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Characterization of lamotrigine inhibition of Na^+ channels in rat hippocampal neurones

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1 Lamotrigine (LTG), a new antiepileptic drug, requires long depolarizations to inhibit Na^+ currents. This suggests either slow binding of LTG to the fast inactivated state or selective binding of LTG to the slow inactivated state of Na^+ channels. To differentiate between these possibilities and to characterize further the action of LTG, we studied the affinity and kinetics of LTG binding to the Na^+ channels in acutely dissociated hippocampal neurones of the rat.

2 LTG inhibited more Na^+ currents at more depolarized holding potentials. The inhibitory effect at various holding potentials could be described by one-to-one binding curves, which yielded an apparent dissociation constant of $\sim 7\ \mu M$ for LTG binding to the inactivated channels (K_I), and a dissociation constant more than 200 times larger for LTG binding to the resting channels. A similar value of K_I ($\sim 9\ \mu M$) was also derived from the LTG concentration-dependent shift of the inactivation curve.

3 The recovery of LTG-bound inactivated Na^+ channels was faster than the recovery of normal (drug-free) slow inactivated channels. Moreover, the binding kinetics of LTG onto the inactivated channels were faster than the development of the slow inactivated state, and were linearly correlated with LTG concentrations, with a binding rate constant of $\sim 10{,}000\ M^{-1}\ s^{-1}$. These findings suggest that LTG chiefly binds to the fast inactivated state rather than the slow inactivated state.

4 We conclude that LTG, in therapeutic concentrations and at relatively depolarized membrane potentials, may potently inhibit Na^+ currents by slow binding to the fast inactivated state of Na^+ channels. Like phenytoin, the slow binding rates may explain why LTG effectively inhibits seizure discharges, yet spares most normal neuronal activities.

Keywords: Lamotrigine; Na^+ channel; inactivation; binding affinity; binding kinetics

Introduction

Lamotrigine (LTG, 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine) is a new antiepileptic drug which shows the same efficacy as carbamazepine (CBZ) and diphenylhydantoin (DPH) when used as monotherapy in newly diagnosed epilepsy (Steiner *et al*., 1994; Brodie *et al*., 1995). LTD also has a similar antiepileptic profile to CBZ and DPH in animal seizure models (Miller, 1986), and shows a similar use-dependent block of neuronal discharges at the cellular level (McLean & McDonald, 1983; 1986; Lees & Leach, 1993; Xie *et al*., 1995). The use-dependent block of discharges is of great mechanistic interest as it readily explains why these nonsedative antiepileptics may effectively inhibit seizure discharges, yet spare most normal activities. The molecular basis of this interesting block has been ascribed to the voltage-dependent inhibition of Na^+ currents in various preparations by DPH, CBZ and LTG (Matsuki *et al*., 1984; Willow *et al*., 1985; Lang *et al*., 1993; Kuo & Bean, 1994a; Xie *et al*., 1995). As in the case of many local anaesthetics (for review see Butterworth & Strichartz, 1990), the inhibition of Na^+ channels by these antiepileptics is more pronounced at more depolarized potentials, and has been similarly envisaged with the 'modulated receptor hypothesis', which proposes that the inactivated state of the channel has a much higher affinity for the drug than the resting state (Hille, 1977; 1993).

However, the steady-state consideration (different binding affinities between the drug and various gating states of the Na^+ channel) cannot completely explain why there should be use-dependent inhibition of neuronal discharges. For example, many Na^+ channels in a neurone would be transiently inactivated after just one single action potential. Obviously DPH, CBZ and LTG do not significantly bind to and inhibit Na^+ channels in this situation, otherwise normal neuronal activities would not be spared with these drugs. Based on the necessity of high-frequency discharges for the inhibition to happen, one may reason that with one single action potential the period of depolarization may be too short for the drug to act on the Na^+ channel. Consistent with this idea, it has been directly demonstrated by voltage clamp studies that long ($\sim$seconds) depolarizations are needed for DPH, CBZ and LTG to exert their inhibitory effect on Na^+ currents (Matsuki *et al*., 1984; Lang *et al*., 1993; Kuo & Bean, 1994a,b; Xie *et al*., 1995). Thus probably both the steady-state effect and the kinetics of the development of the effect are important to understand the use-dependent inhibition of Na^+ channels and neuronal discharges by these antiepileptics.

Why, then, would long depolarizations be needed for potent block of Na^+ currents by these anticonvulsants? There are at least two possibilities. Na^+ channels are voltage-gated molecules whose conformational change is controlled by the membrane potential. At hyperpolarized membrane potentials most Na^+ channels are in the resting (closed or deactivated) state. Upon depolarization the channel quickly opens (activated) and then is quickly inactivated within a few milliseconds (ms). This is the 'fast' inactivated state which has been explained by the 'ball-and-chain' model (Armstrong & Bezanilla, 1977; Armstrong, 1981). If the depolarization is sustained for, say, a few seconds, some Na^+ channels would be driven into another inactivated conformation, the slow inactivated state (Adelman & Palti, 1969; Schauf *et al*., 1976; Rudy, 1978). As the development of slow inactivation and the development of the inhibitory effect of antiepileptics on Na^+ channels occur on a similar time scale, one possibility is that the drug selectively binds to the slow inactivated state rather than the fast inactivated state. This is what has been proposed for the molecular action of LTG (Xie *et al*., 1995). On the other hand, the drug may still act on the fast inactivated state of the channel, but the binding rate is slow. A prolonged depolarization to keep the channel in the high-affinity fast inactivated state is therefore

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necessary for the inhibition to develop. The molecular action of DPH seems to fall into this latter category (Kuo & Bean, 1994a).

To elucidate whether the molecular mechanisms of action are really different between LTG and DPH, we studied the affinity and kinetics of LTG binding to the Na^+ channels in mammalian central neurones. We found that similar to DPH, LTG also inhibited Na^+ currents by slow binding onto the fast inactivated state of the channel. This process can be characterized by simple one-to-one binding with a rate constant of $\sim$10,000 M^{-1} s^{-1} and a dissociation constant of 7–9 μM, well within the clinical therapeutic concentration range of LTG.

Methods

Cell preparation

Coronal slices of the whole brain were prepared from 7 to 14-day-old Long-Evans rats. The CA1 region was dissected from the slices and cut into small chunks. After treatment for 5–10 min at 37°C in dissociation medium (in mM, Na_2SO_4 82, K_2SO_4 30, $MgCl_2$ 3, HEPES 5 and 0.001% phenol red indicator, pH = 7.4) containing 0.5 mg ml^{-1} trypsin (type XI, Sigma, St. Louis, MO), tissue chunks were moved to dissociation medium containing no trypsin but 1 mg ml^{-1} bovine serum albumin (Sigma) and 1 mg ml^{-1} trypsin inhibitor (type II-S, Sigma). Each time when cells were needed, 2–3 chunks were picked and triturated to release single neurones.

Whole-cell recording

The dissociated neurones were put in a recording chamber containing Tyrode solution (in mM NaCl 150, KCl 4, $MgCl_2$ 2, $CaCl_2$ 2 and HEPES 10, pH = 7.4). Whole-cell voltage clamp recordings were obtained with pipettes pulled from borosilicate micropipettes (od 1.55–1.60 mm, Hilgenberg Inc., Germany), fire polished, and coated with Sylgard (Dow-Corning, Midland, MI). The pipette resistance was 1–2 MΩ when filled with the internal solution containing (in mM) CsCl 75, CsF 75, $MgCl_2$ 2.5, HEPES 5 and EGTA 2.5 (pH adjusted to 7.4 by CsOH). Seal was formed and the whole-cell configuration obtained in Tyrode solution. The cell was then lifted from the bottom of the chamber and moved in front of an array of flow pipes (Microcapillary from Hilgenberg Inc., Germany; content 1 μl, length 64 mm) emitting either control or drug-containing external recording solutions (Tyrode solution with or without 10–1000 μM LTG, Wellcome Foundation Ltd., Kent, U.K.). Ex-

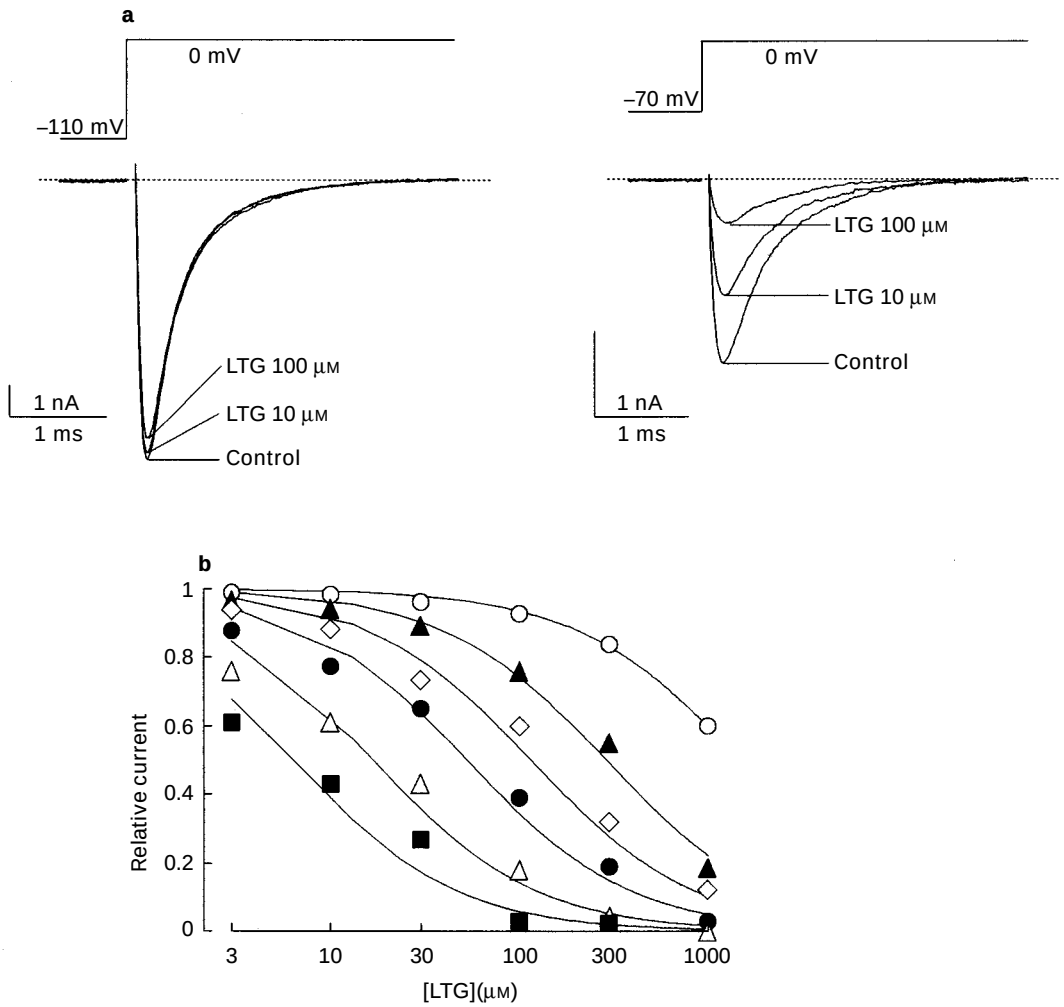


Figure 1 Inhibition of Na^+ currents by lamotrigine (LTG) at different holding potentials. (a) Currents in control, 10 or 100 μM lamotrigine in a cell held at −110 mV or −70 mV and stepped to 0 mV for 5 ms every two seconds. The dashed lines indicate zero current level. (b) Dose-response curves for inhibition of Na^+ currents by 10–1000 μM lamotrigine at different holding potentials (○, −110 mV; ▲, −100 mV; ◇, −90 mV; ●, −80 mV; △, −70 mV ■, −60 mV). The cell, which is typical of 5 cells, was held at −60 to −110 mV and stepped every two seconds to 0 mV for 5 ms. The peak current in the presence of lamotrigine were normalized to the control peak current at each holding potential and plotted against the concentration of lamotrigine in semilogarithmic scale. The lines are best fits for each set of data of the form: relative current = $1/(1+([\text{lamotrigine}]/K_{app}))$, where K_{app} is the apparent dissociation constant and was 1490, 288, 115, 53, 17 and 7 μM at holding potentials of −110, −100, −90, −80, −70 and −60 mV, respectively.

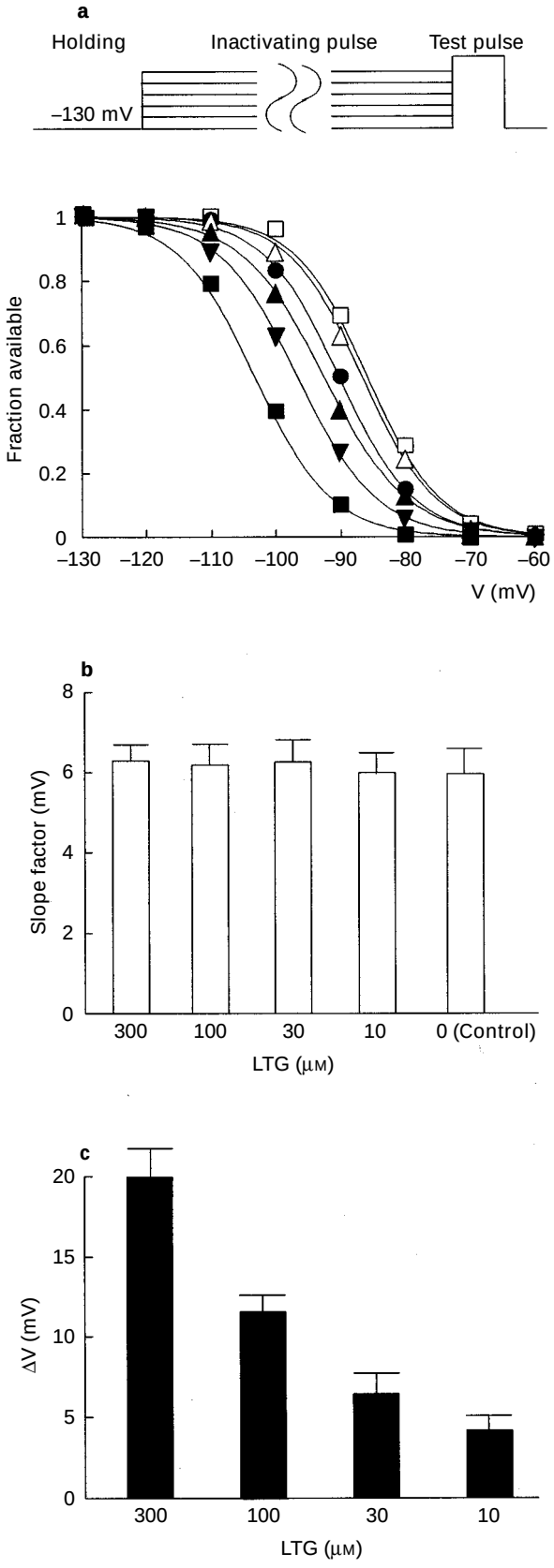


Figure 2 Shift of the inactivation curve by lamotrigine (LTG). (a) The cell was held at −130 mV and stepped every 15 s to the inactivating pulse (−130 to −60 mV) for 9 s. The channels which remained available after each inactivating pulse were assessed by the peak currents during the following short test pulse at 0 mV for 5 ms. The fraction available was defined as the normalized peak current (relative to the current evoked with an inactivating pulse at −130 mV) and is plotted against the voltage of the inactivating pulse. Two sets of control data (□, △) were obtained before and after the four sets of data in 10 (●), 30 (▲), 100 (▼), and 300 (■) μM lamotrigine to demonstrate that there was no significant voltage drift during this long experiment. The lines were fitted with a Boltzmann function 1/(1+exp((V-Vh)/k)), with Vh values (in mV) of −85.8, −86.5, −90.2, −92.5, −96.5, and −103.2; and k values of 5.6, 5.7, 5.8, 6.4, 6.1, and 5.9 for control (before lamotrigine), control (after lamotrigine), 10, 30, 100 and 300 μM lamotrigine, respectively. (b) The slope factor k was not changed by lamotrigine. Average values of k in control, 10, 30, 100 and 300 μM lamotrigine were 6.1±0.5, 6.0±0.5, 6.4±0.4, 6.2±0.5 and 6.4±0.4 (all $n=6$), respectively. (c) Dose-dependent shift of the midpoint of the inactivation curve. The shift (ΔV) was determined in each cell by the difference between V_h in control and in various concentrations of lamotrigine. The values of ΔV (in mV) were 4.3±0.8, 6.5±1.2, 11.5±1.0 and 20.1±1.98 (all $n=6$) for 10, 30, 100 and 300 μM lamotrigine, respectively.

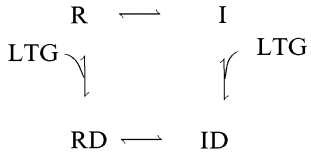
cept for the 1 mM final LTG concentration, which was prepared by directly dissolving LTG in the Tyrode solution, LTG was dissolved in dimethylsulphoxide (DMSO) to make a 100 mM stock solution, and then diluted into Tyrode solution to attain the final concentration desired. The final concentration of DMSO (0.3% or less) was not found to have any significant effect on Na^+ currents. Currents were recorded at room temperature (~25°C) with an Axoclamp 200A amplifier, filtered at 5 kHz with four-pole Bessel filter, digitized at 20–50 μs intervals, and stored by a Digidata-1200 analog/digital interface along with the pCLAMP software (Axon Instruments, Foster City, CA). Residual series resistance is generally smaller than 1 MΩ after partial compensation (typically >90%), and the product of residual series resistance and cell capacitance was <20 μs. All statistics are given as mean±s.d.

Results

Stronger inhibition of Na^+ currents by lamotrigine at more depolarized holding potentials

Figure 1 demonstrates the voltage-dependent effect of LTG on neuronal Na^+ currents. When the holding potential was −110 mV, 10 μM LTG showed negligible effect on the Na^+ currents. Even 100 μM LTG inhibited only 5–10% of the Na^+ currents. However, at a holding potential of −70 mV, both 100 and 10 μM LTG significantly inhibit the Na^+ currents (Figure 1a). Similar experiments were performed at various holding potentials and the dose-response curves are plotted in Figure 1b. For each holding potential the effect of different concentrations of LTG on Na^+ currents could be fitted by a simple one-to-one binding curve. At a holding potential of −60 mV, LTG inhibited neuronal Na^+ currents with an apparent dissociation constant (K_{app}) of ~7 μM. In contrast, the K_{app} was ~1500 μM at a holding potential of −110 mV (a rough estimate since even 1 mM LTG blocked only ~40% of current in this condition), indicating an affinity change by more than 200 times between membrane potentials −60 mV and −110 mV.

As the steady-state inactivation curve of Na^+ channels also showed a steep change between −60 to −110 mV (see the control curves in Figure 2a), the above finding is consistent with the notion that LTG binds to the inactivated channels with a much higher affinity than to the resting channels. This point can be illustrated in a more quantitative manner by the following scheme:



where R and I are the resting and inactivated states of the channel, and RD and ID are the LTG-bound resting and inactivated states, respectively. The activated (open) state was

omitted because most activated Na^+ channels exist so transiently upon depolarization that for steady-state discussion one may consider only the R and I states for simplicity. According to this scheme, the fraction of channels in state R (the channels that may be activated, or therefore the current that may be elicited upon membrane depolarization) would be reduced by LTG with a form expected for simple one-to-one binding and a K_{app} given by (Bean, 1984; Kuo & Bean, 1994a):

$$K_{app} = \frac{1}{(h/K_R + (1-h)/K_I)}$$

where h is the fraction of channels in state R in the absence of drug at a particular holding potential ('fraction available' in the control condition in Figure 2a), and K_R and K_I are the dissociation constants of LTG binding to the resting and inactivated states, respectively. This is as if the overall affinity of LTG toward the channel (which may be envisaged as $1/K_{app}$, since the dissociation constant is inversely proportional to affinity) is a weighted average of the LTG affinity toward each state of the channel (Σ fraction of a state $\times$ the affinity toward that state, or $h/K_R + (1\text{-}h)/K_I$). A K_{app} of $\sim$1500 μM at -110 mV, where h is >0.95, and a K_{app} of ~ 7 μM at -60 mV, where h is <0.05 (Figures 1b and 2a), would therefore indicate that K_R is somewhat larger than 1500 μM, and is much larger than K_I (~ 7 μM).

Measurement of the affinity between inactivated Na^+ channels and lamotrigine by shift of the inactivation curve

As the inactivated state seems to represent the high-affinity conformation of Na^+ channels for LTG binding, it is of great interest to see whether a similar value of K_I can be obtained with other approaches. In the control condition, the fraction of channels in state R at various membrane potentials can be well approximated by a Boltzmann distribution, $1/(1+\exp((V-V_h)/k))$ (the 'inactivation curve', Figure 2a). According to the foregoing scheme, in the presence of LTG the shape of the inactivation curve (the slope factor k) should remain the same, but the midpoint of the curve (V_h) would be shifted by ΔV, with $\exp(\Delta V/k)$ equal to $(1+(D/K_I)/(1+(D/K_R))$, where D is the concentration of LTG (Bean *et al*., 1983; Benoit & Escande, 1991; Kuo & Bean, 1994a). Figure 2a demonstrates the shift of the inactivation curves by LTG. Figure 2b and c further demonstrates that the shift is LTG concentration-dependent, and the slope of the curves is not changed by LTG. Figure 3a shows the cumulative results of $\Delta V/k$, and the exponentials of the mean values of $\Delta V/k$ are plotted in Figure 3b, in which a fitted curve yielded a K_I of ~ 9 μM.

Slowed recovery of the inactivated Na^+ channels by lamotrigine

We subsequently investigated the kinetics of LTG effect on neuronal Na^+ channels. Figure 4 shows that the macroscopic recovery of Na^+ currents after long depolarizations is apparently slower in the presence of 100 to 300 μM LTG (10 to 30 times the estimated K_I). This is as if LTG binding moves the inactivated channel from state I to state ID, a step farther away from state R, and thus slows the recovery from inactivation in Na^+ channels. A closer examination of the slow recovery kinetics in LTG suggests that LTG mainly binds to the fast rather than to the slow inactivated state of Na^+ channels (see Discussion).

Slow binding of lamotrigine onto the fast inactivated Na^+ channels

The binding rates of LTG onto the inactivated Na^+ channels are characterized in Figure 5. According to the shaded area in Figure 4b, most inactivated channels had recovered after 5 ms at the recovery potential, while the majority of LTG-bound channels had not. The binding rate of LTG was thus assessed by a voltage protocol similar to that in Figure 4a, but the prepulse was gradually lengthened while the -120 mV gap was fixed at 5 ms (Figure 5a). The 'contaminating' effect of normal slow inactivation was corrected by taking the difference between the currents in control and in LTG, and it was evident that the macroscopic binding rates increased as the concentration of LTG increased (Figure 5b). Figure 5c and d further demonstrates that the macroscopic binding rates increased linearly with the increased drug concentration, supporting the assumption in Figure 1b that LTG interacts with inactivated Na^+ channels via a simple one-to-one binding reaction. The slope of the line in Figure 5d yielded a binding rate constant of $\sim$10,000 M^{-1} s^{-1}. Further analysis of these binding kinetics also suggested that LTG mainly binds to the fast but not the slow inactivated state of Na^+ channels (see Discussion).

Discussion

LTG, like DPH and CBZ, inhibited repetitive discharges and Na^+ currents in a use-dependent manner, which may be closely related to its mechanism of antiepileptic action. We in-

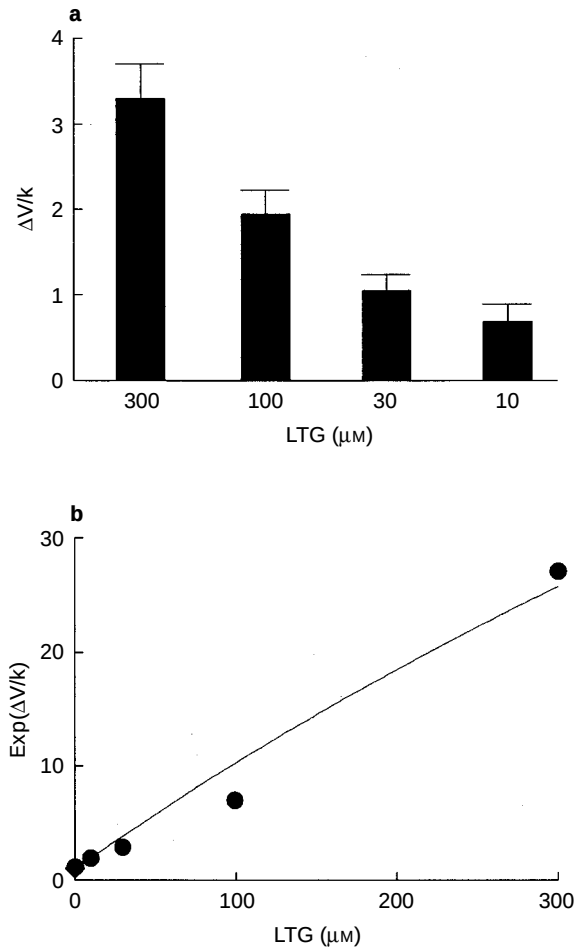


Figure 3 Determination of the affinity between neuronal Na^+ channels and lamotrigine (LTG) by $\Delta V/k$ (for the meaning of ΔV and k please refer to Figure 2 and text). (a) $\Delta V/k$ versus lamotrigine concentration (data from the same cells in Figure 2b and c). $\Delta V/k$ was 0.69 ± 0.19, 1.05 ± 0.20, 1.94 ± 0.31 and 3.3 ± 0.40 for 10, 30, 100 and 300 μM lamotrigine, respectively. (b) $\exp(\Delta V/k)$ versus lamotrigine concentration. The data for lamotrigine were from the mean values in (a). The line was the best fit of the form: $\exp(\Delta V/k) = (1+(D/K_I))/(1+(D/1{,}500))$, where D denotes concentrations of lamotrigine in μM; 1,500 represents the presumed apparent dissociation constant between the resting state of Na^+ channel and lamotrigine (i.e. K_R set at 1500 μM) and K_I was 9.5 μM by the fit.

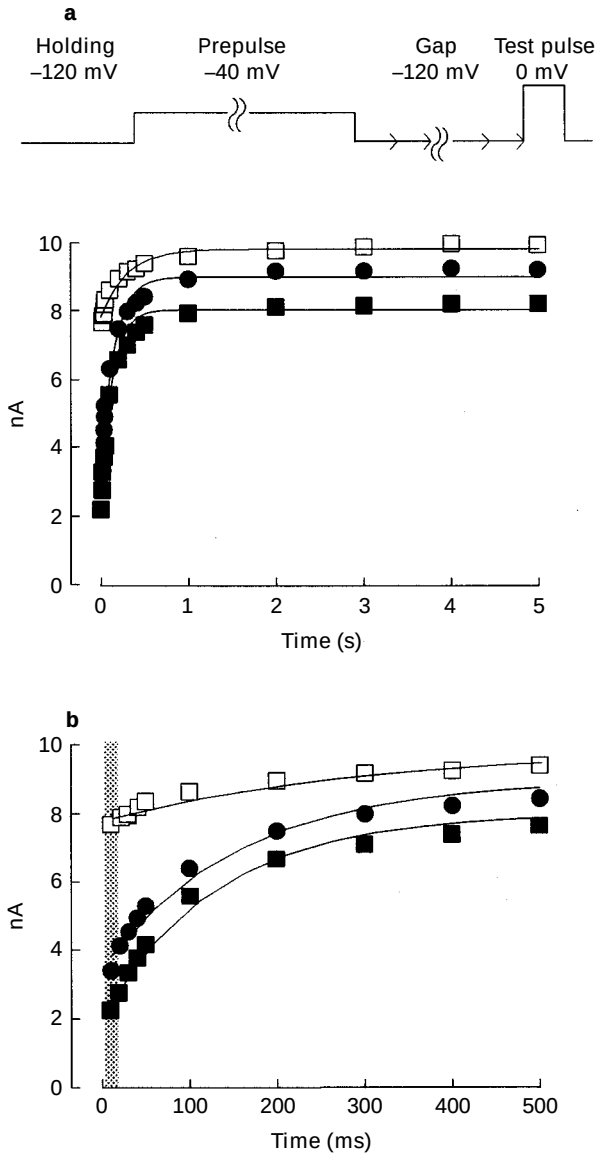


Figure 4 Slowed recovery from inactivation by lamotrigine. (a) In control or in the continuous presence of 100 or 300 μM lamotrigine, the cell was held at -120 mV and then prepulsed to -40 mV for 9 s to reach a maximal (steady-state) block of Na^+ current by lamotrigine. The cell was then stepped back to a recovery gap potential at -120 mV for variable length before being stepped again to a short test pulse at 0 mV for 5 ms to assess the available current. The pulse protocol was repeated every 15 s. The time courses of recovery were obtained by plotting the peak current at the test pulse against the length of the recovery gap potential. The 'steady-state' currents after long recovery periods were slightly reduced in the presence of lamotrigine, possible resulting from mild inhibition of the resting channels (K_R ~1500 μM) by 100–300 μM lamotrigine. The lines are monoexponential fits for the recovery course after 10 ms, and are of the form: current (in nA) = 10.0 – 2.3 × Exp (-t/0.29) for control (□, t denotes length of recovery potential in s), currrent = 9.1 – 5.5 × Exp(-t/0.16) for 100 μM (●), and current = 8.1 – 6.1 × Exp(-t/0.14) for 300 μM (■) lamotrigine, respectively. (b) The first 500 ms of the plot in part (a) was redrawn with an expanded scale of the horizontal axis. The shaded area indicates that the difference between the recovery in control and the recovery in lamotrigine was the most when the recovery period was very short (eg 5–10 ms), when most currents in control have recovered but the majority of currents in lamotrigine have not.

vestigated the molecular events underlying such a use-dependent block and found that the affinity of LTG toward the inactivated Na^+ channels ($1/K_I$) was more than 200 times higher than its affinity toward the resting (deactivated) channels ($1/K_R$). The findings are quantitatively consistent with a simple scheme that LTG preferentially binds to the inactivated state of Na^+ channels via a simple bimolecular, one-to-one binding reaction.

As the inactivated Na^+ channels seem to be the major pharmacological 'target' for LTG, a key issue in understanding the molecular action of LTG would be to clarify the nature of the high-affinity inactivated state. Xie *et al.* (1995) proposed that LTG selectively binds to the slow inactivated state of the Na^+ channel. This proposal is based on the findings that LTG showed no inhibition of Na^+ currents with a train of short-duration pulses (which cause only channel opening and fast inactivation but not slow inactivation), that the inactivation curve was substantially shifted by LTG when the inactivating pulse was long ('slow inactivation curve') but only insignificantly shifted when the inactivating pulse was short ('fast inactivation curve') and that LTG did not change the decaying kinetics of macroscopic Na^+ currents ('the development of fast inactivation'). However, all these findings can be equally well explained if LTG binds to the fast inactivated state of Na^+ channels with slow binding kinetics.

A detailed analysis of the kinetic data in this study revealed interesting information about the inactivated state which LTG binds to. The time constant of recovery from the fast inactivated state is ~1 ms at -120 mV (Kuo & Bean, 1994b), and recovery from the slow inactivated state at similar hyperpolarizing potentials is very much slower (Kuo & Bean, 1994a). As a first approximation, in a time course of recovery from inactivation one may assume that all the recovery happening within 10 ms is from the fast inactivated state, and all that is happening after 10 ms is from the slow inactivated state. If LTG selectively binds to the slow inactivated conformation, then the recovery in LTG should show a major component which is even slower than the recovery from the drug-free slow inactivated state. However, the experimental findings, shown in Figure 4, did not indicate such a component. On the other hand, the exponential fits to the recovery courses after 10 ms yielded smaller time constants in the presence of LTG (Figure 4). This suggests that most Na^+ channels, which are presumably bound by LTG at the end of the long (9 s) prepulse, recover faster than those drug-free slow inactivated channels. The kinetics of recovery thus support a significant binding of LTG to the fast rather than to the slow inactivated state of Na^+ channels.

The binding rates of LTG onto Na^+ channels are also informative in this regard. If LTG significantly binds to the fast inactivated state of Na^+ channels, which develops within a few milliseconds after depolarization, then the requirement of long depolarizations for LTG action most likely indicates slow binding of LTG onto the inactivated Na^+ channels. In other words, if the I state in the foregoing scheme represents the fast inactivated state, then the R to I transition is so fast that the overall slow speed of the R to I to ID must be due to a rate-limiting step I to ID. An important connotation from this argument is that if the I to ID transition is accelerated by increasing LTG concentration, the macroscopic rate of LTG action or LTG binding should be correlatively faster. On the other hand, if LTG selectively binds to the slow inactivated state (i.e. that I state in the scheme represents the slow inactivated state), then the macroscopic effect of LTG should not develop faster than the development of the slow inactivated state. Also, the slow R to I step in this case very likely would be partially or even principally responsible for the slow macroscopic action of LTG. The binding rates of LTG thus probably would not be tightly correlated to the LTG concentration, which presumably changes only the rate of the I to ID step. In Figure 5, we demonstrated that in some conditions the macroscopic binding rates of LTG are remarkably faster than the development of the slow inactivated state (e.g. the ~10 s^{-1} binding rate observed in 1 mM LTG), and that the binding rates are linearly correlated to LTG concentrations. These findings again suggest that LTG mainly binds to the fast inactivated Na^+ channels with slow binding rates rather than

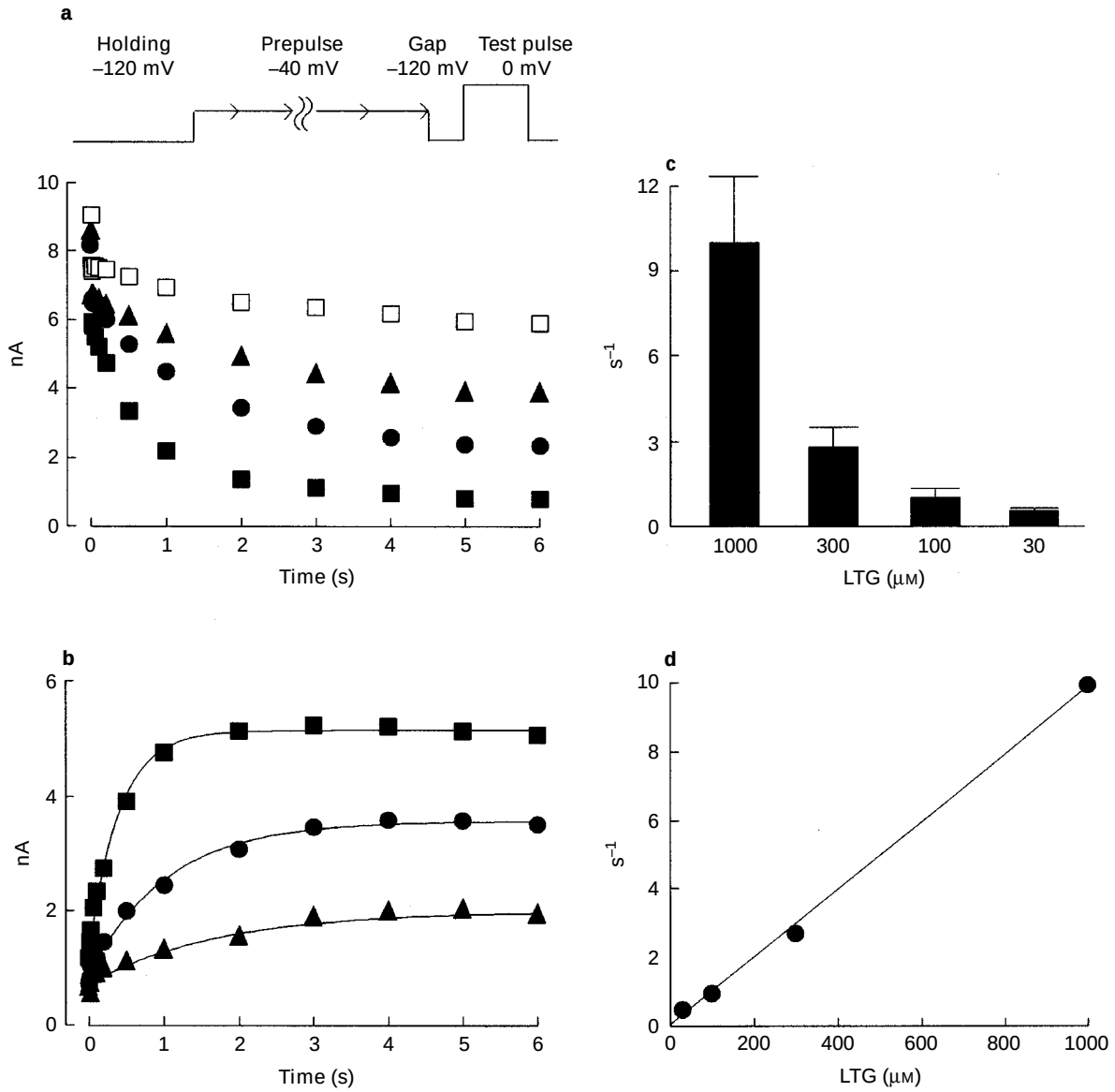


Figure 5 Binding rate of lamotrigine (LTG) onto the inactivated Na^+ channels. (a) In control (□), 30 (▲), 100 (●) or 300 μM (■) lamotrigine, the cell was held at −120 mV and prepulsed to −40 mV for variable length. Immediately following the prepulse, there was a recovery gap potential at −120 mV for 5 ms so that most normal (not lamotrigine-bound) fast inactivated Na^+ channels could recover, and then the available current was assessed by a short test pulse at 0 mV for 5 ms. The pulse protocol was repeated every 15 s. The peak Na^+ current at the test pulse was plotted against the duration the prepulse to demonstrate the decay of the peak currents as the prepulse lengthens. The decrease of the peak Na^+ currents mostly reflects the increase of the drug-bound inactivated channels, possibly with a little 'contamination' from the concomitant increase of the normal slow inactivated channels which is evident by the gradual decrease of the currents in the control condition. (b) To eliminate the contamination from the normal slow inactivation, more accurate time courses of drug binding were obtained by plotting the differences between the currents in lamotrigine and the currents in control (by use of the same experimental data as those in (a)) against the duration of the prepulse. The lines were monoexponential fits of the form: current (nA) = 5.19 − 3.65 × Exp(-t/0.40) for 300 μM (■, t denotes length of prepulse in s, the horizontal axis), current = 3.62 − 2.78 × Exp(-t/1.05) for 100 μM (●) and current = 1.95 − 1.43 × Exp(-t/1.70) for 30 μM (▲) lamotrigine, respectively. (c) The macroscopic binding rates for lamotrigine (the inverses of the time constants as those in (b)) from 5 cells were: $10.0 \pm 2.5\ s^{-1}$ (1000 μM), $2.8 \pm 0.8\ s^{-1}$ (300 μM), $1.05 \pm 0.31\ s^{-1}$ (100 μM) and $0.53 \pm 0.09\ s^{-1}$ (30 μM). (d) Plot of the macroscopic binding rate (the mean values in (c)) against the concentration of lamotrigine. The line was a linear regression fit with a slope of 9,900 $M^{-1}\ s^{-1}$.

selectively binds to the slow inactivated state. We therefore conclude that the major 'target' state of Na^+ channels for LTG binding is the fast but not the slow inactivated state, and the slow recovery and binding rates may represent the key molecular events underlying the use-dependent inhibitory effect of LTG on neuronal Na^+ currents and discharges.

The steady-state block at a holding potential of −60 mV (Figure 1) and the shift of the inactivation curve with 9 s inactivating pulses (Figures 2 and 3) yielded very similar K_I (dissociation constants of LTG binding to the fast inactivated Na^+ channels in mammalian hippocampal neurones) of 7–9 μM. The clinical therapeutic concentrations of LTG in plasma are usually 6–40 μM, but sometimes may be as high as 70 μM, especially during combined therapy with valproic acid (Peck, 1991; Kilpatrick *et al.*, 1996). Because 55% of total LTG is bound to plasma proteins, the free concentration (presumably representing the concentration of LTG in the cerebrospinal fluid) is about 3–18 μM, and may be as high as 30 μM. Thus the measured K_I (7–9 μM) is well within the clinically relevant concentration range of LTG, and significant block of Na^+ current may be expected in appropriate clinical conditions. It should be noted that normally the Na^+ current in hippocampal neurones is very large, and inhibiting part of it may not jeopardize the firing of the cell. However, during ictal

discharges the prolonged depolarization might already drive a large proportion of Na^+ channels into the inactivated state, and a little further reduction of the available Na^+ channels may abolish the generation of action potentials. Perhaps the reduction of Na^+ currents needed to stop ictal discharges is different in different seizure foci, and this may be part of the reason why the effective concentrations of LTG for satisfactory seizure control vary markedly between patients (Kilpatrick *et al*., 1996).

The rate constant for LTG binding to the inactivated channel was estimated to be $\sim$10,000 M^{-1} s^{-1} (Figure 5). With the therapeutic free concentrations 3 to 30 μM, LTG would have a macroscopic binding rate (the product of drug concentration and the binding rate constant) of 0.03 to 0.3 s^{-1}. This means that LTG requires a sustained depolarization of the neurone for at least a few seconds to approach significantly its steady-state blocking effect on Na^+ channels at room temperature. At body temperature the binding should be faster. Also, as many Na^+ channels have been inactivated and become unavailable during ictal depolarization, LTG may not have to reach its steady-state effect to abolish the seizure discharges. It is therefore conceivable that some seizure discharges are probably stopped by LTG before the sustained depolarization has lasted for a few hundred milliseconds. Nevertheless, as such long depolarizations are unusual in normal neuronal activities but are typical of many seizure discharges (for review see Dichter & Ayala, 1987; Lothman & Collins, 1990), the slow binding kinetics of LTG very likely play a key role in its selective effect against seizure discharges without disturbing most normal activities.

It would be interesting to make a comparison between LTG and DPH, the first nonsedative anticonvulsant still widely in use today. Like LTG, DPH also significantly binds to the fast inactivated Na^+ channels with slow kinetics via a simple bimolecular reaction (Kuo & Bean, 1994a), and thus also displays use- or voltage-dependent inhibition of Na^+ currents. Further comparison reveals that the similarities between these two drugs are not only qualitative but also quantitative. The 7–9 μM K_I of LTG is very close to the $\sim$7 μM K_I previously found for DPH. The binding rate constant for LTG binding to the inactivated channel (10,000 M^{-1} s^{-1}) is also very close to that of DPH, which is around 9,000 to 14,000 M^{-1} s^{-1} (Kuo & Bean, 1994a). Thus LTG is very similar to DPH in its pharmacological action on Na^+ channels when compared on an equimolar basis. However, the clinical efficacy of the two drugs could still be different because of differences in their therapeutic concentrations. According to a simple one-to-one binding reaction, high therapeutic concentrations ($\sim$20 μM) of LTG (K_I 7–9 μM) would exert a steady-state inhibition of the available Na^+ channels by 69–74%, whereas high therapeutic concentrations ($\sim$8 μM) of DPH (K_I $\sim$7 μM) would inhibit Na^+ currents by 53%. Thus theoretically LTG would have a higher chance of effective seizure control than DPH, at least when adequate doses are used and high therapeutic concentrations are reached. Moreover, LTG, with its very similar binding rate constant to DPH and its high therapeutic concentration $\sim$3 times higher than DPH, could have a macroscopic binding rate $\sim$3 times faster than DPH (when both are at their high therapeutic concentrations). This might make LTG more effective than DPH in abolishing ictal discharges characterized by relatively short depolarizations or less frequent repetitive bursts. However, the cost of LTG is much higher than DPH. Also, the decrease of Na^+ current necessary for seizure control could be very individual and not necesarily large in a particular patient. Thus it seems reasonable still to try DPH first, and to reserve LTG for those seizures refractory to DPH or other 'traditional' anticonvulsants inhibiting Na^+ currents in a similar fashion. Combined therapy of LTG with DPH or CBZ may also be advisable in the treatment of refractory seizures, as the binding between these antiepileptics and the inactivated neuronal Na^+ channels probably is not saturated in most clinically relevant drug concentrations.

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