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# Effects of new antiepileptic drugs and progabide on the mitogen-induced proliferative activity of mouse splenocytes

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**Abstract:**
Classical antiepileptic drugs are known to affect immune system activity, although the effects of new generation anticonvulsants on T- and B-cell-mediated immunity remain unknown. Therefore, in the present study, we compared a selection of new antiepileptic drugs with classical ones in terms of their effects on the proliferative activity of lymphocytes stimulated by concanavalin A (Con A) and lipopolysaccharide (LPS). Felbamate ($3 \times 10^{-6} – 10^{-4}$ M) was the most potent in inhibiting [$^3$H]-thymidine incorporation in C57BL/6 mouse spleen cells stimulated by Con A and LPS. Treatment of the cells with stiripentol ($3 \times 10^{-5}$ and $10^{-4}$ M) and loreclezole ($10^{-4}$ M) suppressed the proliferative activity of splenocytes both after Con A and LPS stimulation**.** Tiagabine ($3 \times 10^{-5}$ M and $10^{-4}$ M) inhibited the Con A-induced cell proliferation, whereas the effect of LPS was attenuated only by the highest concentration of this drug ($10^{-4}$ M). Progabide showed immunosuppressive effects at concentrations of $3 \times 10^{-5}$ and $10^{-4}$ M or only $10^{-4}$ M after LPS or Con A stimulation, respectively. No effect on the immune parameters was observed after lamotrigine treatment. On the other hand, the Con A-induced proliferation of splenocytes was enhanced by carbamazepine ($10^{-5} – 10^{-4}$ M) and sodium valproate ($5 \times 10^{-4} – 3 \times 10^{-3}$ M). Neither carbamazepine nor sodium valproate affected the LPS-induced proliferation. These data indicate that some new antiepileptic drugs, especially felbamate at pharmacological concentrations, may suppress the mitogen-stimulated proliferative activity of mouse splenocytes. In contrast, two classical anticonvulsants (carbamazepine and sodium valproate) stimulated T-cell-mediated immunity. The above differences in the effects of particular antiepileptic drugs on the immune response may play roles in both their therapeutic efficiency and undesired effects.

**Key words:**
antiepileptic drugs, mitogens, proliferative activity of splenocytes

## Introduction

Epilepsy is a common neurological disorder whose etiopathogenesis has been only partially elucidated. Recent reports have indicated a considerable contribution of humoral and cellular components of the immune system and cytokines to the etiology of epilepsy [1, 18]. Clinical studies have shown changes in Ig production (e.g., a decrease in immunoglobulin (Ig)A and an increase in IgM) in sera of epileptic patients. Furthermore, a decrease in the number of CD4+ lymphocytes, an increase in CD8+ lymphocytes, and a decrease in the CD4+/CD8+ ratio have been detected in various kinds of epilepsy. Other data point to the possible role of cytokines in the pathomechanisms of seizures. In regards to this, proinflammatory cytokines have been shown to provoke epileptic seizures in several experimental models of epilepsy. On the other hand, seizures have been shown to increase the production of proinflammatory and pro-convulsive cytokines [e.g., interleukine (IL)-1β, tumor necrosis factor-α (TNFα), IL-6] and their signaling transducer protein, Gp130, in rats [16]. In line with these findings, an elevated serum level of IL-6 and a decrease in IL-1R was found in patients with drug-resistant epilepsy. On the other hand, antiepileptic treatment is also connected with the modulation of the cellular and humoral response; however, the relevant data are scarce and focus only on a few classical drugs. Experimental studies in rodents have revealed that phenytoin and carbamazepine decrease both the humoral and cellular response, and an involvement of CD8+ cells in these effects has been suggested. It has also been reported that valproate and phenobarbital diminish the humoral response and lymphocyte T cytotoxicity in mice, respectively. Furthermore, the withdrawal of carbamazepine and phenytoin augments an autoimmune response in experimental encephalomyelitis in mice [5]. In line with the results of the animal studies, clinical data have shown that phenytoin, carbamazepine, and valproate predominantly possess immunosuppressive activity, inhibit protein synthesis in lymphocytes, decrease the CD4+/CD8+ ratio and IgA concentration, and decrease or elevate the levels of IgG and IgM [2, 7, 10, 27]. On the other hand, some reports indicate that carbamazepine and its metabolites can also stimulate T-cell proliferation *via* a direct interaction with major histocompatibility complex (MHC) and specific T-cell receptors [32].

Antiepileptic drugs also affect proinflammatory cytokine production, especially when used in polytherapy. In epileptic patients treated with carbamazepine or phenytoin, blood levels of IL-2 or IL-1, respectively, were increased. Furthermore, carbamazepine elevates IL-1α, IL-1β, IL-2 and IL-6 serum concentrations, whereas valproate decreases IL-2, IL-6 and TNF-α levels [14, 22, 30]. *In vitro* studies have revealed that carbamazepine inhibits IL-2 and IL-4 but enhances IL-10 and transforming growth factor-β production. On the other hand, valproate inhibited TNF-α and IL-6 production *in vitro*, probably *via* its action on nuclear factor-κB, whereas this drug enhanced IL1, IL-6 and IL-5 levels in patients. Hypersensitivity of the immune system has been observed in some human subjects treated with lamotrigine, carbamazepine, phenobarbital and phenytoin. The suggested mechanism of this phenomenon involves the activation of drug-specific CD4+ and CD8+ cells, an increase in IL-4 and IL-5 levels, T-cell receptor polymorphism, or a direct effect of drugs on T lymphocyte receptors [17, 20, 21].

In recent decades, a number of new-generation antiepileptic drugs with various chemical structures have been introduced to clinical practice. However, their influence on the immune system activity remains largely unknown. The only report dealing with the effects of new-generation anticonvulsants on peripheral parameters of the immune system revealed that topiramate reversed the kainate-induced decrease in T lymphocyte proliferative activity in rats [15]. Therefore, in the present study, we compared selected new antiepileptic drugs with two classical drugs in terms of their effects on the proliferative activity of lymphocytes stimulated by concanavalin A (Con A) and lipopolysaccharide (LPS). To this end, we chose new antiepileptic drugs with different mechanisms of action (e.g., tiagabine, stiripentol, loreclezole, felbamate, lamotrigine) and compared their effects with those exerted by carbamazepine and sodium valproate. In addition, progabide was included in this study.

## Materials and Methods

### Animals

The experiments were performed on male C57BL/6 mice, about 3 months of age. The animals were housed

in groups of five per cage under standard animal housing conditions (e.g., a room temperature of 23°C and a 12/12h light/dark cycle, with the light on at 08:00) in the animal facility of the Department of Experimental Neuroendocrinology. The mice had free access to food and water. All the tests were approved by the Animal Care and Use Committee of the Institute of Pharmacology, Polish Academy of Sciences in Kraków, and met the requirements of the International Guide for the Care and Use of Laboratory Animals.

### Immunological experiments

The animals were sacrificed by cervical dislocation. Their spleens were removed under laminar flow, sterile homogenized in a glass homogenizer, suspended in RPMI-1640 medium (Sigma, USA) and centrifuged at 500 × g for 5 min. Cell pellets were resuspended in medium supplemented with antibiotics (50 μg/ml of penicillin, 50 μg/ml of streptomycin), 10% fetal bovine serum and 2 mM L-glutamine (all reagents from Sigma, USA) for proliferative activity study.

### Proliferative response of splenocytes to mitogen stimulation *in vitro*

The proliferative response of spleen cells has been described earlier by Basta-Kaim et al. [3]. Briefly, $4 \times 10^5$ cells per ml were stimulated with 2.5 μg/ml of concanavalin A (Con A) or 5 μg/ml of LPS (Sigma, USA). The cells were incubated in 96-well plates at 37°C at a final volume of 0.2 ml (0.1 ml of the splenocyte suspension and 0.1 ml of medium or respective mitogens) for 72 h. Cell proliferation was determined by adding 10 μl (0.5 μCi) of [$^3$H]-thymidine per well (ICN Pharmaceuticals, USA; 6.7 Ci/mmol) 16 h before the end of the incubation. The cultures were harvested with an automatic cell harvester (Scatron, Norway) and [$^3$H]-thymidine was estimated using a liquid scintillation counter (Beckman LS 335).

### Drug treatments

In order to determine the influence of antiepileptic drugs on splenocyte proliferative activity, splenocytes were cultured in the presence of progabide, stiripentol, loreclezole, lamotrigine, tiagabine and carbamazepine at the final concentrations of 3, 10, 30 or 100 μM, with felbamate at concentrations of 1, 3, 10, 30 or 100 μM and sodium valproate at concentrations of 0.01, 0.1, 0.5, 1 or 3 mM for 3 days. The control cultures were supplemented with the same amount of an appropriate vehicle. Progabide, topiramate, stiripentol, loreclezole, lamotrigine, tiagabine, carbamazepine and sodium valproate were dissolved in a small amount of ethanol, followed by dilution in water (the final concentration of ethanol was below 0.5%). Felbamate was dissolved in DMSO (below 0.5%) followed by dilution in water. All reagents were sterilized by filtration. Drug sources have been listed elsewhere [4].

### Effect of antiepileptic drugs on cell viability

Splenocytes were treated with the vehicle or antiepileptic drugs (at concentrations used in the proliferation study) for 3 days. The effect of drugs on cell viability was determined by counting viable and non-viable (blue) cells in a hemocytometer. Briefly, the cell suspensions were mixed (at a 1:1 ratio) with a 0.4% trypan blue, and the number of non-viable cells per a total of 100 cells was counted.

### Statistical analysis

The data are presented as the means ± SEM of five to seven independent experiments (in triplicate wells) and the significance of differences between the means was evaluated by Duncan's test following a one-way analysis of variance (ANOVA).

## Results

Treatment of the splenocytes with antiepileptic drugs and progabide (in concentrations used in our study) for 3 days had no statistically significant effect on the cell viability (data not shown).

Felbamate ($3 \times 10^{-6}$ – $10^{-4}$ M) showed the most potent effect in inhibiting the [$^3$H]-thymidine incorporation into C57BL/6 mouse spleen cells stimulated by Con A and LPS (Fig. 1A, 2A). Treatment of the cells with stiripentol ($3 \times 10^{-5}$ and $10^{-4}$ M) and loreclezole ($10^{-4}$ M) suppressed the proliferative activity of splenocytes after both Con A and LPS stimulation (Fig. 1A, 2A). Tiagabine at $3 \times 10^{-5}$ M and $10^{-4}$ M inhibited the Con A-induced cell proliferation (Fig. 1A), whereas the effect of LPS was attenuated only by the highest concentration of this drug ($10^{-4}$ M) (Fig. 2A).

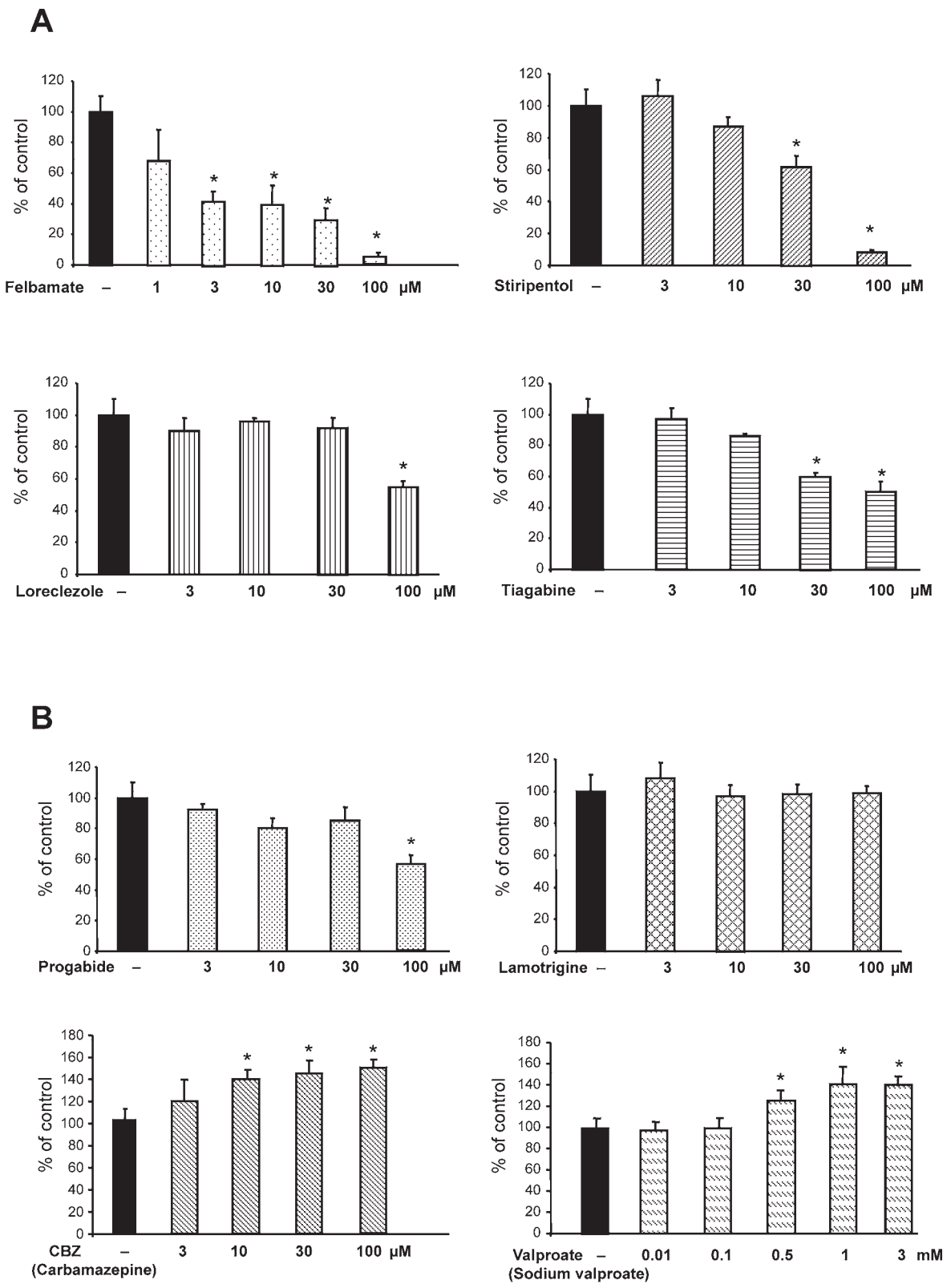


**Fig. 1. (A, B)** The effect of antiepileptic drugs applied at the indicated concentrations for 3 days on the proliferative activity of splenocytes stimulated by concanavalin A (Con A, 2.5 µg/ml). All results are shown as the mean ± SEM of percentage of the control from 5–7 independent experiments (in triplicate wells). The percentage of the control is a ratio of the mean counts of [$^3$H]-thymidine incorporated in the presence of the drug to the mean counts incorporated without the drug, × 100. The significances of differences between the means was evaluated by the Duncan's test following a one-way analysis of variance (* $p < 0.01$ *vs.* control group)

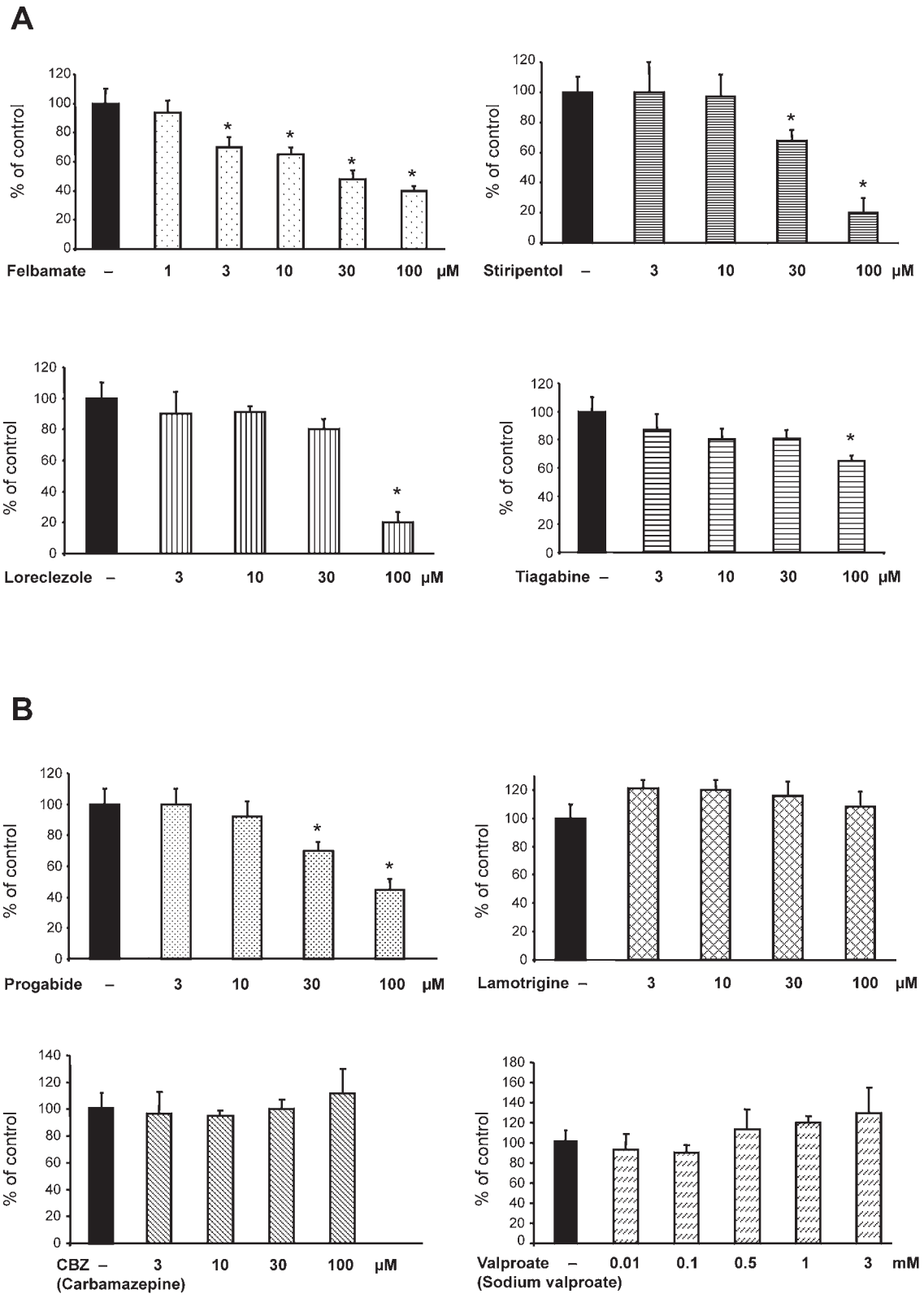


**Fig. 2. (A, B)** The effect of antiepileptic drugs applied at the indicated concentrations for 3 days on the proliferative activity of splenocytes stimulated by lipopolysaccharide (LPS, 5 μg/ml). All results are shown as the mean ± SEM of the percentage of the control from 5–7 independent experiments (in triplicate wells). The percentage of the control is a ratio of the mean counts of [$^3$H]-thymidine incorporated in the presence of the drug to the mean counts incorporated without the drug, × 100. The significances of differences between the means was evaluated by the Duncan's test following a one-way analysis of variance (* $p < 0.01$ *vs.* control group)

Progabide showed immunosuppressive effects at concentrations of $3 \times 10^{-5}$ and $10^{-4}$ M or only $10^{-4}$ M after LPS or Con A stimulation, respectively. No effect on the immune parameters was observed after lamotrigine administration (Fig. 1B, 2B). On the other hand, the Con A-induced proliferation of splenocytes was enhanced by carbamazepine ($10^{-5} – 10^{-4}$ M) and sodium valproate ($5 \times 10^{-4} – 3 \times 10^{-3}$ M) (Fig. 1B). Neither carbamazepine nor sodium valproate affected the LPS-induced proliferation (Fig. 2B).

## Discussion

The new antiepileptic drugs generally possess a better pharmacological profile in experimental studies than the conventional drugs do and show predictable pharmacokinetics [6, 9], but their mechanisms of action and possible undesired effects have not been fully investigated. This is the first report showing that some new antiepileptic drugs can suppress *in vitro* proliferative activity of splenocytes. We found that treatment of C57BL/6 mouse spleen cells with felbamate, tiagabine, stiripentol and loreclezole suppressed the proliferative activity of these cells after both Con A and LPS stimulation. Furthermore, the antiproliferative effects of the new antiepileptics on splenocytes can be observed at micromolar concentrations; i.e., the pharmacologically relevant antiepileptic drug concentrations in the brain tissue. The most potent in this respect was felbamate, which inhibited cell proliferation at a concentration as low as 3 μM. Despite the efficiency of this drug in the treatment of partial refractory epilepsy, its clinical use is limited since it might induce aplastic anemia and hepatic failure [25]. Interestingly, these fatal though rare undesired effects of felbamate may be observed in patients with preexisting immune disorders [23], supporting the notion of a possible interaction of this drug with the immune response. Stiripentol is a well-tolerated drug, but it can sometimes induce transient aplastic anemia and leukopenia [29]. There is only one report on the interaction of tiagabine with the immune system. It has been reported that, in the olfactory bulbectomized rat model of depression, tiagabine normalized the reduced lymphocyte and increased neutrophil counts [24]. No data on the effect of loreclezole or progabide on the immune system has been reported so far. It must be underlined that a majority of studies describe the immune effects of traditional antiepileptics, such as phenytoin, carbamazepine, phenobarbital or valproate [12]. An early clinical report showed that phenytoin, and to a lesser extent carbamazepine, depressed the cellular and humoral immune responses due to their direct effect on the metabolism of lymphoid cells in epileptic patients [27]. However, a more recent study has indicated that the administration of carbamazepine is associated with the activation of T-cells, probably *via* a direct binding to MHC molecules [32]. Our present data show that Con A-induced proliferation of splenocytes *in vitro* is enhanced by both carbamazepine and sodium valproate. It should also be mentioned here that the LPS-induced proliferation has been affected neither by carbamazepine nor sodium valproate, which indicates that the effects of these antiepileptics on splenocytes depend on a specific proliferation-inducing agent and do not involve B lymphocytes. This may partly explain why other investigators have failed to observe the *in vivo* and *in vitro* effects of valproate on immunoreactivity in mice [8, 26]. The ability of carbamazepine and valproate to block voltage-dependent sodium channels is unlikely to be responsible for the inhibition of splenocyte proliferation, since other blockers of these channels either had no effect (e.g., lamotrigine) or even stimulated proliferation (e.g., topiramate) [15]. Although these data suggest that lamotrigine should be a safer drug than carbamazepine as far as the immune system is concerned, some clinical observations indicate that both drugs may induce serious, though rare, hypersensitivity reactions in patients. In fact, the patients with hypersensitive cutaneous responses to lamotrigine were reported to have a highly enhanced number of CD4+ and CD8+ T cells, moderately elevated B lymphocyte (CD19) counts and increased serum IgE levels [13]. The above-mentioned facts clearly indicate that there is no simple relationship between the neuronal mechanism of antiepileptic drug action and the *in vitro* effects on immune cells. It cannot be excluded that conventional antiepileptic drugs affect the proliferative response of splenocytes *via* their interaction with glucocorticoid receptors (GRs). GRs are ligand-dependent transcription factors that regulate the expression of many target genes involved in neurotransmission, metabolism, plasticity and immune system activity. It is commonly accepted that corticosteroids mainly inhibit immune system activity, especially the Th-1 cell-mediated immunity [15, 18, 31]. Recently, we have found that carbamazepine

and valproate decrease functional activity of GR in fibroblast cells *in vitro*, whereas lamotrigine and topiramate showed no activity in this model [4]. Assuming that antiepileptics may exert similar inhibitory actions on GR located on the immune cells, this may explain the stimulatory effect of carbamazepine and valproate on splenocyte proliferation. Indeed, there is a linear relationship between the GR binding and splenocyte proliferation. For example, in transgenic mice showing a decreased GR density, splenocytes have a higher level of proliferative activity and the inhibitory effect of glucocorticosteroids is weaker than in control animals [19]. This mechanism, however, cannot explain the inhibitory effects of new antiepileptic drugs on splenocyte proliferation, since these drugs also inhibit the transcriptional activity of GR [4]. It has been reported that T cells possess functional γ-aminobutyric acid $(GABA)_A$ receptors, activation of which leads to the inhibition of proliferative response of T cells [28]. Among the new antiepileptic drugs under study, felbamate, progabide and loreclezole are all positive modulators of $GABA_A$ receptors and could inhibit splenocyte proliferation *via* this mechanism. On the other hand, tiagabine and stiripentol enhance GABA neurotransmission indirectly *via* the inhibition of GABA uptake, and in the case of the latter drug, also by the inhibition of GABA transaminase. Since GABA transaminase activity in lymphocytes has been proven, it is not unlikely that stiripentol may inhibit splenocyte proliferation by interaction with this enzyme [11]. However, the presence of a GABA uptake mechanism in splenocytes has not been demonstrated so far; thus, the mechanism of action of tiagabine on cell proliferation remains unknown.

Summing up, our data show that some new antiepileptic drugs and progabide at pharmacological concentrations may suppress mitogen-stimulated proliferative activity of mouse splenocytes. In contrast, two classical antiepileptics, carbamazepine and sodium valproate, stimulate T-cell-mediated immunity. The above differences in the immune response to particular antiepileptic drugs may play a role in both their therapeutic efficiency and undesired effects.

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