Lamotrigine is an open-channel blocker of the nicotinic acetylcholine receptor

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Lamotrigine is an antiepileptic drug employed in the treatment of partial epilepsies. We studied its possible interaction with channels other than its known therapeutic target, the voltage-gated sodium channel, using the adult muscle nicotinic acetylcholine receptor as a model system. At the single-channel level, lamotrigine caused a dose-dependent (a) diminution in mean open time, (b) increase in mean burst duration and (c) increase in the area of a new closed-time component. A simple linear channel blocking mechanism accounts for these results. Thus, lamotrigine exerts a blocking action on the muscle nicotinic acetylcholine receptor. *NeuroReport* 18:45–50 © 2007 Lippincott Williams & Wilkins.

Keywords: antiepileptic drug, cholinergic receptor, single-channel

Introduction

It has long been established that both peripheral and neuronal nicotinic acetylcholine receptors (AChRs) are pentameric oligomers [1–3]. In muscle, AChRs occur in the combination $2\alpha_1\beta_1\gamma\delta(\varepsilon)$ subunits (ε replacing γ in adulthood). Neuronal AChRs are assembled by combining α and β subunits or, alternatively, a functional receptor can be constituted from the homomeric or heteromeric combination of α subunits. The neuronal AChR subunits described to date are $\alpha_2-\alpha_{10}$ and $\beta_2-\beta_4$ (for a review see [4,5]). A significant homology exists between the different AChR subtypes, all consisting of a large hydrophilic amino-terminal domain, a compact hydrophobic domain divided into three segments, each of 19–27 amino acids, termed M1–M3, a small highly variable hydrophilic domain and finally a hydrophobic C-terminal domain of approximately 20 amino acids, termed M4 [3].

Although considerable advances have been made in understanding the structural and functional properties of neuronal AChRs, little is known about their physiological role in humans. AChRs are involved in a great number of central nervous system disorders and are thus suitable potential targets for rational drug therapy [4–7].

Lamotrigine (LTG) is a triazine compound chemically unrelated to any other antiepileptic drug (see Fig. 1). The major pharmacological effect reported to date is the blockage of voltage-dependent sodium channel conductance [8–11].

The overall aim of this study was to determine whether LTG acts as a direct modulator of AChR function. To this end, we investigated the effect of the drug on the best characterized and most representative AChR subtype, the peripheral muscle-type adult AChR, heterologously expressed in the clonal cell line CHO-K1/A5 in our laboratory [12]. Using the patch-clamp technique [13], we characterized the effect of LTG on the AChR at the single-channel level. The drug behaved as a typical open-channel blocker. These findings on the prototype AChR open up new avenues for studying other possible targets of the anticonvulsive drug LTG, including neuronal AChRs, whose involvement in some forms of epilepsy is well documented [6,7].

Methods

Materials

Lamotrigine (6-(2,3-dichlorophenyl) 1,2,4-triazine-3, 5-diamine) was purchased from GlaxoSmithKline (Co. Durham, UK). Acetylcholine (ACh) was purchased from Sigma Chemical Co. (St Louis, Missouri, USA) and stored at -20°C in a 10 mM aqueous stock solution.

Cell culture

CHO-K1/A5 cells, expressing in a stable manner adult muscle AChR [12], were cultured in Ham F12 medium supplemented with 10% bovine fetal serum and 40 μg/ml of the selective antibiotic G418 (Sigma) in the cell medium.

Single-channel recordings

Single-channel currents were recorded in the cell-attached configuration [13] at a membrane potential of −70 mV and 20°C using an Axopatch 200B patch-clamp amplifier (Axon Instruments, Inc., Foster City, California, USA), digitized at 94 kHz with an ITC-16 interface (Instrutech Corporation,

Long Island, New York, USA) and transferred to a computer using the program Acquire (Bruxton Corporation, Seattle, Washington, USA). The bath and pipette solutions contained 142 mM KCl, 5.4 mM NaCl, 1.8 mM $CaCl_2$, 1.7 mM $MgCl_2$ and 10 mM *N*-2-hydroxyl piperazine-*N*′-2-ethane sulfonic acid, pH 7.4. Patch pipettes were pulled from Kimax-51 capillary tubes (Kimble Products, Vineland, New Jersey, USA), coated with Coat D (M-Line accessories, Measurements Group, Raleigh, North Carolina, USA) and fire-polished. Pipette resistances ranged from 5 to 7 MΩ. ACh at a final concentration of 1 μM was present in the pipette solution. LTG was prepared from a 10 mM stock solution in ethanol, stored at −20°C. Following ethanol evaporation under N_2 stream, the drug was dissolved in the bath solution (1–400 μM) and then applied to the cell from the pipette tip together with 1 μM ACh.

Detection of single-channel events using the program TAC (Bruxton Corporation) followed the half-amplitude threshold criterion at a bandwidth of 5 kHz. Open, burst and closed-time histograms were plotted using a logarithmic abscissa and a square-root ordinate and fitted to the sum of exponential functions by the maximum likelihood criterion using the program TACFit (Bruxton Corporation). In control recordings, bursts were defined as a group of opening events separated by closed times briefer than 1 ms. In the experimental recordings, the burst resolution was obtained from the delay between the main closed-time component and the succeeding one.

Data analysis

Data are expressed as mean ± SD from independent experiments. Statistical analysis was performed using Student's *t*-test.

Results

Lamotrigine modifies acetylcholine receptor channel gating kinetics

The effect of the antiepileptic drug LTG (Fig. 1) on AChR single-channel kinetics was evaluated using the patch-clamp technique in the cell-attached configuration.

Kinetic parameters of the control recordings, obtained in the presence of ACh in the pipette solution, were in agreement with the previously reported data [14,15]. At low agonist concentration (1 μM), the channel opened sparsely, exhibiting isolated openings, occasionally interrupted by brief closures (Fig. 2a, control). The distribution of single-channel openings and burst durations could be well fitted with a single exponential component in both cases. The mean open duration, τ_{open}, and mean burst duration, τ_{burst}, were 0.95 ± 0.11 and 1.11 ± 0.19 ms (n=9), respectively (Figs 2b and 3a and b). The observed amplitude was 5.2 ± 0.27 pA. In most of the recordings, the distribution of the closed-time duration was described by two components (Fig. 2b, 0 μM LTG); the longer component was variable and depended on the number of AChR channels in the patch, as described by Sine and Steinbach [16]. The briefest component was minor and lasted 0.2 ± 0.08 ms (area=0.10 ± 0.05) and most likely corresponds, as previously reported, to reopening of the closed channel [16].

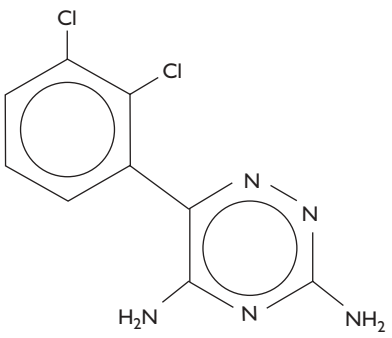


Fig. 1 Chemical structure of lamotrigine (6-(2,3-dichlorophenyl) 1,2,4-triazine-3, 5-diamine).

When ACh (1 μM) and LTG (50 μM) were both present in the pipette solution, the traces showed that AChR channels opened either individually or in groups separated by silent periods. Such groups of openings consisted of individual apertures often interrupted by brief closures (Fig. 2a). The analysis of the recordings revealed statistically significant differences between control and LTG-exposed channels. The channel open-state duration diminished to 0.70 ± 0.07 (n=4, P<0.001). Two components contributed equally to the τ_{burst} (Fig. 2b). The first one was similar to τ_{open} (τ_{burst1}=0.74 ± 0.1 ms), thus reflecting individual openings, and the second one, τ_{burst2}, lasting 2.14 ± 0.25 ms, indicates that the opening events occurred in bursts in the presence of LTG (Fig. 3b).

Analysis of the closed-time distributions provided additional diagnostic criteria. The area of the briefer component (0.26 ± 0.02 ms) was significantly larger (0.42 ± 0.04). A new minor component of 1.61 ± 0.26 ms (area=0.11 ± 0.03) was systematically observed (Figs 2a and b and 4). Both components are suggestive of the reopening of the AChR channel in the presence of LTG. The component related to the number of channels in the patch was also present in the experimental recordings, and a fourth closed component, in the range of seconds, was frequently observed. The channel amplitude, 4.97 ± 0.14 pA, was not significantly modified by exposure to 50 μM LTG.

The inhibitory effect of lamotrigine on the muscle acetylcholine receptor is concentration dependent

Using a fixed ACh concentration (1 μM) and LTG concentrations below 50 μM (1 and 20 μM), no differences were observed with respect to the control condition (data not shown). At higher LTG concentrations (100–400 μM), we observed that single-channel duration became shorter as the drug concentration increased, whereas the occurrence of opening events in bursts became more evident (Figs 2a and b). Quantitative analysis of the recordings indicated that the decrease in the duration of the channel open state was dose dependent (Fig. 3a). The two components of burst duration described for 50 μM LTG were also observed at higher LTG concentrations (Fig. 3b). Again, the duration of τ_{burst1} was similar to the observed τ_{open} and comprised half of the burst events (Figs 2b and 3b). Interestingly, the duration of the τ_{burst2} increased with drug concentration (Fig. 3b).

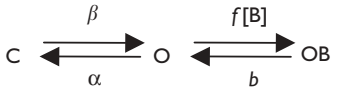
When the distribution of channel closed times was studied as a function of LTG concentration, two distinctive features became apparent: (a) the new component of about 2 ms detected at 50 μM LTG was conserved along the drug range tested (Figs 2a, b and 4), and (b) the area of this closed-time component exhibited concentration dependence

(Figs 2a and b and 4). We interpret this new component as reflecting channel blocking events, and hence refer to it hereafter as $\tau_{blocked}$.

Finally, at the membrane potential of −70 mV, changes in channel amplitude were detected (100 μM LTG=4.89 ±0.28 pA; 200 μM LTG=4.54±0.11 pA; 300 μM LTG=4.52 ±0.38 pA; 400 μM LTG=4.41±0.10 pA).

The effect of lamotrigine can be accounted for by a simple kinetic model

Given the resemblance between the effects of LTG and some local anesthetics on AChR single-channel currents [17–19], we evaluated next whether the action of LTG could be interpreted in terms of the classic linear kinetic mechanism (shown below), typically applicable to channel blocking compounds:



where C is the closed, O is the open and OB is the open-blocked state of the AChR channel in the presence of LTG, α and β are the apparent closing and opening rate constants for ACh-activated channels, repectively, b is the apparent dissociation rate constant for the unblocking and f[B] is the forward rate constant for channel blocking in the presence of compound B. According to this scheme, LTG would bind to the open channel and dissociate quite rapidly, blocking and unblocking the channel several times before entering into the closed state. In fact, as predicted by this model, in the presence of LTG, opening durations became shorter and occurred in bursts of increased duration, whereas the appearance of a new component in the closed-time distribution reflected the blocked state of the channel.

LTG adequately fulfills the requirements for an open-channel blocker of the AChR channel because at the concentration range tested here, the area of $\tau_{blocked}$ increased whereas its duration remained constant and the open-state duration decreased in a concentration-dependent manner. Moreover, its effect upon $\tau_{blocked}$ was voltage dependent (data not shown). Assuming that the effective concentration of the drug was the one present in the pipette solution, linear regression analysis of the reciprocal of the mean open time versus LTG concentration yields a value of f of 6.2×10^6/M s (Fig. 3a, inset). The value of α in the absence of LTG was determined to be 1086/s, whereas b,

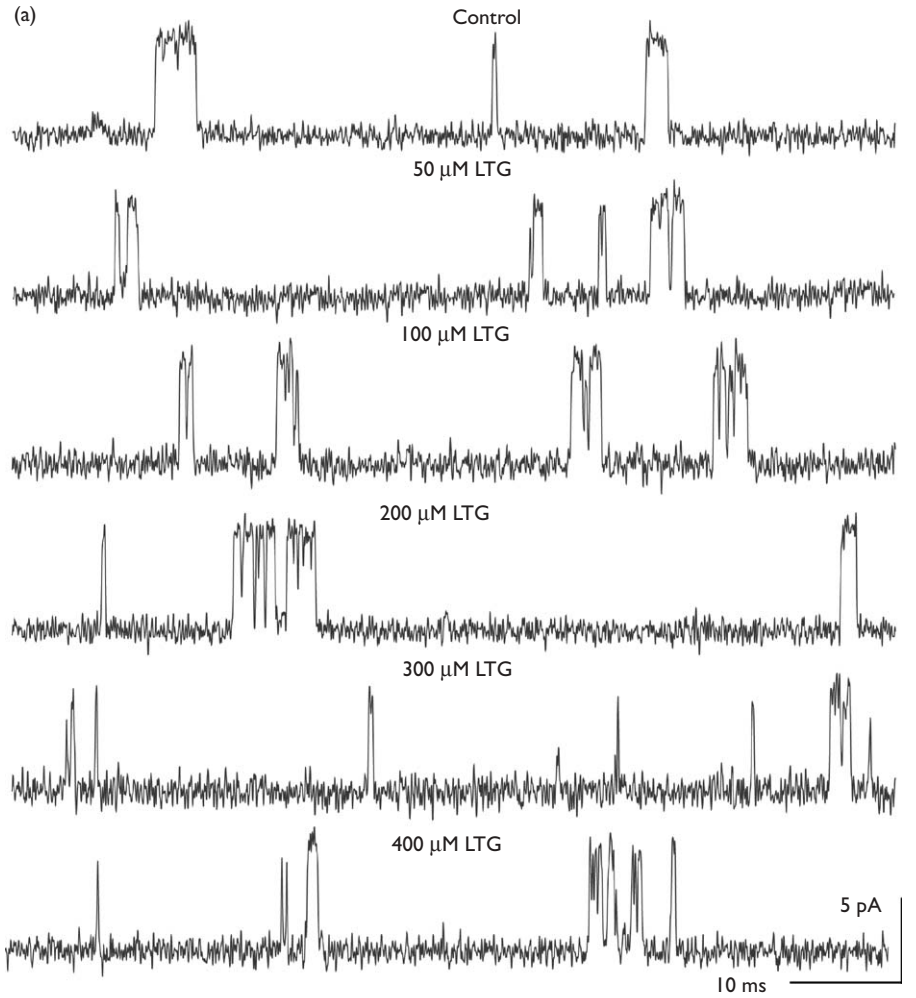


Fig. 2 (***Continued***)

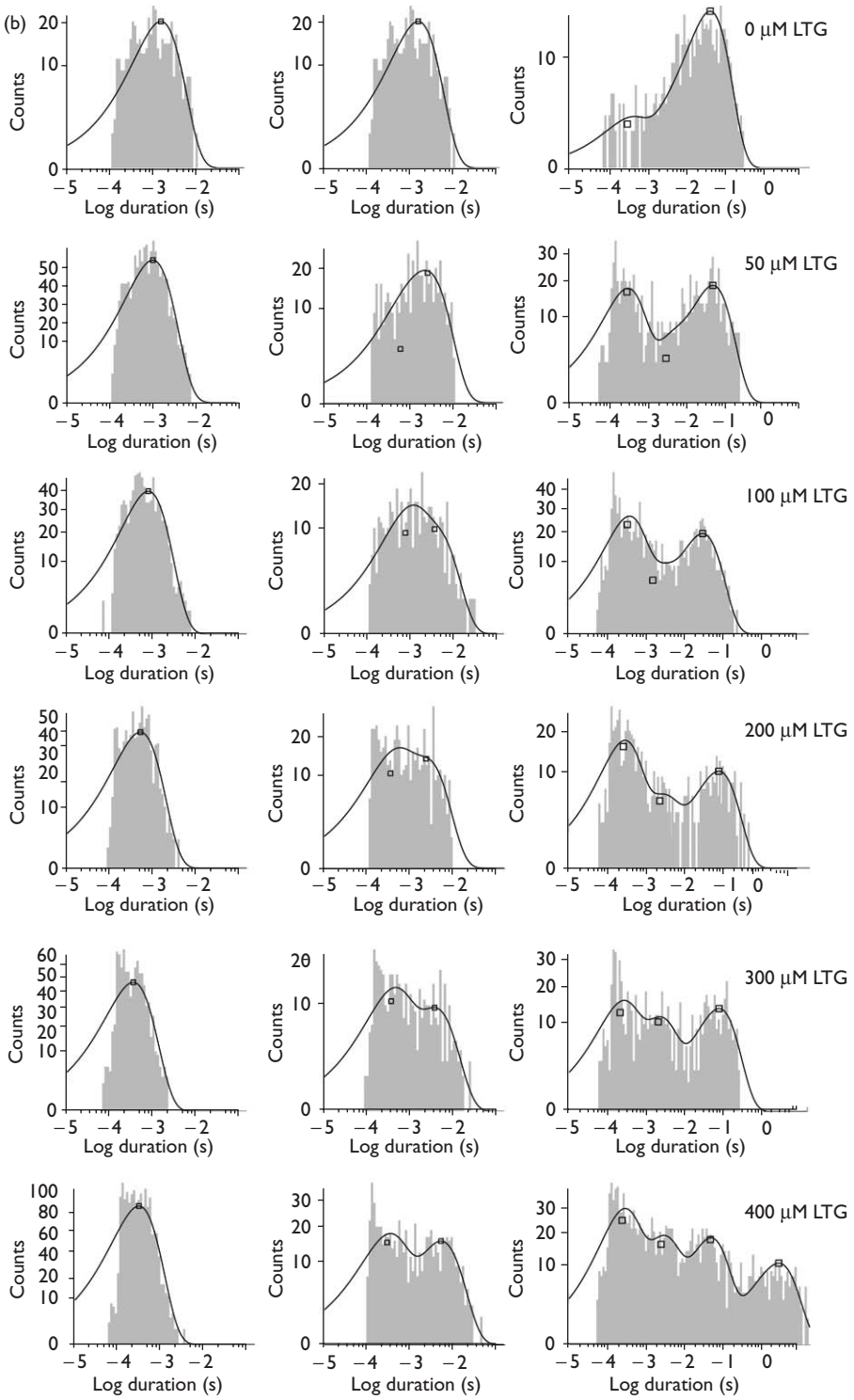


Fig. 2 Acetylcholine (ACh) and lamotrigine (LTG) pharmacological activity on muscle acetylcholine receptor (AChR). (a) Raw traces of single-channel recordings obtained in the cell-attached configuration from CHO-K1/A5 cells expressing adult AChR activated by ACh (1 μM) in the absence (first row) or the presence (subsequent rows) of 50, 100, 200, 300 and 400 μM LTG. (b) Open (left), burst (center) and closed (right) time histogram resulting from the analysis of the recordings are shown. Membrane potential: −70 mV. Filter: 5 kHz.

the reciprocal of the $\tau_{blocked}$, was 575/s. The apparent dissociation constant for the blocking process, K_d, obtained as the ratio between b and f[B], was 92.7 μM.

Discussion

A wide range of compounds have the ability to modulate AChR function. This modulation, in turn, can be accomplished through different mechanisms involving specific

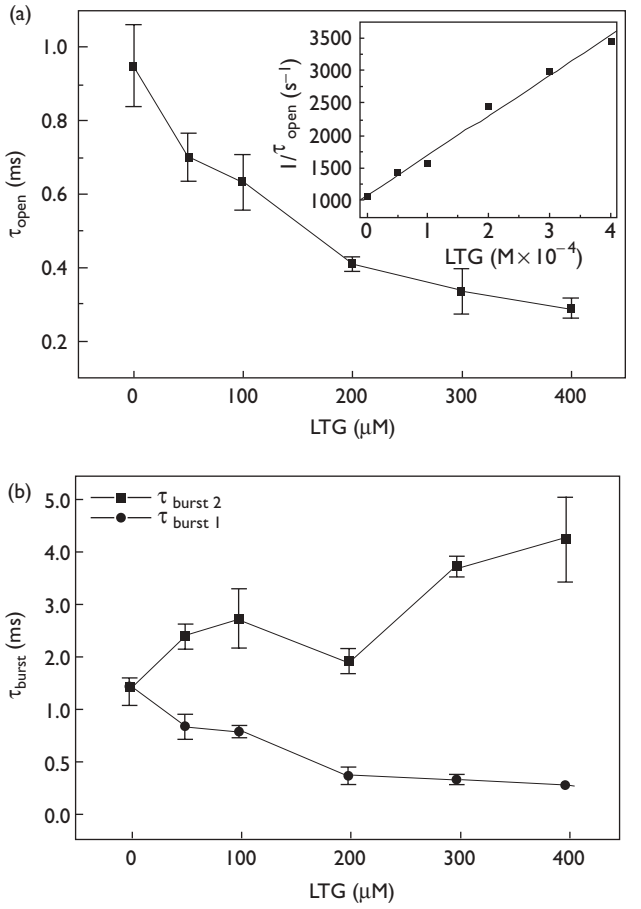


Fig. 3 Dependence of mean open time (τ_{open}) and burst duration (τ_{burst}) on lamotrigine (LTG) concentration. Data values were extracted from the dwell-time histograms obtained from the single-channel recordings shown in Fig. 2. (a) Dependence of mean open duration, τ_{open}, on LTG concentration. Inset: reciprocal of τ_{open} plotted against the concentration of LTG. (b) Relationship between burst time distribution components, τ_{burstI} and τ_{burst2}, and LTG concentration.

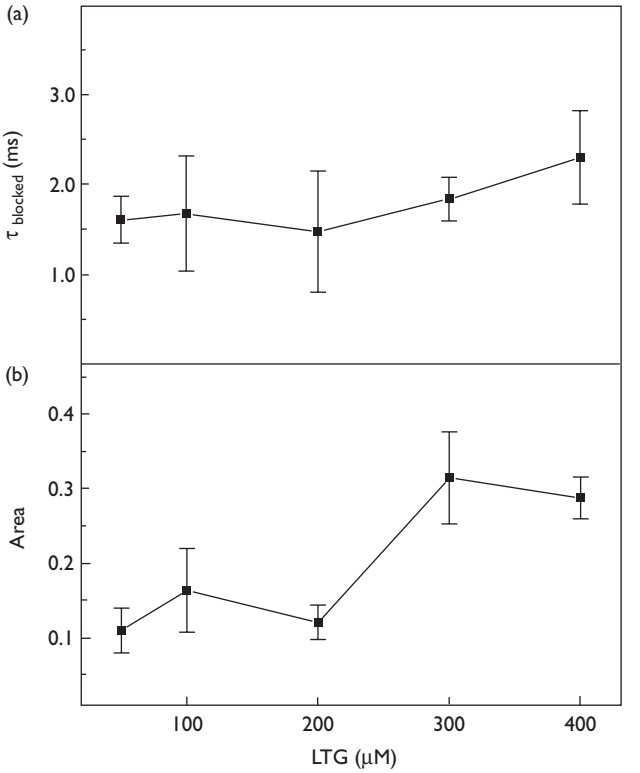


Fig. 4 Dependence of $\tau_{blocked}$ on LTG concentration. The figure illustrates the effect of LTG on $\tau_{blocked}$ duration (a) and area (b). Data values were extracted from the dwell-time histograms obtained from the single-channel recordings shown in Fig. 2.

binding to the agonist site, to the pore itself, or to amino-acid residues in the transmembrane moiety of the AChR, the latter resulting in allosteric regulation [1].

We characterized the effect of the antiepileptic drug LTG on muscle AChR using one of the most reliable tools for elucidating molecular aspects of drug–receptor interactions, the single-channel recording technique. Using this approach, we showed that LTG affected AChR channel function. The main effects caused by the drug were a concentration-dependent decrease in channel mean open time (Fig. 3), an increase in mean burst duration (Fig. 3) and a diminution in channel amplitude – the latter observed for LTG concentrations of 100 μM and above. A salient feature of the LTG inhibition was the appearance of a new closed-channel component, which we termed 'blocked time' (Fig. 2). The duration of this component remained constant in the range of concentrations tested, although its relative contribution showed concentration-dependent behavior (Fig. 4). Moreover, we found that the effect on blocked time increased with hyperpolarization of the membrane (data not shown). Thus, LTG behaves as an inhibitor of the AChR channel.

The effect of LTG is satisfactorily explained by a simple open-channel blocking mechanism (shown above) [20,21]. This model predicts that the rapid dissociation of the drug shortens the channel open state and lengthens burst duration because blockage delays AChR closure, preventing transition of the channel to the closed-blocked state. The dissociation rate of LTG from the AChR, estimated to be 575/s, is slow enough for LTG to block and unblock the channel several times before it finally closes, thus resulting in an observable flickering behavior.

The LTG blocking rate constant was found to be similar to those reported for other AChR blockers (e.g. local anesthetics and alcohols [19,22,23]). Although changes in channel amplitude are not predicted by this scheme, the model is compatible with the apparent amplitude decrease observed in the present work, which may be caused by fast blockage of the channel.

Even though under therapeutic conditions plasma and cerebrospinal fluid concentrations of LTG are usually 10–40 μM, values above 100 μM have been reported in overdose [24]. Although AChRs are not the specific targets for the anticonvulsive action of LTG, their blockage by the drug may be related to side effects such as aching of the joints and muscles reported in some cases (http://www.answers.com/topic/lamotrigine). Muscle-type AChR was chosen for this initial study because of the wealth of information on its functional properties, and in particular the thorough characterization of its channel kinetics. The actions of LTG reported in the present work cannot be directly applied to neuronal AChRs, targets of some forms of hereditary

epilepsies. We can only surmise that given the strong similarity between various members of the AChR family, some of the pharmacological effects of LTG may apply to the neuronal AChR subtype, a subject of future investigations.

Conclusions

On the basis of the analysis of single-channel patch-clamp data, we can conclude that the anticonvulsive drug LTG blocks the AChR channel, allowing it to reopen quickly, through a mechanism that is compatible with that of open-channel blockers.

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