Journal of Neuroscience Research 86:1781–1788 (2008)

Implications of Antiinflammatory Properties of the Anticonvulsant Drug Levetiracetam in Astrocytes

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There is accumulating evidence that epileptic activity is accompanied by inflammatory processes. In the present study, we evaluated the effect of levetiracetam (Keppra), an anticonvulsant drug with decisive antiepileptic features, with regard to its putative antiinflammatory potential. We previously established an in vitro cell culture model to mimic inflammatory conditions: Primary astrocytic cultures of newborn rats were cocultured with 30% (M30) microglial cells. Alternatively, cocultures containing 5% microglia (M5) were incubated with the proinflammatory mediator, the cytokine interleukin-1β (IL-1β), and lipopolysaccharide (LPS), a potent bacterial activator of the immune system. For the M30 cocultures, we observed reduced expression of connexin 43 (Cx43), the predominant gap junction protein. Impaired functional dye coupling and depolarized membrane resting potential (MRP) were monitored in M30 cocultures as well as in M5 cocultures treated with IL-1β and LPS. We could show that the Cx43 expression, the coupling property, and the membrane resting potential on which we focused our inflammatory coculture model were normalized to noninflammatory level under treatment with levetiracetam (Keppra). Cumulatively, our results provide evidence for antiinflammatory properties of levetiracetam in seizure treatment.

Key words: levetiracetam; epilepsy; inflammation; astrocyte/microglial coculture; gap junction communication

Recent reports underscore the idea that inflammatory conditions are present during and after seizure activity (Vezzani, 2005). Previous investigations could detect an increase of proinflammatory cytokines, in particular interleukin-1 (IL-1), in experimental epilepsy models (De Simoni et al., 2000). A prolonged inflammatory condition was emphasized, demonstrating a role of inflammation in chronic epilepsy rather than in acute seizures. Moreover, inflammatory mediators were detected in the cerebrospinal fluid from patients with recent epileptic seizures (Peltola et al., 2000). Microglial cells among other cells, such as astrocytes, represent the main source of cytokines in the CNS (Hanisch, 2002), so we assume that the occurrence of inflammatory cytokines in epileptic seizures involves activation of microglia. The overexpression of cytokines triggers disturbance of the neuronal and glial enviroment.

More detailed knowledge about the link between inflammatory responses and the etiopathogenesis of epilepsy would help in developing novel and effective therapeutic modalities. Here we focused on the novel drug levetiracetam (LEV; Keppra; ucb L059; [S]-alpha-ethyl-2-oxo-1-pyrrolidine acetamide), which has been shown to possess antiepileptic efficacy and good tolerability in the treatment of refractory partial seizures in several clinial trials (Ben-Menachem and Falter, 2000; Cereghino et al., 2000). However, many studies devoted to the detailed molecular actions of LEV have suggested that LEV is devoid of impact on many targets accepted as accounting for classical antiepileptic drugs (AEDs; Klitgaard et al., 1998; Zona et al., 2001). Thus, the knowledge about the mechanisms responsible for its antiepileptic activity remains limited. The aim of the present study was to evaluate the ability of LEV to restrain the effects of inflammation on astrocytes, focusing on astrocytic connexin 43 (Cx43) expression, gap junction-mediated intercellular

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Received 3 June 2007; Revised 21 November 2007; Accepted 30 November 2007

Published online 11 March 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.21639

communication, and membrane resting potential within inflammation.

To mimic inflammatory conditions, we used a defined in vitro bioassay consisting of primary astrocytic cultures of newborn rats that were cocultured with 30% (M30) microglial cells. Astroglial cocultures containing 5% (M5) microglial cells served as controls, which corresponds to the physiological situation in healthy brain. Previously, we had demonstrated that M30 cocultures revealed a high fraction of activated round phagocytic-type (RPT) microglia, whereas the M5 cocultures contained mainly microglial cells of resting ramified type (RRT; Faustmann et al., 2003). The change of the morphological phenotype from RRT to RPT is considered to occur in line with enhanced phagocytic activity of the microglia and the capability to secrete inflammatory cytokines (Stoll et al., 1998; Ledeboer et al., 2000). In addition to the M30 coculture model, activation of microglia was also observed after exposure of M5 cocultures to diverse proinflammatory cytokines, including IL-1β (Hinkerohe et al., 2005).

Astrocytes have multiple functions, e.g., cell proliferation, metabolic and trophic support of neurons and the control of extracellular ion and neurotransmitter homoeostasis (Dermietzel and Spray, 1998; Reuss and Unsicker, 1998). These properties can be influenced by a well-functioning intercellular communication based on gap junction channels, which in the case of astrocytes are predominantly constituted by the gap junction protein Cx43 (Dermietzel et al., 1991; Giaume and Venance, 1998). Impairment of the astrocytic syncytium and subsequent disturbance of the dissipation and buffering capacity have been reported to occur in diverse pathophysiological processes, such as inflammatory conditions (John et al., 1999; Faustmann et al., 2003).

Here, we first evaluated the astroglial Cx43 epression in the M30 cocultures and the strength of gap junction-mediated intercellular communication (GJIC) within the astroglial syncytium with and without LEV. Furthermore, the astrocytic membrane resting potential (MRP) was measured by means of the patch-clamp technique. These parameters were studied in parallel using M5 cocultures pretreated with the proinflammatory cytokine IL-1β and lipopolysaccharide (LPS), which is a cell wall component of gram-negative bacteria and contributes to a distinct production of inflammatory cytokines by activation of immune component cells, such as astrocytes and microglia (Saukkonen et al., 1990; Lee et al., 1993).

MATERIALS AND METHODS

Cell Culture

Primary astroglial cell cultures were obtained from whole brain hemispheres of postnatal (P0–P2) Wistar rats based on a modified protocol reported by Dermietzel et al. (1991). Briefly, after removal of meninges and choroid plexuses, brains were treated with 0.1% trypsin/1% DNAse I (Invitrogen, Karlsruhe, Germany; Serva, Heidelberg, Germany) and passaged through a 60-μm nylon mesh. Cells (40,000 cells/coverslip) were then incubated in culture flasks (Becton Dickinson, Heidelberg, Germany) for 4–5 days in 87% Dulbecco's minimal essential medium (DMEM; Gibco, Karlsruhe, Germany), 10% fetal calf serum, 1% nonessential amino acids, 1% penicillin (50 μg/ml) streptomycin (50 μg/ml), and 1% glutamine (2 mM; Invitrogen) until confluence in humidified carbon gas (5% CO_2/95% a.a.) at 37°C. Non-adjacent microglia were removed by vigourously shaking the flask, followed by subsequent washing procedures. Depending on the extent of shaking, an astrocyte/microglia coculture containing about 5% microglia (M5) was acchieved, comparable to the concentration found in healthy brain tissue or left to obtain a coculture containing about 30% microglia cells (M30). To confirm whether the cocultures contatined a 5% or 30% fraction of migroglia, immunohistochemical staining and subsequent counting were performed as described below.

The study was approved by the Bioethical Committee of the Ruhr-University Bochum, and experiments were performed in accordance with accepted guidelines for care and use of animals in research. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Immunocytochemistry

Astrocytes/microglia cocultures (M5 and M30) on poly-L-lysine-coated glass coverslips (12 mm^2 diameter) were washed and fixed in 100% ethanol and treated with PBS blocking solution containing 10% horse serum and 1% bovine serum albumin (BSA) before incubating with the primary antibodies for 60 min at room temperature. Monoclonal mouse antiglial fibrillary acidic protein (GFAP; 1:100; G9269; Sigma; Taufkirchen, Germany) was used; microglia was labeled by using a monoclonal antibody to the ED1 marker (1:250; MCA 341R; Serotec, Eching, Germany), which allowed classification of microglia as resting ramified (RRT) or activated, rounded phagocytic (RPT) phenotypes (Faustmann et al., 2003); and Hoechst 33342 (1:2,500; B2261; Sigma, München, Germany) was used to counterstain the cell nuclei. Conjugated Alexa-Fluor 488 (green) or 568 (red) goat anti-rabbit and goat anti-mouse IgG were applied for 30 min (1:1,000; Molecular Probes, Leiden, The Netherlands) as secondary antibodies. Processed coverslips were observed via confocal scanning microspocy (Zeiss LSM 510 inverted confocal microscope; Zeiss, Göttingen, Germany) at ×630 magnification.

Enzyme-Linked Immunosorbent Assay

Cytokine levels in cell culture supernatants (M5, M30, M30 + LEV) were quantified by ELISA. The Quantikine-ELSIA-Kits were applied (R&D Systems, Minneapolis, MN) for quantification of IL-1β (RLB00), according to the R&D Systems protocol. Fifty microliters of dilutant and 50 μl of each supernatant sample were given to prepared wells and incubated for 2 hr at room temperature. After washing procedures, 100 μl of substrate solution was added to each well. This reaction was stopped after 30 min by adding 100 μl hydrochloric acid solution. Optical densities of each well were

determined by using a microplate reader (Bio-Rad 550) set to 450 nm. Wavelength correction was set to 570 nm.

Concentrations of cytokines were calculated by normalized standard twofold diluted series. All samples were determined in triplicate. All values are medians of three independent experiments. The same statistical analysis was performed as described for functional coupling and MRP (see below).

Administration of the Anticonvulsive Substance LEV, the Inflammatory Cytokine IL-1β, and LPS

Based on clinical findings of Grim and coworkers (2003), LEV at a concentration of 50 μg/ml was used to mimic serum concentrations that were found after 4 weeks of treatment with the sufficient dose of LEV. However, this concentration might be less than that found in the extracellular space in vivo, taking into account the findings of Rambeck et al. (2006), who showed that, similarly to the mechanisms in serum, protein binding play a crucial role in AED concentrations in affected brain sites. In the first set of experiments, the human recombined cytokine IL-1β (500 pg/ml; Pepro-Tech, Rocky Hill, NY) or LPS (100 ng/ml; 026:B6; L2654; Sigma) was added to the primary M5 cocultures for 2 hr. M30 cocultures that had been pretreated with LEV (50 μg/ml; Keppra; UCB Pharma) for 24 hr and M5 cocultures that had been pretreated the same way for 22 hr and additionally with 2 hr of IL-1β (500 pg/ml) or LPS (100 ng/ml) were assessed for the experiments.

Functional Coupling and MRP

The whole-cell patch clamp technique (Axon 200-B patch clamp amplifier; Axon Instruments, Burlingame, CA) was used for simultaneous measurement of MRP and functional coupling, which allows concurrent monitoring of GJIC. For this purpose, patch pipettes (GB 150-8P; Science Products, Hofheim, Germany) with 2–4 MOhm resistance were filled with intracellular solution (135 mM K-gluconate, 20 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES, 10 mM EGTA, pH 7.2) containing 1% (w/v) Lucifer yellow (LY). The number of coupled cells was counted 10 min after dye transfer using a Zeiss Axioskop with an FITC filter set. Significant differences between medians of coupled cells were analyzed by using the Mann-Whitney test (one-tailed; significance was determined at $\star P = 0.05$ and high significance at $\star\star P = 0.001$ or $\star\star\star P = 0.0001$). GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA) was used for statistical analyses and graph design.

Protein Analysis

Astroglial M5 and M30 cocultures as well as M5 cocultures pretreated with IL-1β (500 pg/ml; Pepro-Tech) or LPS (100 ng/ml) treated with LEV 50 μg/ml (LEV 50) for 24 hr were subjected to Western blotting. Cells were lysed in Laemmli lysis buffer, and total protein content was determined by Bio-Rad's Bradford assay. Samples containing 5 μg of total protein were loaded on 10%-SDS gels and transferred onto nitrocellulose membranes by semidry blotting. The membranes were preincubated in 0.5% blocking reagent (catalog No. 1096176; Boehringer, Mannheim, Germany) in PBS for 1 hr and probed with a primary polyclonal affinity-purified antibody (diluted 1:1,000 in 0.2% blocking solution) from rabbit directed to the carboxy terminus of Cx43 (amino acids 360–382; Hofer et al., 1996) at 4°C overnight. The membranes were then washed twice in PBS + 0.05% Tween 20 (PBS-T) for 10 min and once in PBS for 5 min, followed by incubation with a horseradish peroxidase-coupled secondary anti-rabbit IgG antibody for 1 hr. After four washes in PBS-T, signals were visualized by using an ECL Kit (Amersham Pharmacia, Freiburg, Germany) according to the manufacturer's directions.

The membranes were rinsed with stripping buffer (62.5 mM Tris-HCl, pH 6.8, 100 mM β-mercaptoethanol, 2% (w/v) sodium dodecyl sulfate) and probed with anti-β-actin antibody (catalog No. A5441; Sigma, Taufenkirchen, Germany, 1:1,000 in 0.2% blocking solution) as described above to verify equal protein content in the blotted samples. The exposed X-ray films were scanned (Arcus II scanner, Agfa, Taiwan) and densitometrically evaluated with TINA software, version 2.09 (Raytest GmbH, Straubenhardt, Germany).

RESULTS

Definition of the Culture Conditions

As we described previously (Faustmann et al., 2003), the activation of microglia in a microglia/astrocyte coculture system can be achieved through variation of the amount of microglial cells. Whereas, in cultures with a low fraction of microglia (M5), the RRT dominates (Fig. 1A), M30 cocultures are characterized by an increase in the activated RPT (Fig. 1B).

ELISA

In M5 coculture supernatants, low concentrations of IL-1β were found (41.87 ± 6.3 pg/ml, n = 3). In M30 cocultures, IL-1β was almost six times higher than in M5 cocultures (242.4 ± 28.2 pg/ml, n = 3, $P < 0.05$), whereas M30 cocultures treated with levetiracetam at a concentration of 50 μg/ml for 24 hr revealed a decreased level of IL-1β (140.46 ± 15.2 pg/ml, n = 3, $P = 0.05$; Fig. 2). The increased release of IL-1β in M30 cocultures strengthens the assumption of inflammatory characterics of astroglial/microglial cocultures containing a high fraction of microglia.

GJIC

The number of coupled astrocytes in the cocultures was quantified at 10 min after LY application. M5 cocultures revealed a significant decrease of coupled cells when incubated with the proinflammatory cytokine IL-1β (M5: 36.9 ± 4.2, n = 30; M5 + IL-1β: 3.1 ± 0.7, n = 11, $P < 0.0001$) and LPS (2.25 ± 0.6, n = 12, $P < 0.0001$). Similar results were found for the M30 cocultures (13.6 ± 12.3, n = 19, $P < 0.0001$), demonstrating the effects of activated microglia on the astrocytic syncytium (Figs. 3, 4). The application of 50 μg/ml LEV for 24 hr to the M5 cocultures significantly

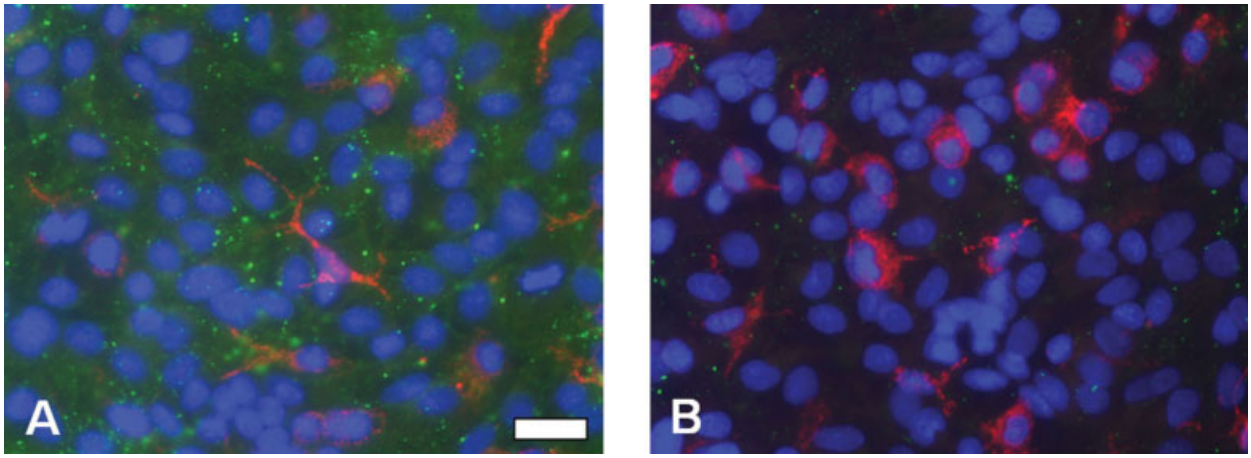

Fig. 1. Astroglial/microglial cocultures containing either 5% (M5) or 30% (M30) microglial cells. As shown in the ED1 immunostaining, the M5 cocultures contained mainly microglial cells of resting ramified type (RRT; **A**), whereas the M30 cocultures revealed a high fraction of activated round, phagocytic type (RPT) microglia (red; **B**). Scale bar = 40 μm.

restored the impaired coupling efficiency caused by incubation with IL-1β (14 ± 3.6, n = 10, $P = 0.0062$) and LPS (7.1 ± 2.6, n = 9, $P = 0.0345$). The incubation of the M30 cocultures with 50 μg/ml of LEV for 24 hr revealed an increase in coupled cells to 55.4 ± 12.3 (n = 16, $P = 0.0006$; Fig. 4). For M5, the incubation with 50 μg/ml LEV did not have any significant effects on the astrocytic coupling (39.6 ± 7.7, n = 12, $P = 0.3797$; Fig. 4).

MRP of Astrocytes

In M5 cocultures incubated with PBS, which served as controls, MRP was –74.9 ± 1.6 mV (n = 30), which is in the range of normal astrocytic MRP. A significant MRP depolarization to –48.4 ± 3.0 mV was induced by IL-1β (n = 11, $P < 0.0001$) and –62.7 ± 2.9 mV measured in M30 cocultures (n = 19, $P = 0.0001$; Fig. 5). M5 cocultures incubated with LEV at a concentration of 50 μg/ml showed no significant alteration vs. control samples (–74.7 ± 2.3 mV, n = 12, $P = 0.4889$; Fig. 5).

Prevention of the inflammatory effects on MRP of astrocytes could be shown under pretreatment with 50 μg/ml LEV for 24 hr: in M30 cocultures, an MRP of –76.2 ± 3.1 mV (n = 16, $P = 0.0023$) could be observed (Fig. 5). In M5 cocultures incubated with IL-1β, the MRP was –66.7 ± 2.7 mV (n = 10, $P = 0.0004$) when pretreated with LEV (50 μg/ml; Fig. 5).

Quantitative Analysis of Cx43 Protein Expression

Cx43 revealed a typical three-banded pattern on Western blots, which originates from its differently phosphorylated isoforms (Laird et al., 1991). To quantify the Cx43 concentration, density profiles of the three bands of treated cultures were obtained by assessing their densitometric values and set in ratio to the signals of the three bands from control cultures. PBS-treated cultures served as controls. Control medians were set to the hypothetical value of 100%, to which values for stimulated samples were normalized. The Cx43 concentration was found to decrease for M30 primary astrocyte/microglia cocultures to 49.2% ± 5.5% (n = 3). Cx43 concentration appeared at control level after pretreatment of the cocultures with LEV 50 (103.8% ± 9.0%; n = 3) for 24 hr (Fig. 6). No significant changes in Cx43 expression were observed after LEV treatment of M5 cocultures preexposed to IL-1β (500 pg/ml) or LPS (100 ng/ml; data not shown).

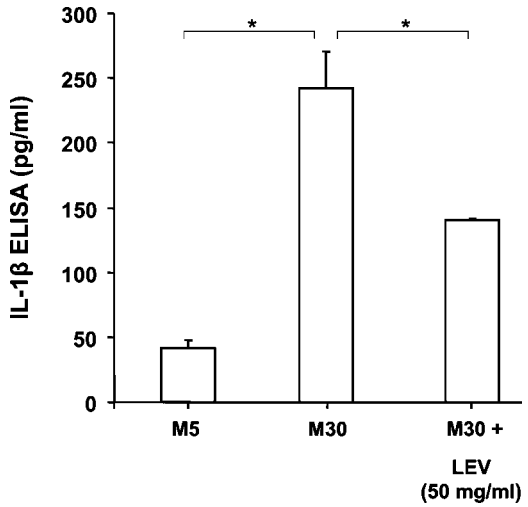

Fig. 2. ELISA IL-1β concentration in M5 vs. M30 cocultures. In comparison with M5 cocultures, supernatants of M30 cocultures revealed a six times higher concentration of IL-1β. The enhanced IL-1β level in M30 cocultures was decreased by LEV. *$P < 0.05$.

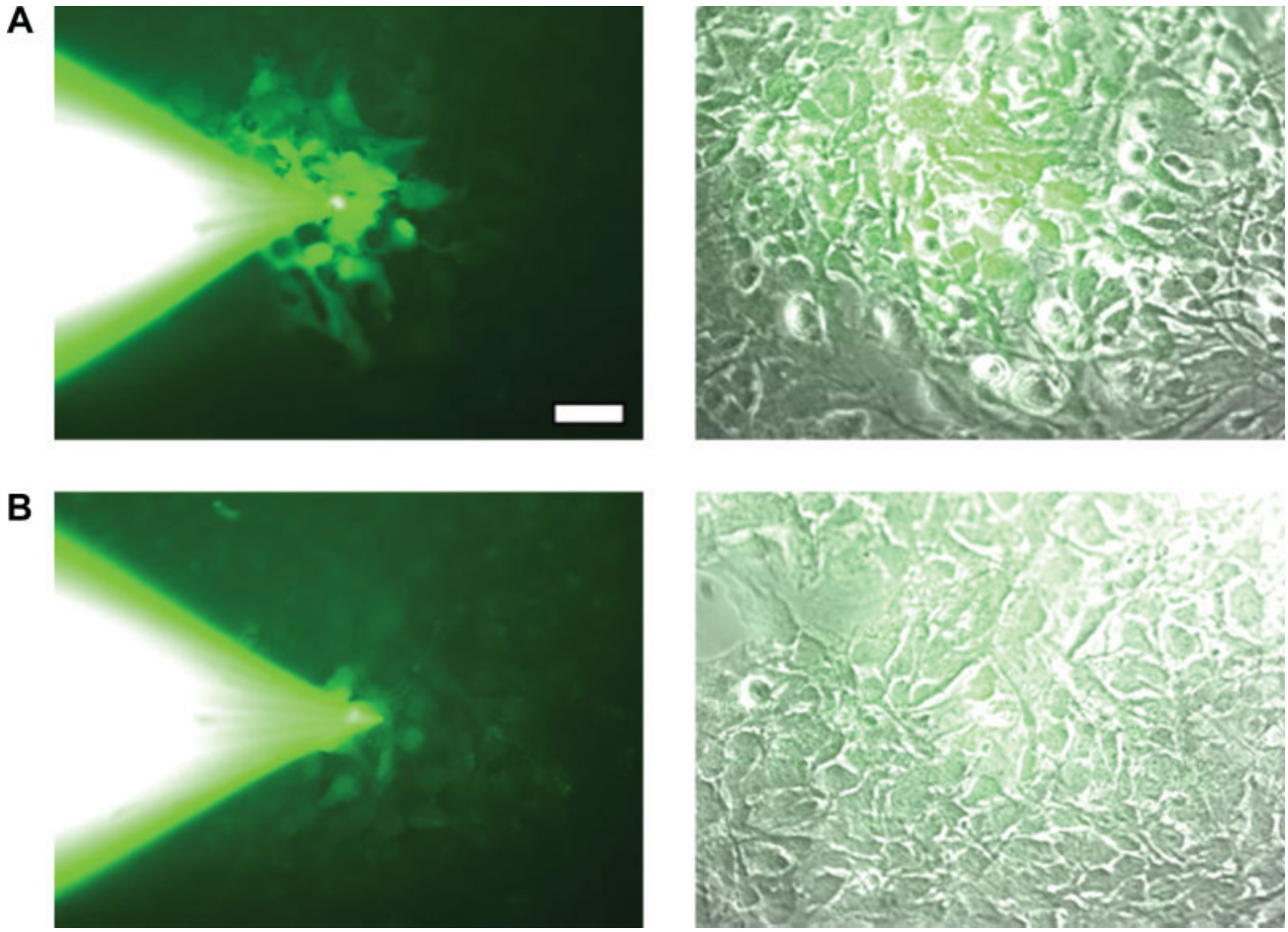

Fig. 3. Astroglial coupling in M5 and under inflammatory conditions in M30 cocultures (left, fluorescent; right, phase contrast). A high level of Lucifer yellow transfer was found under noninflammatory condotions in M5 (**A**) compared with low coupling under inflammatory conditions in M30 (**B**). Scale bar = 40 μm.

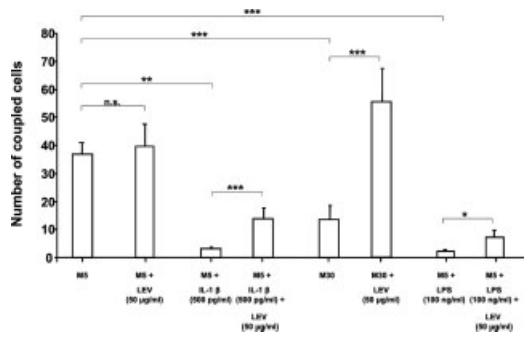

Fig. 4. Coupling efficiency after Lucifer yellow application using the patch-clamp technique in astroglia/microglia cocultures. The number of coupled cells was significantly reduced in the M5 cocultures treated with IL-1β (500 U/ml) and LPS (100 ng/ml) and in the M30 cocultures. Preincubation of the M5 cocultures with LEV (50 μg/ml) partially reversed the impaired coupling efficiency of astroglial cells elicited by IL-1β and LPS. The number of coupled cells in the M30 cocultured was also strongly increased after treatment with LEV (50 μg/ml). $\star P < 0.05$, $\star\star P < 0.001$, $\star\star\star P < 0.0001$.

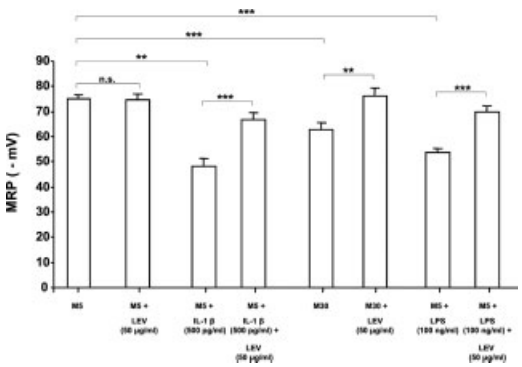

Fig. 5. Astroglial membrane resting potential under noninflammatory and inflammatory conditions. Addition of LEV at 50 μg/ml to the M5 cocultures did not significantly affect the astroglial MRP under noninflammatory conditions. The astroglial MRP was significantly depolarized in the IL-1β (500 U/ml)- and LPS (100 ng/ml)-preincubated M5 cocultures and in the M30 cocultures. Depolarization of MRP under the inflammatory conditions—IL-1β and LPS exposure and activated culture conditions (M30)—could be significantly reduced by application of LEV (50 μg/ml). $\star\star P < 0.001$, $\star\star\star P < 0.0001$.

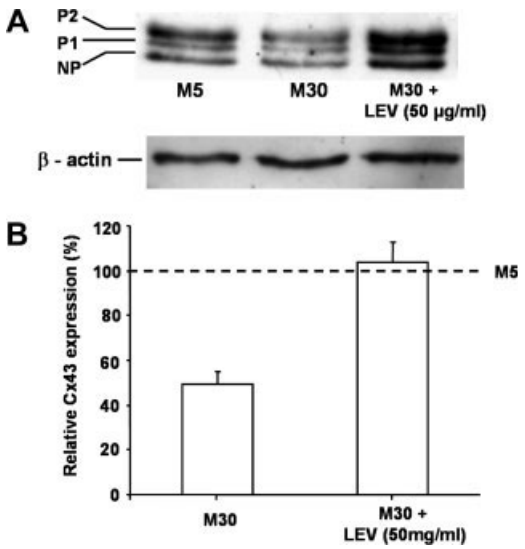

Fig. 6. The expression of Cx43 is reduced in the M30 coculture but can be restored by treatment with LEV. **A:** Western blot analysis of Cx43 expression was performed in the M5 cocultures and M30 cocultures with and without application of LEV (50 µg/ml). **B:** Densitometric evaluation of immunoblotting revealed a significant downregulation of the astroglial Cx43 expression under activated culture conditions (M30), which was raised to control level (M5) by application of LEV (50 µg/ml).

DISCUSSION

Several clinical trials of major study groups have demonstrated that adjunctive therapy with LEV was superior to placebo in suppressing seizures in patients with refractory partial epilepsy (Ben-Menachem and Falter, 2000; Cereghino et al., 2000). More recent studies even suggested that the use of LEV as monotherapy is safe and effective for partial seizures (Ben-Menachem, 2003). However, the anticonvulsive mechanisms of LEV are not completely understood, and many experimental studies performed so far have distinguished LEV from other AEDs in its structure, pharmakokinetics, and molecular effects (Patsalos, 2000; Klitgaard, 2001).

Some AEDs, such as phenytoin, carbamazepine, valproate, and Lamotrigine, are known to act on neuronal excitatory Na^+ channels (Macdonald and Kelly, 1995), whereas LEV failed to modify the profile of voltage-gated tetrodotoxin-sensitive inward Na^+ current in rat neocortical neurons. Likewise, a lack of effect on low-voltage-gated (T-type) Ca^{2+} current in hippocampal neurons has been reported (Zona et al., 2001), whereas an incomplete inhibition of high-voltage-activated Ca^{2+} current of N-type Ca^{2+} has been ascribed to LEV (Niespodziany et al., 2001; Lukyanetz et al., 2002).

As far as the GABAergic system is concerned, the existing results remain obscure. On the one hand, LEV appears to be devoid of impact on GABA metabolism and transport (Sills et al., 1997; Fraser et al., 1999) and fails to interact with the benzodiazepine site of the $GABA_A$ receptor (Klitgaard et al., 1998). On the other hand, it has also been reported that systemic administration of LEV induces alterations in GABA metabolism and turnover and that LEV reverses the action of negative allosteric modulators of neuronal GABA currents, such as zinc (Rigo et al., 2002).

With regard to the type of seizure activity, LEV lacks potent anticonvulsant activity in the acute maximal electroshock seizure test and in the maximal pentylenetetrazol seizure test in rodents (Loscher and Honack, 1993; Klitgaard et al., 1998) but shows potent protection against generalized epileptic seizures in electrically and pentylenetetrazol-kindled (Gower et al., 1995; Klitgaard et al., 1998).

Given these findings, LEV appears to have selective anticonvulsant activity in animal models of chronic epilepsy rather than in acute seizure models and exerts its effect through a distinctive profile of a mechanism that does not involve direct influence on synaptic neurotransmission via conventional ligand–receptor interaction with the classical receptors that are known to be targeted by other AEDs (Noyer et al., 1995; Klitgaard, 2001). Thus, recent studies were devoted to exploring alternative pathways that may account for the effects of LEV. For example, Lynch and coworkers (2004) identified the synaptic vesicle protein SV2A as the brain binding site of LEV. This membrane glycoprotein has been suggested to act as a modulator of vesicle fusion and thus to be of major importance for the neuronal release propabilitiy of synaptic vesicles (Xu and Bajjalieh, 2001). Another example of alternative effects of LEV is its impact on the synthesis of brain-derived neurotrophic factor (BDNF) and inducible nitric oxide synthase (iNOS) in astrocytes as possible candidates for targets of antiepileptic treatment, which has only rarely been considered so far (Cardile et al., 2003; Pavone and Cardile, 2003).

To explore the impact of LEV on glial cells, the focus of the present study was to investigate whether the antiepileptic profile of LEV involves modulation of impaired astroglial properties under inflammatory conditions. We had previously shown that primary astrocytic cultures of newborn rats that are cocultured with 30% (M30) microglial cells or incubated with IL-1β provide suitable environment for such investigations as demonstrated by the high fraction of activated microglia indicative for inflammatory responses (Faustmann et al., 2003; Hinkerohe et al., 2005). This model is characterized by a decrease in astroglial Cx43 expression, an impaired functional coupling within the astroglial syncytium, and a depolarized astrocytic MRP. The treatment of this inflammatory culture model with LEV displayed a modification of the functional properties of astrocytes.

LEV at therapeutic concentration (50 µg/ml) enhanced Cx43 expression in M30 cocultures and coupling strength of astrocytes and normalized the MRP to physiological levels. The ELISA results showed an increase of the inflammatory cytokine IL-1β in the supernatants of the M30 cocultures, which provides clear evidence that the morphological transformation of the microglia in these cultures is accompanied by functional changes (Fig. 2). Moreover, the ability of LEV to

decrease the enhanced IL-1β level in M30 cocultures shows an antiinflammatory mechanism. The finding that GJIC (Fig. 4) in IL-1β-treated M5 cocultures at the concentration of 500 pg/ml was only partially recovered after LEV treatment most likely is due to the twofold concentration of IL-1β compared with IL-1β measured in M30 cocultures (Fig. 2). Similarly, LEV had only limited effect on GJIC of LPS-treated M5 cocultures. This can be explained by a stronger and a broader range of LPS-induced cytokine sekretion (Saukkonen et al., 1990; Lee et al., 1993). The missing effect of IL-1β and LPS on Cx43 expression, which does not meet the results observed in M30 cocultures, suggests that mechanisms other than Cx43 expression are involved in altered GJIC. A feasible mechanism is the modification of Cx43 in astrocytic gap junctions, e.g., through phosphorylation, which results in impaired GJIC. It has been described that phosphorylation of Ser or Tyr residues in the C-terminal domain of Cx43 regulates the gating of gap junction channels and that this effect is achieved through activation of protein kinase C (PKC; Lampe et al., 2000). In this context, PKC has been shown to be among the distal effectors of IL-1β in modulating GJIC (Zvalova et al., 2004).

Epilepsy and Inflammation

The finding that LEV exhibits reversal of affected astroglial properties from inflammatory conditions supports the idea of a link between inflammatory processes and seizure activity. Indeed, in a number of recent studies, it has been suggested that activation of the innate immune system, such as the production of proinflammatory cytokines, accompanies the molecular and structural changes that take place during and after seizure activity (Vezzani, 2005). This is supported by clinical findings in which elevated levels of proinflammatory cytokines were observed in the cerebrospinal fluid from patients with recent epileptic seizures (Peltola et al., 2000) and in surgically resected human brain tissue from patients with intractable epilepsy (Sheng et al., 1994). Also, in various experimental seizure models, a rapid increase of proinflammatory cytokines on protein level as well as upregulated expression of messenger RNA has been observed after seizure induction (Gahring et al., 1997; Oprica et al., 2003). In particular, the accumulation of IL-1β persisting for 60 days (De Simoni et al., 2000) implies a prolonged activation of the immune system, which strengthens the idea that inflammation is linked to epileptic activity rather than being a mere event with minor implications. Furthermore, immunohistochemical studies demonstrated the enhancement of IL-1β accompanied by an increase of activated microglial cells after kainic acid-induced seizures (Vezzani et al., 1999).

Among the effector cells to respond to activated microglia, atrocytes are of major importance, insofar as they undergo loss of multiple regulatory properties, such as ion and neurotransmitter uptake and dissipation, which are crucial for the maintenance of efficient interneuronal signalling in normal brain. For example, glutamate uptake by the astrocytes is a well-known mechanism that provides low extracellular levels of glutamate, which are essential for proper neuronal activity (Oprica et al., 2003). There is clear evidence for a direct link between coupling capacity of astrocytes and uptake of glutamate. It was recently shown that decoupling of astrocytes with gap junction blockers resulted in decreased expression of the astrocytic glutamate transporter 1, which constitutes the major glutamate transporter subtype in the cortex (Figiel et al., 2007). Hence, impairment of GJIC, e.g., by IL-1β, possibly promotes proconvulsant effects through inhibition of glutamate uptake, leading to an increase in glutamate available for the activation of N-metyl-D-aspartate (NMDA) and non-NMDA receptors (Scimemi et al., 2005).

In conclusion, the results of our study suggest that the efficacy of LEV derives in part from its ability to prevent impairment of astroglial regulatory properties under inflammatory conditions. This observation contributes to a better understanding of how glial cells participate in seizure disorder. In this regard, astrocytes may serve as candidates for potential targets of future anticonvulsant therapeutics in consideration of antiinflammatory and neuroprotective aspects, an issue that has been largely ignored so far.

ACKNOWLEDGMENTS

The authors thank Sabine Schreiber-Minjoli, Bernhard Vornefeld, Monika Birkelbach, and Markus Wüthrich for excellent technical assistance. Levetiracetam was kindly provided by UCB Pharma.

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