, indicating a transient, putatively functional but not demyelinating effect on the optic nerve. An alterations of glial cell function, like a loss of membrane potential and an increase of intracellular $Ca^{2+}$ (as reported by our group, Köller *et al.*, 1996b) — with or without edema — may represent the underlying mechanism (see also Table 3).

### 4.2. HIV-associated Encephalopathy (HIVE)

#### 4.2.1. *Cytokines in HIVE*

In HIVE, neurological symptoms such as dementia appear, despite a relatively low number of productively infected cells within the brain (Glass *et al.*, 1993; Epstein and Gendelman, 1993). This paradox has been explained by indirect mechanisms that would involve cytokines. The HIV-infected macrophages produce cytokines, which in turn further augment cytokine production by uninfected macrophages, microglia and astrocytes. This amplification of cytokine production, especially of TNFα, IL-1β and arachidonic acid metabolites, is the suggested underlying mechanism of HIVE (Genis *et al.*, 1992; Epstein and Gendelman, 1993; Vitkovic *et al.*, 1994; but see also Tardieu *et al.*, 1992). Levels of TNFα mRNA from subcortical white matter correlated with clinical symptoms. Patients suffering from HIVE showed elevated TNFα levels compared to non-demented HIV patients (Glass *et al.*, 1993). The CSF analysis of HIV patients also showed an elevation of TNFα levels (Grimaldi *et al.*, 1991; Perrella *et al.*, 1992; Tyor *et al.*, 1992).

Table 3. Putative Immuno-electric Coupling in Multiple Sclerosis

| Immune mediator | Effect | Reference |
|---|---|---|
| TNFα | Hyperpolarization and inhibition of invertebrate neurons | Sawada *et al.* (1991a, 1991b); Mimura *et al.* (1994) |
| | Depolarization and increase of intracellular $Ca^{2+}$ in rat astrocytes | Köller *et al.* (1995b, 1996b) |
| | Induction of increase in VEP delay after intraocular injection in rabbits | Brosnan *et al.* (1989) |
| | Inhibition of LTP induction in rat hippocampal slices | Tancredi *et al.* (1992) |
| Unknown factor(s) in CSF | Increase of $Na^+$ current inactivation in rat neurons | Köller *et al.* (1996a) |
| Unknown factor(s) in sera | Neuroelectric blocking activity (frog spinal cord) | Schauf *et al.* (1976, 1978, 1981) |

#### 4.2.2. *Evidence for Putative Effects of Cytokines on Neuronal or Glial Function in HIVE*

There are several lines of evidence which suggest that virus proteins from HIV interfere with neuronal $Ca^{2+}$ currents and neuronal transmitter receptors, especially the NMDA–glutamate receptors, as discussed above (see Sections 2.1.1.2, 2.1.2.2 and Table 4). In addition to the effects of virus proteins, cytokines generated by macrophages or astrocytes might also affect neuronal or glial membrane properties as discussed for TNFα and others. Furthermore, in CSF of HIV patients, neurotoxic substances were detected, which seemed to interfere with neuronal function. Heyes *et al.* (1991) reported that quinolinic acid, which acts via an activation of the NMDA receptor, is present in the CSF of HIV patients. Levels of quinolinic acid correlated to clinical symptoms and were found to be elevated more than 10-fold in patients with HIVE compared to non-demented HIV patients in the same Walter Reed stage group. Zidovudine treatment reduced quinolinic acid concentrations in the CSF, further indicating a correlation of the HIV infection and quinolinic acid production. Zidovudine treatment also led to an improvement of neuropsychiatric dysfunction (Schmitt *et al.*, 1988) and motor performance (Arendt *et al.*, 1992) in patients with HIVE.

### 4.3. Meningitis and Encephalitis

#### 4.3.1. *Cytokines and Bacterial Toxins in Meningitis and Encephalitis*

In meningitis, in particular of bacterial origin, cytokines have been identified in the CSF (Frei *et al.*, 1988; Leist *et al.*, 1988; Mustafa *et al.*, 1989; Waage *et al.*, 1989; Arditi *et al.*, 1990; Moller *et al.*, 1991; Weller *et al.*, 1991; Glimaker *et al.*, 1993; Seki *et al.*, 1993; Stearman and Southgate, 1994) and cytokine elevation correlated with neurological outcome (Mustafa *et al.*, 1989). In addition to cytokines, lipopolysaccharides were found to be elevated in bacterial meningitis. The LPS titers also correlated with neurological outcome (Dwelle *et al.*, 1987; Arditi *et al.*, 1989; Mertsola *et al.*, 1991).

#### 4.3.2. *Evidence for Putative Cytokine Effects on Neuronal and Glial Function in Meningitis and Encephalitis*

Meningitis patients mostly suffer from psychomotor slowing and loss of consciousness which can be regarded as a consequence of reduction of neuronal excitability. Epileptic seizures occur in about 40% of meningitis patients and can be the presenting symptom (Anderson, 1993). The EEG abnormalities, such as generalized slowing or focal spikes or slowing, were recorded in about two-thirds of meningitis patients (Pomeroy *et al.*, 1990). The probability of experiencing seizures positively correlated with titers of bacterial endotoxins (Dwelle *et al.*, 1987; Arditi *et al.*, 1989) and with cytokine titers (TNFα and PAF, Arditi *et al.*, 1990). This may rather reflect the severity of intracerebral infection than an etiologic role of these molecules in generating epileptic convulsions. However, LPS, applied by intraperitoneal injection in rats, led to alterations of EEG activity (Lancel *et al.*, 1995), which may support the hypothesis of an effect of LPS on neuronal activity. Data from the *in vitro* studies described above showed that neuronal excitability, as well as glial electrophysiological function, can be affected by these immune mediators. LPS induced a depolarization of astrocytes just in the same range of concentrations (1.0–10.0 μg/ml, Köller *et al.*, 1994a) that were measured in the CSF of meningitis patients suffering from seizures. Arditi *et al.* (1989) noted a significantly elevated risk for development of seizures in patients with LPS titers above 1.0 μg/ml CSF. TNFα also affects neuronal and glial function and PAF has been reported to increase neuronal excitability in slices (Wieraszko *et al.*, 1993).

#### 4.3.3. *Glutamate Receptor Autoantibodies in Rasmussen's Encephalitis*

Rasmussen's encephalitis is an inflammatory childhood disease characterized by intractable epilepsy, hemiplegia and dementia. Its etiology is unknown (Larner and Anderson, 1995). Recently, it was reported that the sera of patients with Rasmussen's encephalitis contained autoantibodies which bind to and activate glutamate receptors (Rogers *et al.*, 1994; Twyman *et al.*, 1995). Rabbits which have been immunized with a portion of the

Table 4. Effect of Virus Proteins in HIVE

| Virus protein | Effect | Reference |
|---|---|---|
| gp 120 | Increase of intracellular $Ca^{2+}$ in neurons | Lipton (1991) |
| | Modulation of NMDA receptors in neurons with the effect of increased intracellular $Ca^{2+}$ | Lipton (1992a); Dawson *et al.* (1993); Diop *et al.* (1994); Lannuzel *et al.* (1995) |
| | Activation of $Na^+/H^+$ exchanger in astrocytes | Benos *et al.* (1994a, b); Bubien *et al.* (1995) |
| | Increase of intracellular $Ca^{2+}$ in astrocytes | Ciardo and Meldolesi (1993); Codazzi *et al.* (1995) |
| | Impairment of glutamate uptake system | Dreyer and Lipton (1995) |
| | Depolarization of cockroach interneurons | Sabatier *et al.* (1991) |
| tat | Kynureate-sensitive depolarization of human neurons | Magnuson *et al.* (1995) |

extracellular domain of a glutamate receptor (GluR3) developed high titers of GluR3 antibodies, associated with a disease mimicking the clinical picture of human Rasmussen's encephalitis, including seizures (Rogers *et al.*, 1994). Autoantibodies against this glutamate receptors were found in sera of Rasmussen's encephalitis patients and one patient transiently improved with a reduction of seizure frequency upon plasma exchange therapy, which also reduced the glutamate receptor antibodies (Rogers *et al.*, 1994). Kainate responsive neurons cultured from fetal mouse exhibited an inward current after stimulation with sera from the GluR3 immunized rabbits as well as after stimulation with sera from Rasmussen's encephalitis patients (Twyman *et al.*, 1995). This inward current could be blocked by CNQX, indicating that it was elicited by activation of a non-NMDA type of glutamate receptors.

The hypothesis of a pathogenic role of circulating factors like autoantibodies in Rasmussen's encephalitis is further confirmed by the beneficial effect of plasmapheresis in 3 of 4 patients treated (Andrews *et al.*, 1996). The authors suggest a "vicious circle" hypothesis for the pathogenesis of Rasmussen's encephalitis: focal disruption of the blood–brain barrier allows the entry of circulating pathogenic antibodies, e.g. against glutamate receptors, which induce neural injury leading to an increase of focal seizures and thereby, locally, further disturb the blood–brain barrier. Autoantibodies against glutamate receptor subunits also seem to play a pathogenic role in paraneoplastic syndromes (Gahring *et al.*, 1995) and in nervous system degeneration such as olivopontocerebellar degeneration (Gahring *et al.*, 1997).

#### 4.3.4. *Evidence for Putative Effects of Immune Mediators in the Bing Neel Type of Waldenström's Macroglobulinemia*

Waldenström's macroglobulinemia belongs to the neoplasms of the immune system with an excessive production of immunoglobulins and cytokines (Solary *et al.*, 1992). Apart from cerebral ischemia due to a hyperviscosity syndrome, neurological symptoms occur as a consequence of aberrant immune responses within the CNS. Logothetis *et al.* (1960) described a patient with seizures and gait disturbance without cerebral lesions on necropsy. They proposed a 'toxic–metabolic mechanism' as origin of the functional deficit and the seizures. Spencer and Moench (1980) reported on a patient with progressive cerebellar ataxia without morphological lesion on imaging who improved upon chlorambucil treatment for WM. In their review of the literature, Logothetis *et al.* (1960) found a small group of patients without focal neurological symptoms but with diffuse neurological dysfunction, which seemed to be generated by soluble factors released from the tumor masses. In our experimental study using the CSF of a WM patient without cerebral lesions but suffering from seizures and psychomotor deficits, we provided evidence that soluble factors in the CSF–WM may indeed affect the electrophysiological functions of astrocytes (Köller *et al.*, 1995b; see also Section 2.2.6).

### 4.4. Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a motoneuron disease of unknown etiology leading to progressive paresis of muscles of arms and legs but also compromising speech, swallowing and breathing. Two hypotheses for the sporadic type of ALS have been discussed in the literature: one favoring the excitotoxic theory, according to which glutamate is elevated in spinal cord and motor cortex, producing neuronal cell death via increases of intracellular $Ca^{2+}$ (Rothstein *et al.*, 1992; Appel, 1993). The other theory favors an autoimmune origin of the disease (Appel *et al.*, 1993). Immunoglobulins from animal models of motor degeneration and from ALS patients have been reported to bind to motoneurons and neuromuscular junctions in mice (Appel *et al.*, 1991). Electrophysiological investigation of the muscle showed an increased frequency of miniature endplate potentials in the animal models and in animals injected with human immunoglobulins from ALS patients. Immunoglobulin G from ALS patients reduced the peak $Ca^{2+}$ current in muscle fibers (Delbono *et al.*, 1991) and the amount of ALS IgG in serum correlated with the disease progression (Smith *et al.*, 1992). Recently, the binding antigen in sera from ALS patients was identified as the $\alpha_1$ subunit of the voltage-gated $Ca^{2+}$ channel (Kimura *et al.*, 1994). Binding of the ALS immunoglobulins to skeletal muscle fibers resulted in a 50% reduction in $Ca^{2+}$ activation currents. Beside the reduction of $Ca^{2+}$ currents induced by IgG from ALS patients, an increase of $Ca^{2+}$ inward current was also reported (Llinas *et al.*, 1993). In cerebellar Purkinje cells IgG from ALS patients evoked an increased ion flux through P-type $Ca^{2+}$ channels, mediated by an increased open time of the channel. However, the relevance of these antibodies for the pathogenesis of ALS has been questioned (Vincent and Drachman, 1996). Using immunoprecipitation assays, Arsac *et al.* (1996) did not find autoantibodies against L-type $Ca^{2+}$ channels in sera from ALS patients and autoantibody titers against N-type $Ca^{2+}$ channels were present only in 2 of 25 sera at low levels.

## 5. IMPLICATIONS FOR PNS DISEASES

### 5.1. Guillain-Barré Syndrome and Miller-Fisher Syndrome

Guillain-Barré syndrome (GBS) is an inflammatory demyelinating disease of the peripheral nervous system which results from aberrant immune responses (for review, see Hartung *et al.*, 1995b, 1995c). In addition to demyelination of nerve fibers, symptoms result from a direct interaction between immune mediators and electrophysiological nerve functions. Brinkmeier and coworkers tested this hypothesis by investigating the effects of CSF from GBS patients on ion currents in human myoballs (Brinkmeier *et al.*, 1992a; Würz *et al.*, 1995). They found a reversible reduction of the current maximum and a shift of the $Na^+$ current inactivation curve to more hyperpolarized potentials, induced by CSF from GBS patients (Brinkmeier *et al.*, 1992a). Both led to a reduction of excitability. The $Na^+$ current

activation, however, was unchanged. The underlying factor(s) which interfered with the muscular $Na^+$ channel are unknown. Interleukin-2, a cytokine which is elevated in GBS (Hartung *et al.*, 1991), induced a very similar effect in this myoball preparation (Brinkmeier *et al.*, 1992b; Kaspar *et al.*, 1994) and an increased $Na^+$ current inactivation induced by IL-2 also was detected in cardiac muscle cells (Proebstle *et al.*, 1995). In contrast, serum of GBS patients, in which IL-2 can be present in concentrations of nearly 100 U/ml (Hartung *et al.*, 1991), did not induce an increase but rather a decrease of $Na^+$ current inactivation (Würz *et al.*, 1995). To our knowledge, the IL-2-induced decrease of $Na^+$ currents has not been confirmed for neuronal $Na^+$ currents so far and in our cultures of cortical neurons from rat, IL-2 was ineffective (Köller *et al.*, 1996a).

The blocking activity could be eliminated by CSF filtration in experimental conditions (Wollinsky *et al.*, 1991) and in patients (Hülser *et al.*, 1991). However, the number of patients was small, the study was not controlled and hence results need to be confirmed in larger trials.

The Miller-Fisher syndrome (MFS) is a variant of GBS with autoantibodies directed to the ganglioside $GQ_{1b}$ (Hartung *et al.*, 1995b). Roberts and coworkers tested the effect of sera from MFS patients on miniature endplate potential (MEPP) frequency and evoked muscular responses after nerve stimulation in the phrenic nerve/diaphragm preparation (Roberts *et al.*, 1994). Sera from MFS patients induced a reduction of MEPP frequency after a short increase and MEPPs ceased completely after 3 hr of incubation. Then, stimulation of the phrenic nerve did not evoke a muscle response, whereas the muscle was still excitable after direct stimulation. This indicates that a factor in sera from MFS patients, possibly the antibody against $GQ_{1b}$ as suggested by the authors, directly acted at the motor nerve terminal, first increasing acetylcholine release and then abolishing it. Since the amplitudes of MEPPs were constant during the experiment, unless MEPPs disappeared, it is likely that the factor acted at the presynaptic site of the nerve terminal. This has been further supported by recent findings which showed that IgG from a MFS patient reversibly depressed quantal release of acetylcholine at the neuromuscular junction (Buchwald *et al.*, 1995).

### 5.2. Multifocal Motor Neuropathy

Multifocal motor neuropathy (MMN) is an acquired immune-mediated motor neuropathy, characterized by asymmetrical, slowly progressive weakness, motor conduction block in electrodiagnostic studies and the presence of serum antibodies against gangliosides (for review, see Kornberg and Pestronk, 1995). In passive transfer experiments, sera from MMN patients induced a conduction block in injected animals (Santoro *et al.*, 1992; Uncini *et al.*, 1993; Arasaki *et al.*, 1993; Roberts *et al.*, 1994). Santoro *et al.* (1992) and Uncini *et al.* (1993) injected serum of a patient with MMN with anti-$GM_1$ antibodies into rat sciatic nerve, together with human complement, and found an induction of conduction blocks within 3 days. Deposits of immunoglobulin could be detected at the node of Ranvier. Preabsorption with $GM_1$ abolished the ability of the serum to induce conduction blocks, suggesting that the anti-$GM_1$ antibody directly interfered with ion channels of the nerve fiber. By contrast, according to Arasaki *et al.* (1993), the effect seemed to be limited to myelinated nerves, since conduction in demyelinated nerves was undisturbed. Roberts *et al.* (1995) showed that serum from MMN patients directly applied to a mouse phrenic nerve/diaphragm preparation led to an increase in stimuli, necessary to evoke a muscle contraction within 30 min. In some experiments, the nerve-evoked muscle contraction was blocked completely while depolarization-induced MEPPs still could be evoked. The effect was not reversible upon washing the preparation and could not be abolished by plasma heating.

Anti-$GM_1$ antisera from rabbits immunized against $GM_1$ increased the depolarization-evoked $K^+$ currents in isolated nerve fibers of rat (Takigawa *et al.*, 1995). The $Na^+$ currents were affected only if complement was applied additionally. The authors concluded that the anti-$GM_1$ antibody directly uncovered $K^+$ channels and formed an antibody–complement complex which blocked $Na^+$ channels.

### 5.3. Acquired Neuromyotonia

The immunopathogenetic origin of neuromyotonia (Isaacs' syndrome) was described by Newsom-Davis and colleagues (Sinha *et al.*, 1991; Newsom-Davis and Mills, 1993), who found oligoclonal bands in CSF of neuromyotonia patients and observed clinical improvement upon plasma exchange therapy. Recently, Shillito *et al.* (1995) provided evidence that neuromyotonia is caused by blocking antibodies against $K^+$ channels, which they identified in the sera of three patients. Neuromyotonia IgG preparations increased the quantal release of acetylcholine at endplates in mice diaphragms and the peroneal nerve compound action potential also markedly increased. Applied to dorsal root ganglion neurons in culture, neuromyotonia IgG preparation produced an increase in repetitive firing of action potentials. Similar changes of neuronal excitability were elicited by 3,4 diaminopyridine, a $K^+$ channel blocker, suggesting that antibodies in neuromyotonia similarly may reduce $K^+$ conductances.

Sonoda *et al.* (1996) tested the effect of sera from patients with Isaacs' syndrome on potassium currents of the clonal cell line PC-12. They found a reduction of $K^+$ currents after incubation of the cells for 3 to 6 days during development in culture media containing 2% of patients' sera. When sera of these patients were added directly to the perfusion solution in order to investigate acute effects, the $K^+$ currents of PC-12 cells were unaffected.

The putative effects of immune mediators, autoantibodies, bacterial toxins or viral proteins on electrophysiological functions of astrocytes and neurons in various diseases are summarized in Table 5.

Table 5. Synopsis of Putative Effects of Immune Mediators, Bacterial and Viral Products on Electrophysiological Functions

| Disease | Agent | Effect | *In vitro* | Clinical study |
|---|---|---|---|---|
| MS | Cytokines | Increase correlates with transient symptoms | | X |
| | Unknown factor(s) in CSF | Inactivates neuronal $Na^+$ current | X | |
| | TNFα | Depolarizes and alters intracellular $Ca^{2+}$ in astrocytes | X | |
| HIVE | Virus proteins | Affect neuronal excitability | X | |
| | | Affect glial intracellular $Ca^{2+}$ | X | |
| Meningitis | LPS and TNFα | Increased CSF titers correlate with seizure probability | | X |
| | | Depolarizes astrocytes | X | |
| | TNFα | Depolarizes astrocytes, alters intracellular $Ca^{2+}$ | X | |
| Rasmussen's encephalitis | Autoantibodies | Activate glutamate receptors | X | |
| | | Improvement upon plasma exchange therapy | | X |
| Waldenström's macroglobulinemia | Unknown factor(s) in CSF | Depolarize astrocytes | X | |
| Amyotrophic lateral sclerosis | Autoantibodies (IgG) | Bind to $Ca^{2+}$ channels | X | |
| Guillain-Barré syndrome | Unknown factors in CSF | Inactivates muscular $Na^+$ currents | X | |
| Miller Fisher syndrome | Autoantibodies (Anti-$GQ_{1b}$) | Reduce MEPP frequency | X | |
| Multifocal motor neuropathy | Autoantibodies (Anti-GM1) | Depress quantal acetylcholine release | X | |
| | | Induce conduction block | X | |
| | | Increase $K^+$ currents | X | |
| Issacs' syndrome | Unknown factors | Improvement upon plasma exchange therapy | | X |
| | Autoantibodies | Increase quantal acetylcholine release | X | |
| | | Increase repetitive firing in neurons | X | |

## 6. INHIBITION OF ELECTRICAL ACTIVITY — POSSIBLE ROLE IN THE IMMUNOLOGICAL CASCADE

There is convincing evidence from cell culture, *in vitro* and *in vivo* studies, as well as from clinical data, that immunological active molecules can directly affect neuronal excitability. In many cases, the immune mediators induce a reduction of neuronal excitability, e.g. by increasing $Na^+$ current inactivation in CSF–MS (Köller *et al.*, 1996a) or the effect of IL-1α and TNFα (Mimura *et al.*, 1994) on mollusc neurons. Astrocytes may be even better candidates for mediating electrophysiological dysfunction by immunological activation, which indirectly disturbs neuronal function (Fig. 4). These effects on the electrophysiological properties of neurons and astrocytes may be side-effects of cytokines or effects of immunoglobulins binding the 'wrong' antigen, e.g. the binding of autoantibodies to glutamate receptors in Rasmussen's encephalitis, which can explain the pathogenesis of symptoms in these patients. However, according to recent results from Neumann and colleagues (Neumann *et al.*, 1995, 1997), it may also play a role in the immunological cascade, promoting further immunological damage. Neumann *et al.* reported that the expression of MHC class I antigen in hippocampal neurons depended on their electrical activity. In electrically silent neurons, they demonstrated the expression of $\beta_2$ microglobulin and MHC class I genes after IFNγ stimulation, whereas, in electrically active neurons, the expression of MHC class I genes was suppressed. After paralyzing the cultures with TTX, MHC class I genes were expressed in all neurons after application of IFNγ. Loss of bioelectric activity therefore renders them susceptible to the action of cytotoxic T cells. Interestingly, this effect of electrically paralyzing of the neurons by TTX could be reverted by the application of glutamate (Neumann *et al.*, 1997). The addition of 2 μm glutamate in presence of TTX reduced the expression of MHC class I protein to baseline levels (without TTX). Therefore, the authors raise the question whether electrical activity of neurons control the MHC gene expression within these neurons or in synaptically coupled neurons via glutamatergic synapses.

The hypothesis of downregulation of immunological susceptibility of brain cells by neuronal electrical activity was even extended on astrocytes and microglia (Neumann *et al.*, 1996). In the neighbourhood of functionally active neurons the IFN-γ induced MHC class II expression of astrocytes and microglia was suppressed. Blockade of neuronal activity by TTX led to an increase of IFN-γ induced MHC class II expression.

With respect to these results, inhibition of neuronal excitability by immune mediators also may be part of the immunological cascade by facilitating the expression of MHC class I or II antigen at the surface of neurons or glial cells and thereby contributing to the immunological attack.

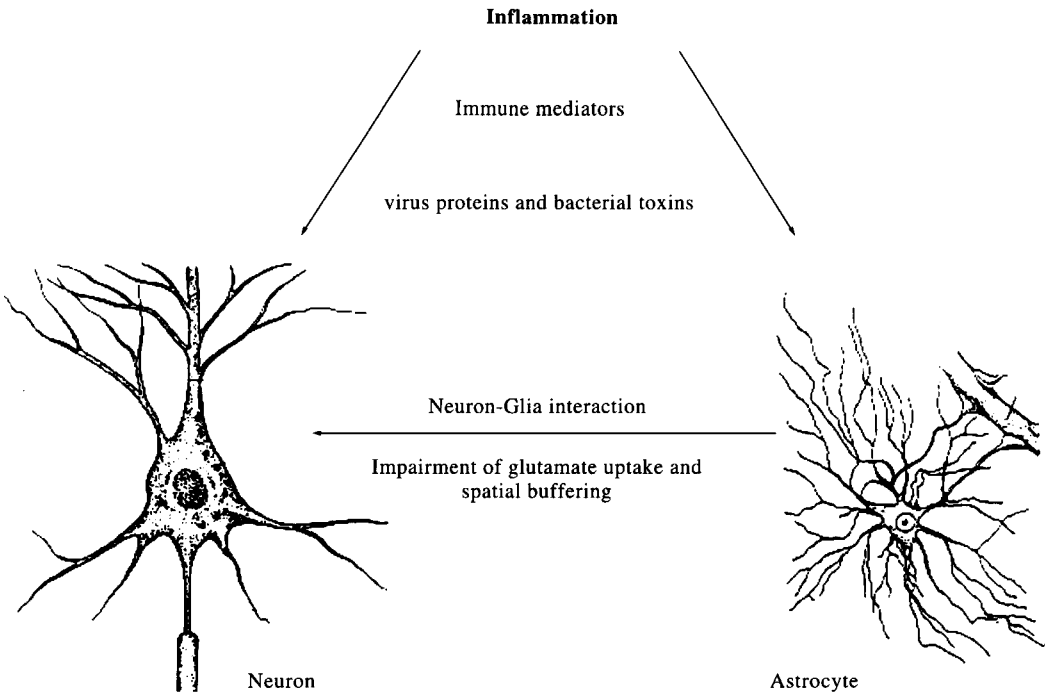


Fig. 4. Immunological–electrophysiological coupling via soluble immune mediators. In the CNS, immune mediators released during the inflammatory process can either directly affect neuronal membrane properties, leading to neuronal dysfunction, or indirectly, via disturbed glial electrophysiological properties. The latter is especially striking, since astrocytes belong to the local immunocompetent cells within the CNS and, according to their electrophysiological function, play a crucial role in maintaining local ion microenvironment. Disturbance of glial functions, such as spatial buffering or glutamate uptake, may result in a disturbance of neuronal function.

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