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Effects of some new antiepileptic drugs and progabide on glucocorticoid receptor-mediated gene transcription in LMCAT cells

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Abstract:
Antiepileptic drugs affect endocrine and immune system activity, however, it is not clear whether these effects are indirect, *via* interference with neurotransmitters, membrane receptors and ion channels or maybe independent of neuronal mechanisms. In order to shed more light on this problem, in the present study, we evaluated effects of some new-generation antiepileptic drugs and progabide as a GABA-mimetic on the corticosterone-induced chloramphenicol acetyltransferase (CAT) activity in mouse fibroblast cells stably transfected with mouse mammary tumor virus (MMTV)-CAT plasmid. Treatment of cells with felbamate for five days inhibited in a concentration-dependent manner (3–100 μM) the corticosterone-induced reporter gene transcription. Progabide and loreclezole also inhibited the corticosterone-induced CAT activity, but with lower potency, and significant effects were observed at 10 to 100 μM concentration. Tiagabine and stiripentol showed less potent inhibitory effect on functional activity of glucocorticoid receptors (GR). In contrast, topiramate and lamotrigine (3–100 μM) failed to affect the corticosterone-induced gene transcription. These data indicate that some new antiepileptic drugs and progabide may suppress glucocorticoid effects via the inhibition of GR-mediated gene transcription. In turn, attenuation of GR function could influence antiepileptic drug effect on seizures, neuronal degeneration and immune system activity.

Key words:
antiepileptic drugs, progabide, glucocorticoid-mediated gene transcription, fibroblast cells

Introduction

It has been firmly established that glucocorticosteroids are important modulators of seizure-related neuronal damage, especially that observed in the hippocampus [13, 16, 28, 29]. Seizures enhance release of glucocorticoids, which may aggravate neurodegenerative processes e.g. kainate-evoked seizures, an animal

model of temporal lobe epilepsy, are associated not only with extensive hippocampal neuronal loss in CA1 and CA3 field but also with enhanced corticosterone plasma level [9]. Glucocorticoids are known to potentiate neurotoxic effects exerted by various insults such as hypoxia, hypoglycemia, antimetabolites and excitatory amino acids (glutamate, kainate) [24, 25, 28]. The mechanism of glucocorticoid neurotoxicity has been only partially unraveled, and it involves the inhibition of glutamate uptake to astrocytes and suppression of glucose uptake to both neurons and astrocytes in the hippocampus [10, 11, 26]. Interestingly, some antiepileptic drugs, besides seizure-suppressing properties, show also neuroprotective activity [8, 15, 30]. Mechanism of neuroprotective effects of anticonvulsants predominantly involves antagonistic action on glutamate receptor and potentiation of GABAergic transmission, whereas their potential interaction with glucocorticosteroid receptors (GR) remains unknown. GRs are ligand-dependent transcription factors, which bind to a specific DNA sequences (glucocorticoid responsive element – GRE) and regulate expression of many target genes involved in neurotransmission, metabolism, neurodegenerative processes and immune system activity. Recently, we found that two classical antiepileptics, carbamazepine and valproate, decreased transcriptional activity of GR *in vitro* [3]. The aim of the present study was to evaluate whether some new-generation antiepileptic drugs may also directly affect GR function. To this end, we chose new antiepileptic drugs with various mechanism of action i.e. topiramate, stiripentol, loreclezole, felbamate, lamotrigine and tiagabine. Progabide, as a GABA-mimetic drug, was also selected. Their effects were determined in fibroblast cells, stably transfected with mouse mammary tumor virus-chloramphenicol acetyltransferase (MMTV--CAT) plasmid (LMCAT cells). Reporter gene transcription was induced by high concentration of corticosterone.

Materials and Methods

Cell culture conditions and treatments

The glucocorticoid receptor-mediated gene expression was determined in mouse fibroblast cells (L929), stably transfected with mouse mammary tumor virus-chloramphenicol acetyltransferase (MMTV-CAT) plasmid (LMCAT cells). The LMCAT cell line was generously provided by Dr. E.R. Sanchez (Department of Pharmacology, Medical College of Ohio, Toledo, OH). The cells were grown in DMEM (Gibco-BRL) with a 10% heat-inactivated fetal bovine serum (Gibco-BRL) and a 0.02% geneticin (Gibco-BRL) at 37°C, at a 5% CO_2/95% air atmosphere.

LMCAT cells, at final confluence of about 80%, were treated for 5 days with progabide (Sanofi-Synthelabo, Paris, France), topiramate (RW Johnson Pharmaceutical, Spring House. PA, USA), stiripentol (a gift from Dr. J. Vincent, Biocodex Laboratoires, Gentilly, France), loreclezole (Janssen Research Foundation, Beerse, Belgium), lamotrigine (Lamictal^R, Glaxo Wellcome, Kent, UK), and tiagabine (Sanofi Winthrop, Gentilly Cedex, France); all the above drugs at concentrations of 3, 10, 30 and 100 μM, and with felbamate (Taloxa^R, Schering Plough, Levallois Paret, France) at concentrations of 1, 3, 10, 30 and 100 μM. The control cultures were supplemented with the same amount of an appropriate vehicle. The medium and drugs were changed once during 5-days culture, on the third day. The CAT activity was stimulated by adding 1 μM corticosterone for 2 h. Progabide, topiramate, stiripentol, loreclezole, lamotrigine, tiagabine and corticosterone 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 followed by dilution in water.

Chloramphenicol acetyltransferase (CAT) activity assay

Cell lysates were prepared by a freezing/thawing procedure [6, 21]. To determine CAT activity, aliquots of lysate (after heating for 10 min at 60°C) were incubated in a 0.25 M Tris-HCl buffer (pH = 7.8) with 0.25 μCi D-threo-[dichloroacetyl-1-^{14}C]-chloramphenicol and 0.2 mM n-butyryl coenzyme A for 1 h at 37°C. The butyrylated forms of chloramphenicol (in direct proportion to the CAT gene expression) were extracted twice with xylene, washed with 0.25 M Tris-HCl buffer, and radioactivity was measured in a β-counter (Beckmann LS 335 liquid scintillation counter). The results are calculated as dpm of a butyrylated fraction of chloramphenicol per 10 μg of protein per hour of incubation, and are expressed as a percentage of the control value (compared to sam-

ples with corticosterone and appropriate vehicle but without the drug). The protein concentration in cell lysates was determined by the method of Lowry et al. [17].

Statistical analysis

The data are presented as the mean ± SEM of five independent experiments (in duplicate wells), and the significance of differences between the means was evaluated by the Dunnett's test following one-way analysis of variance.

Results

Corticosterone added at a concentration of 1 μM for 2 h increased CAT activity about 30-fold (basal CAT activity was 595 ± 55 dpm/10 μg of protein/h; while corticosterone-induced CAT activity was 12,500 ± 1,100 dpm/10 μg of protein/h). As described previously, the effect of corticosterone was blocked by RU-38486 – a specific antagonist of the type II GR [6].

None of the antiepileptic drugs under study or progabide given alone affected the low, non-stimulated CAT activity (data not shown). Treatment of cells with felbamate for five days inhibited in a concentration-dependent manner (3–100 μM) the corticosterone-induced reporter gene transcription (Fig. 1). Lower concentration of felbamate (1 μM) was inactive. Progabide and loreclezole also inhibited the corticosterone-induced CAT activity, but with lower potency, and significant effects were observed at 10 to 100 μM concentration (Fig. 2). Tiagabine and stiripentol showed less potent inhibitory effect on functional activity of GR. Tiagabine significantly decreased CAT activity at concentrations of 30 and 100 μM, whereas stiripentol was active only at the highest (100 μM) concentration. In contrast, topiramate and lamotrigine (3–100 μM) failed to affect the corticosterone-induced gene transcription.

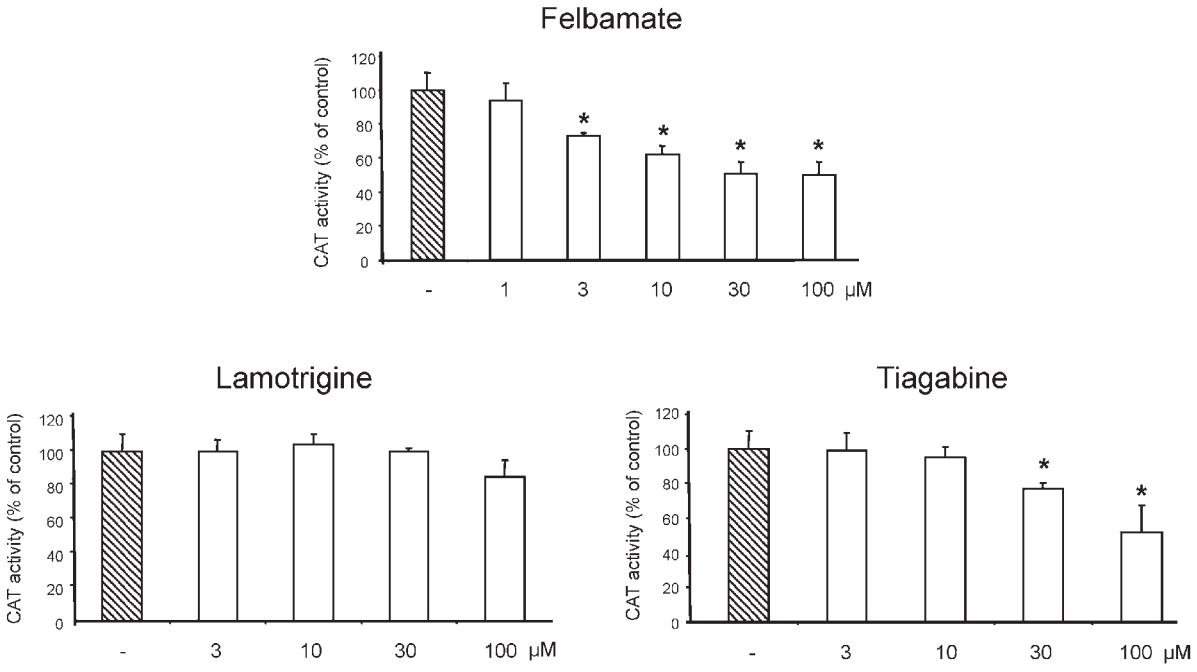

Fig. 1. The effect of felbamate, lamotrigine and tiagabine on the CAT gene transcription induced by corticosterone in LMCAT cells. Antiepileptic drugs were applied at the indicated concentration for 5 days. Corticosterone (1 μM) was added 2 h before harvesting the cells for CAT activity assay. The data were calculated as dpm of the butyrylated fraction of chloramphenicol per 10 μg of protein per hour of incubation averaged over five separate experiments and presented as percentage (± SEM) of control culture (with appropriate vehicle only). The significance of differences between the means was evaluated by the Dunnett's test following a one-way analysis of variance (* $p < 0.001$ *vs.* control group; n = 10)

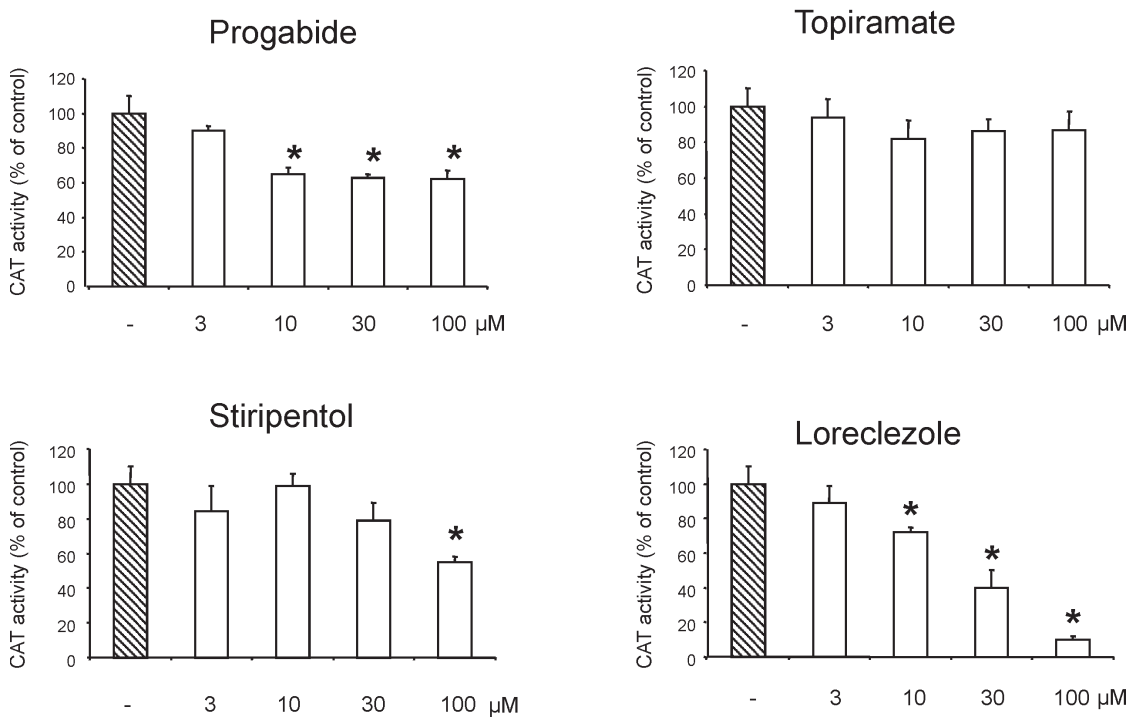

Fig. 2. The effect of progabide, topiramate, stiripentol and loreclezole on the CAT gene transcription induced by corticosterone in LMCAT cells. Antiepileptic drugs were applied at the indicated concentrations for 5 days. Corticosterone (1 μM) was added 2 h before harvesting the cells for CAT activity assay. The data were calculated as dpm of the butyrylated fraction of chloramphenicol per 10 μg of protein per hour of incubation averaged over five separate experiments and presented as percentage (± SEM) of control culture (with appropriate vehicle only). The significance of differences between the means was evaluated by the Dunnett's test following a one-way analysis of variance (* $p < 0.001$ *vs.* control group; n = 10)

Discussion

Ion channels, membrane receptors and enzymes involved in synthesis or metabolism of neurotransmitters are the primary targets of antiepileptic drugs, however, these drugs also affect endocrine and immune systems [7, 19]. The present study demonstrated that some new antiepileptic drugs and progabide at pharmacologically relevant concentrations could inhibit functional activity of GR. It should be emphasized that the interaction of antiepileptic drugs with GR was not connected with their neuronal mechanism of action since the experiments were performed on fibroblast cells. Felbamate, loreclezole and progabide had the most profound inhibitory effects on GR function, moderate attenuation of GR transcriptional activity was seen after tiagabine and stiripentol, whereas lamotrigine and topiramate showed no activity. A question arises whether modification of GR activity may influence pharmacological action of anticonvulsant drugs. Assuming that the antiepileptic drugs will influence the GR receptors in similar way in the brain as they do in LMCAT cells, one should consider an interference with their anticonvulsant, neuroprotective and immunomodulatory action. Glucocorticosteroids have been shown to possess both pro- and anticonvulsant activity, depending on seizure model. The decrease in GR activity may play a beneficial role in absence epilepsy and in suppressing kainate-induced epileptic activity, but not in West syndrome [27, 31, 36]. On the other hand, ability to promote seizure-related brain damage by glucocorticoids is rather firmly established. Felbamate, an NMDA receptor antagonist, possesses well-recognized neuroprotective properties [30], so the ability to decrease transcriptional activity of GR may be an adjunctive mechanism by which this drug prevents neuronal damage [35]. Furthermore, it has been reported that felbamate attenuates stress-induced rise in corticosterone release [22], which also may contribute to its anti-glucocorticoid effect. Loreclezole and progabide, that are

essentially GABA-mimetic anticonvulsants, potently decreased transcriptional activity of GR in LMCAT cells, in fact, the maximal inhibition was detected following 100 μM of loreclezole. No data are available on putative neuroprotective effects of loreclezole or progabide nor on an interaction of these drugs with GR. Interestingly, acute administration of progabide was reported to enhance corticosterone secretion in rats, thus inhibition of GR function may counteract this effect [18]. Another GABA-mimetic, tiagabine, with moderate inhibitory effect on GR function was reported to decrease both seizure- and ischemia-related pyramidal cell damage in the rat hippocampus [32]. Although the authors indicate that GABAergic mechanism is mainly responsible for the tiagabine neuroprotective action, inhibition of GR activity may support survival of the hippocampal neurons. Stiripentol acts through $GABA_A$ receptors in the central nervous system and, like tiagabine, produces a modest effect on GR function in LMCAT cells. No data were published on stiripentol effect on neuronal survival or its interference with glucocorticosteroids. On the other hand, lamotrigine, a voltage-dependent sodium channel blocker and topiramate, an AMPA receptor antagonist, $GABA_A$ receptor positive modulator and sodium channel blocker, did not interfere with the GR activity. Moreover, topiramate did not elevate corticosterone level [12] and no reports on lamotrigine effect on this hormone level have been published.

Besides neuroprotection, the ability of some antiepileptic drugs to attenuate GR function may influence also the immune system activity [14, 15, 19]. A serious undesired effect of anticonvulsant therapy, hypersensitivity syndrome, can be successfully treated with systemic corticosteroids [20, 23]. Since the attenuation of the immune system activity is one of the main roles of corticosteroids, it cannot be excluded that the inhibitory effect of anticonvulsants on the GR function, as demonstrated in the present study, may participate in the pathomechanism of hypersensitivity syndrome. On the other hand, the inhibitory effect of antiepileptic drugs on GR function may explain their therapeutic efficacy in the treatment of corticosteroid-induced mood disorder and psychosis [1, 5, 33, 34]. It should be mentioned here that, like in depression and schizophrenia, an impairment of inhibitory control of the hypothalamic-pituitary-adrenal axis activity also occurs in epileptic patients [38].

Molecular mechanism of antiepileptic drug effect on GR activity has not been studied. However, it is known that transcriptional activity of GR depends on several factors such as synthesis and degradation of these receptors, their phosphorylation status, the amount and activity of GR-associated proteins and efficacy of GR translocation from cytosol to the nucleus. Our previous studies with psychotropic drugs suggest importance of protein kinases such as protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) in the regulation of GR function in LMCAT cells [2, 4, 6]. It was found that also the classical antiepileptic drug valproate, but not carbamazepine, decreased the number of GR, diminished the level of PKCα isoenzyme in the membrane fraction and elevated the activity of c-Jun N-terminal kinase (JNK)-MAPK [4]. Other investigators found that valproate could inhibit GR receptor function by affecting synthesis of cochaperone protein BAG-1 [37]. In contrast to valproate and carbamazepine, no data are available on the influence of new antiepileptics on activity of protein kinases or GR-associated proteins, therefore, the mechanism of their action on GR has to be elucidated in future studies.

Summing up, our data showed for the first time that several new antiepileptic drugs, though with different potency might decrease functional activity of GR in fibroblast cells in a concentration-dependent manner. Assuming that similar interaction may be exerted on GR located on neuronal and immune cells these effects may play a role in effects of antiepileptic drugs on seizures, neurodegeneration and immune system activity.

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