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Levetiracetam does not modulate neuronal voltage-gated Na^+ and T-type Ca^{2+} currents

CRISTINA ZONA[†], ISABELLE NIESPODZIANY[‡], CATERINA MARCHETTI[†], HENRIK KLITGAARD[‡], GIORGIO BERNARDI[†] & DORU GEORG MARGINEANU[‡]

[†]*Department of Neuroscience, University of Rome "Tor Vergata" and IRCCS Fondazione "S. Lucia", Via Ardeatina 306, Rome, Italy;* [‡]*Preclinical CNS Research, UCB S.A. Pharma Sector, Chemin du Foriest, B-1420 Braine-l'Alleud, Belgium*

Correspondence to: D.-G. Margineanu, UCB S.A. Pharma Sector, R & D, Chemin du Foriest, B-1420 Braine-l'Alleud, Belgium. *E-mail*: doru.margineanu@ucb-group.com

This study investigated whether the mechanism of action of levetiracetam (LEV) is related to effects on neuronal voltage-gated Na^+ or T-type Ca^{2+} currents. Rat neocortical neurones in culture were subjected to the whole-cell mode of voltage clamping under experimental conditions designed to study voltage-gated Na^+ current. Additionally, visually identified pyramidal neurones in the CA1 area of rat hippocampal slices were subjected to the whole-cell mode of voltage clamping under experimental conditions designed to study low-voltage-gated (T-type) Ca^{2+} current. LEV (10 μM–1 mM) did not modify the Na^+ current amplitude and did not change (200 μM) the steady-state activation and inactivation, the time to peak, the fast kinetics of the inactivation and the recovery from the steady-state inactivation of the Na^+ current. Likewise, LEV (32–100 μM) did not modify the amplitude and did not change the steady-state activation and inactivation, the time to peak, the fast kinetics of the inactivation and the recovery from the steady-state inactivation of the T-type Ca^{2+} current. In conclusion, neuronal voltage-gated Na^+ channels do not appear directly involved in the antiepileptic mechanism of action of LEV, and LEV was devoid of effect on the low-voltage-gated (T-type) Ca^{2+} current in hippocampal neurones.

Key words: levetiracetam; antiepileptic drugs; sodium current; low-voltage-gated calcium current; rat.

INTRODUCTION

Levetiracetam (LEV : (S)-α-ethyl-2-oxo-pyrrolidine acetamide) is a new antiepileptic drug (AED) with a unique pharmacological profile. LEV is devoid of anticonvulsant activity in the two classical screening models for AEDs in mice and rats, the maximal electroshock seizure test and the pentylenetetrazol test[1,2]. This contrasts potent seizure suppression, a wide safety margin and antiepileptogenic properties in kindling models of epilepsy[2,3]. Clinical studies have shown that LEV is effective as an add-on therapy in the management of partial-onset seizures in adults[4]. A specific binding site in rat brain has been described for LEV[5] and several studies have demonstrated an ability to antagonize epileptiform responses in rat hippocampal slices *in vitro*[6,7], but the mechanism(s) of action of LEV remain to be characterized.

Inhibitory effects exerted on neuronal excitatory voltage-gated currents, either Na^+ currents or low-voltage-activated (T-type) Ca^{2+} currents, are cellular mechanisms which are commonly accepted as accounting for the anti-seizure activities (e.g. Reference 8) of several established and newer AEDs. Several reference AEDs—chiefly phenytoin and carbamazepine, but also valproate[9]—as well as the newer AEDs lamotrigine[10] and topiramate[11] have been shown to reduce voltage-gated Na^+ currents in rat central neurones in culture. The established AEDs ethosuximide, active against absence epilepsy, and dimethadione, have been shown to reduce low-voltage-activated (T-type) Ca^{2+} currents in rodent thalamic neurones *in vitro*[12].

The aim of the present study was to establish whether the antiepileptic mechanism(s) of action of LEV involve a modulation of Na^+ or T-type Ca^{2+} channels in mammalian central neurones. Here we report the results of whole-cell patch-clamp record-

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ings *in vitro*, investigating possible effects of LEV on voltage-gated Na^+ currents in rat cortical neurones in culture and on low-voltage-activated (T-type) Ca^{2+} currents in the CA3 pyramidal neurones in rat hippocampal slices. Some of these results have been communicated in abstract form[13, 14].

MATERIALS AND METHODS

Na^+ currents

Experiments were performed at room temperature on neocortical neurones removed from 14 day old Wistar rat embryos and grown in dissociated cell culture up to 4 weeks in BME (Gibco) with the addition of 10% foetal bovine serum (Gibco), 6 mM glucose, 2 mM glutamine and 100 μg ml^{-1} of gentamicin (Gibco). The results were obtained from 12 cortical culture preparations.

Membrane currents were recorded from cell somas of fusiform cells, with soma diameters of 20–26 μm, in the whole-cell configuration of the patch-clamp method. The resistance of the patch pipettes was approximately 4 MΩ. The currents were filtered at 3 KHz, digitized by computer at 10 KHz and stored on a hard disc. Leakage currents were subtracted automatically. Test pulses were usually applied at 3 second intervals.

The standard saline bath solution (ACSF) contained (in mM): NaCl, 120; KCl, 3; $CaCl_2$, 2; $MgCl_2$, 2; glucose 20; HEPES / NaOH, 10; pH 7.3. K^+ and Ca^{2+}currents were blocked by addition of 4-aminopyridine (2 mM) and cadmium (200 μM), respectively. The recording pipettes used for recording sodium currents contained (in mM): CsCl, 130; TEA-Cl, 20; $MgCl_2$, 1; $CaCl_2$, 0.24; EGTA, 5; ATP, 2; glucose, 10; HEPES/CsOH, 10; pH 7.3. Neurones were perfused with different concentrations of LEV, freshly dissolved in the saline solution and applied by a gravity perfusion system with small (diameter <1 mm) tubes, placed at less than 0.2 cm from the patched cell. During the experiments, the cells were continuously superfused at a flow rate of 0.5–1 ml per minute.

Fitting with Boltzmann sigmoids the plots of the mean normalized Na^+ current amplitude (I_{Na}/I_{Na}^{max}) against either the step potential, or the holding potential indicated the potentials $V_{1/2}$, of either half-activation, or half-inactivation, respectively. The rate of Na^+ current activation was expressed by the time to peak (TTP), defined as the duration of the rising phase, between the capacitive current and the peak of the Na^+ current. TTP exponentially decays as a function of the step voltage: TTP $\sim \exp(-V/\upsilon_{act})$, with a voltage constant υ_{act}. To characterize the rate of inactivation of the Na^+ currents—elicited from a holding potential of −80 mV with step voltages increasing from −30 to +30 mV—their fast falling phase was fitted with a single exponential function of time (t): $\sim \exp(-t/\tau_{inact})$, leading to the time constants τ_{inact}. The exponential decay of τ_{inact} values as a function of the step voltage (V): $\tau_{inact} \sim \exp(-V/\upsilon_{inact})$, indicates the voltage constant υ_{inact}. The rate of recovery from inactivation of the Na^+ currents, having an obvious impact on repetitive neuronal firing, has also been assessed. Na^+ current was elicited in a two-pulse protocol, by repeated depolarization from a holding potential of −80 to −10 mV, with two 10 ms duration depolarizing steps, separated by inter-pulse intervals (Δt) ranging from 0 to 30 ms. Since the Na^+ current reached a steady-state inactivation over the 10 ms duration of the first depolarizing step, the second step elicited a Na^+ current whose amplitude increased asymptotically as a function of Δt: $I_{Na} = I_{Na}^{max}[1 - \exp(-\Delta t/\tau_{rec})]$, with τ_{rec} representing the time constant of the recovery from inactivation.

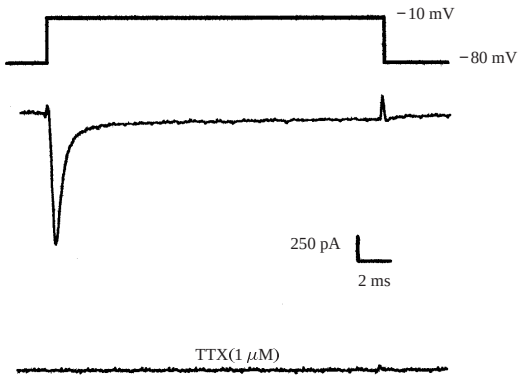

Fig. 1: Inward Na^+ current, elicited from a holding potential of −80 mV to a test potential of −10 mV, in a rat cortical neurone, 8 days old in culture. The current was completely blocked when the perfusion fluid contained 1 μM TTX (lower trace).

Low-threshold Ca^{2+} currents

Whole-cell patch-clamp recordings were performed, at about 28 °C, on pyramidal neurones in the CA1 area of hippocampal slices, visualized by near-infrared differential interference contrast video microscopy. The slices, 200 μm thick, were prepared from male Sprague–Dawley rats weighing 20–50 g, according to standard procedures, and were kept in oxygenated ACSF containing (in mM): NaCl, 126; KCl, 3; $CaCl_2$, 2.4; $MgCl_2$, 1.3; NaH_2PO_4, 1.24; $NaHCO_3$, 26; D-glucose, 10; pH 7.4. Patch pipettes (with a resistance of around 5 MΩ) were filled with a solution containing (in mM): CsF, 110; NaCl, 15; $CaCl_2$, 1; $MgCl_2$, 2; TEA-Cl, 10; HEPES, 10; EGTA, 10; Mg-ATP, 2; pH 7.2. To isolate the T-type current, Na^+ current and K^+ currents were inhibited by ad-

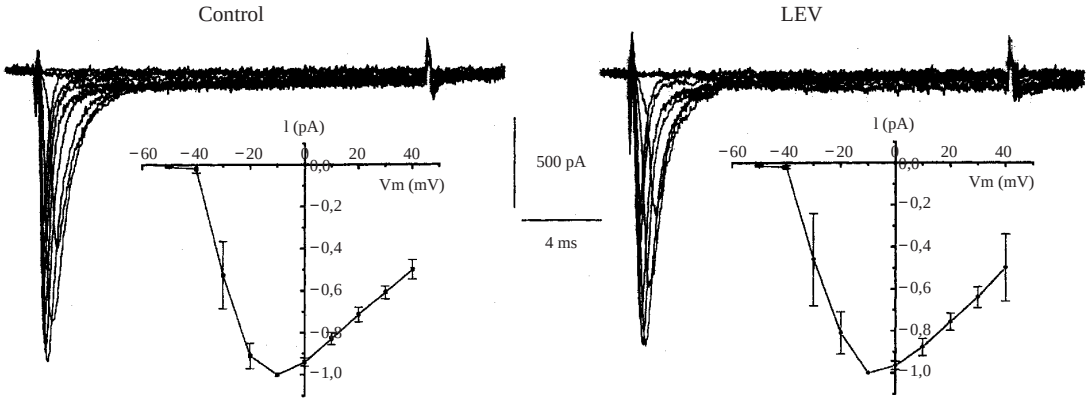

Fig. 2: Na^+ currents elicited in rat cortical neurones in culture with step depolarizations from a holding potential of -80 mV to test potentials increasing from -50 to $+40$ mV, in standard perfusion fluid (control, at left) and after 60 seconds perfusion with levetiracetam 200 μM (LEV, at right). The current–voltage curves show the Na^+ current amplitude (given as mean $\pm$ SEM), normalized with respect to its maximal value, as a function of the test potential (Vm) in a group of 18 cultured neurones, recorded in both control saline and under LEV.

junction of tetrodotoxin (TTX; 0.75 μM in ACSF), and Cs^+ (5 mM in ACSF and 110 mM internally) plus TEA (10 mM internally), respectively. High-threshold Ca^{2+} currents were cancelled by their fast run-down. Voltage-clamped recordings were collected with sampling rates of either 0.625 or 1 KHz. The T-type currents, isolated as shown in Fig. 3, did not require any further correction of leak conductance and capacitive transients, but were corrected for the liquid junction potential.

Upon entering into whole-cell configuration, the zero time recording was taken only after complete stabilization of the current, which usually took 20 minutes. After control recordings in normal ACSF, the recordings were performed over 40 minutes perfusion (flow rate 1 ml per minute) of the slices in LEV, either 32 or 100 μM, freshly dissolved in ACSF, or in ACSF alone. These (clinically relevant) concentrations of LEV were used because previous studies have shown that LEV exerts clear-cut *in vitro* effects in this concentration range, between 10 and 100 μM[6,7].

The steady-state activation of the T-type current was studied with voltage steps from -100 to -30 mV. It was characterized by fitting the normalized amplitude of the current with a rising Bolzmann sigmoid: $I_T/I_T^{max} = 1/\{1 + \exp[(V_{1/2} - V)/k]\}$, which gives the half-activation potentials ($V_{1/2}$) and the activation slopes (k). The steady-state inactivation was studied with 1 s conditioning steps increasing from -100 to -30 mV, followed by a step to -30 mV. It was similarly analysed with a falling Bolzmann sigmoid: $I_T/I_T^{max} = 1/\{1+\exp[(V-V_{1/2})/k]\}$, which gives the half-inactivation potentials ($V_{1/2}$) and the slopes (k).

The rising (activation) and the falling (inactivation) phases of the T-type current have been characterized by the same parameters, as defined above for the Na^+ current: the 'time to peak' (TTP) and the time constant τ_{inact}. Also, the recovery from inactivation has been described by the time constant τ_{rec}, as defined for the Na^+ current.

Statistics

The quantitative results are expressed as mean $\pm$ SEM, for the number of neurones recorded in each group. The statistical significance of differences between experimental groups was assessed with Student's *t*-test.

RESULTS

Na^+ currents

When cortical neurones were voltage clamped at -80 mV, a depolarization step to -10 mV elicited an inward current that was completely blocked by 1 μM TTX in the external solution (Fig. 1). Extracellular perfusion of different concentrations of LEV, from 10 μM to 1 mM, for time intervals from 3 seconds to 10 minutes, did not modify the amplitude of this TTX-sensitive Na^+ current (I_{Na}) in 25 neurones, 7–15 days old in culture. In view of the lack of effect of LEV on Na^+ current amplitude, a high concentration of 200 μM LEV was consistently used in further investigations of the biophysical characteristics of the Na^+ current, in order to avoid overlooking an effect of the drug which might only appear at the high limit of clinically relevant concentrations. Indeed, a meta-analysis on pharmacokinetic data from the four phase-III studies performed so far with adjunctive therapy of LEV in adults with refractory partial epilepsy reveals

that clinically effective doses (1–3 g per day) result in trough plasma levels between 35 and 100 μM and C_{1hour} (close to C_{max}) levels between 90 and 250 μM (unpublished results of UCB Pharma[15]).

In both control ACSF and that containing 200 μM LEV, Na^+ currents started to activate at a membrane potential of -40 mV and peaked at -10 mV (Fig. 2). Moreover, LEV did not change the steady-state activation of the Na^+ currents, elicited from a holding potential of -80 mV. The fit of the mean normalized Na^+ current amplitude against the step potential, indicated similar half-activation potentials ($V_{1/2}$) in both control conditions and in the presence of LEV (Table 1). In addition, LEV did not modify the steady-state inactivation of the Na^+ currents, elicited with voltage steps applied every 5 seconds, from different holding potentials to -10 mV. In both control ACSF and that containing LEV, Na^+ currents were similarly reduced when the holding potential was more positive than -90 mV and hardly any current could be elicited from holding potentials more positive than -40 mV. The fit of the normalized Na^+ current amplitude against the holding potential indicated quite similar half-inactivation potentials ($V_{1/2}$), in control conditions and in the presence of LEV (Table 1).

Neither TTP values, nor their voltage constant υ_{act}, have been influenced by the perfusion of LEV (Table 2). Likewise, LEV did not influence either the τ_{inact} values, or their voltage constant υ_{inact}, (Table 2). Also, the recovery from inactivation of the Na^+ currents, a phenomenon having an obvious impact on the repetitive neuronal firing, was not influenced by LEV, as shown by the time constant of the recovery from inactivation (τ_{rec}), which was almost identical, irrespective of the presence of LEV (Table 2).

Low-threshold Ca^{2+} currents

From a holding potential of -50 mV, voltage steps between -100 and -10 mV activate the low-threshold (T-type) Ca^{2+} current in CA1 pyramidal neurones, together with the leak current and, possibly, a residual high-threshold voltage-activated Ca^{2+} current, when the steps are preceded by a 2 seconds pre-step to -120 mV. However, when the steps are preceded by a 2 seconds pre-step to -60 mV, only the leak current and the residual high-threshold Ca^{2+} current are activated. Accordingly, the T-type Ca^{2+} current is isolated by subtraction of the two families of traces (Fig. 3). This T-type Ca^{2+} current was blocked by Ni^{2+} (200 μM; see the inset in Fig. 3) and by Co^{2+}

Table 1: Voltages of half-activation and half-inactivation of Na^+ currents ($V_{1/2}$), in rat cortical neurones, 7–21 days old in culture, recorded in control perfusion fluid and under 60 seconds perfusion of levetiracetam 200 μM (LEV). $V_{1/2}$, arising from the fit with Boltzmann sigmoids of the voltage dependencies of the normalized amplitudes of Na^+ currents, are given as mean $\pm$ SEM, for the number of neurones indicated between parentheses.

	$V_{1/2}$(mV)	
	Control	LEV
Activation (18)	30 ± 3.4	28.5 ± 4.6
Inactivation (13)	-45.6 ± 2.7	-44.5 ± 2.5

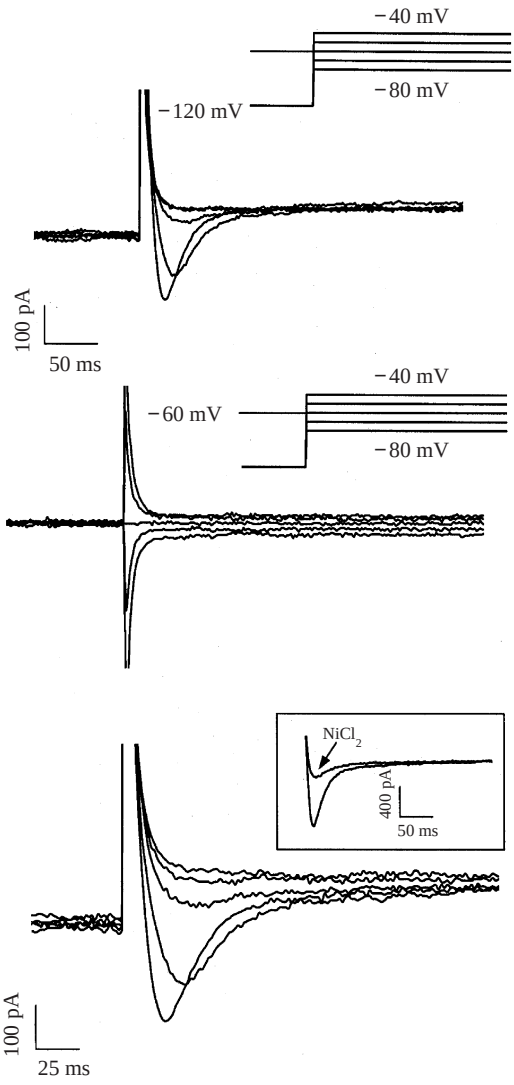

Fig. 3: Isolation of low-voltage gated (T-type) Ca^{2+} current in a pyramidal CA1 neurone from a rat hippocampal slice. From top, downward: activation of the T-type current together with the leak current and, possibly, a residual high-threshold voltage-activated Ca^{2+} current, when the depolarization steps from a holding potential of -50 mV are preceded by a pre-step to -120 mV (upper), activation of only the leak current and the residual high-threshold Ca^{2+} current, when the depolarisation steps from the same holding potential are preceded by a pre-step to -60 mV (middle) and the isolated T-type currents, obtained by subtraction of the two families of traces (lower). The inset shows the blocking of this current by $NiCl_2$ (200 μM).

Table 2: Kinetic parameters of Na^+ currents in rat cortical neurones, recorded in control perfusion fluid and under 60 seconds, perfusion of levetiracetam 200 μM (LEV). TTP is the 'time to peak', defined in the text; τ_{inact} is the time constant of the exponential decay of Na^+ currents; υ_{act} and υ_{inact} are the voltage constants arising from exponential fits of the voltage dependencies of TTP and τ_{inact}, respectively; τ_{rec} is the time constant of the recovery from inactivation, arising from the exponential fit of the inter-pulse dependence of Na^+ current amplitude (see Methods). All parameters are given as mean ± SEM, for the number of neurones given between parentheses. No difference between control and LEV-treated groups is significant.

	Activation (rising phase)			Inactivation (falling phase)			Recovery from inactivation
	TTP (ms)		υ_{act}	τ_{inact} (ms)		υ_{inact}	
	at −30 mV	at −10 mV	(mV)	at −30 mV	at +30 mV	(mV)	τ_{rec} (ms)
Control	2.47 ± 0.54 (9)	1.460 ± 0.021 (9)	14.6 ± 2.7 (9)	1.20 ± 0.20 (17)	0.300 ± 0.003 (17)	23.9 ± 2.5 (17)	3.5 ± 0.8 (8)
LEV	2.63 ± 0.42 (11)	1.470 ± 0.055 (11)	15.6 ± 1.8 (11)	1.20 ± 0.19 (21)	0.350 ± 0.005 (21)	22.5 ± 1.9 (21)	3.7 ± 0.7 (11)

Table 3: Voltages of half-activation and half-inactivation ($V_{1/2}$) and slopes (k) of the voltage dependencies of the normalized T-type current amplitudes, fitted with Boltzmann (rising for the activation, falling for the inactivation) sigmoids. The values are mean ± SEM for groups of neurones recorded under perfusion with either control ACSF, or levetiracetam (LEV) 32 μM, or LEV 100 μM. The number of neurones in each group is given between parentheses. No difference between the groups is statistically significant.

	Activation				Inactivation			
	$V_{1/2}$ (mV)		k (mV)		$V_{1/2}$ (mV)		k (mV)	
Time of recording (min)	0	40	0	40	0	40	0	40
Control (6)	−50.5 ± 2.5	−53.2 ± 2.6	2.6 ± 0.2	2.9 ± 0.5	−64.2 ± 2.1	−68.7 ± 1.6	−5.1 ± 0.3	−5.4 ± 0.1
LEV 32 μM (9)	−49.3 ± 0.6	−51.5 ± 0.6	2.5 ± 0.3	3.2 ± 0.3	−64.2 ± 1.0	−68.1 ± 0.7	−5.1 ± 0.1	−5.3 ± 0.2
LEV 100 μM (7)	−48.8 ± 1.1	−50.7 ± 1.3	3.1 ± 0.3	3.4 ± 0.3	−67.0 ± 1.0	−69.5 ± 0.1	−5.4 ± 0.3	−5.3 ± 0.7

(2.5 mM). However, LEV (32 μM, 100 μM) was without effect on the amplitude of the T-type current (Fig. 4).

The data in Table 3 show that neither the steady-state activation, nor the steady-state inactivation of the T-type current, characterized by the potentials of half-effects ($V_{1/2}$) and the slopes (k), were influenced by LEV. Likewise, none of the kinetic parameters of the T-type current in CA1 pyramidal neurones, the TTP and the time constants τ_{inact} and τ_{rec}, were influenced by LEV (Table 4).

DISCUSSION

This study revealed a complete lack of effect of LEV on the neuronal (TTX-sensitive) Na^+ current, studied with the same methodology with which some of us have previously shown clear-cut inhibitory effects of both established[9] and newer[10,11] AEDs, and of riluzole[16]. This appears to corroborate pharmacological observations with LEV in animal models of seizures and epilepsy. LEV is distinct from Na^+ channel blockers like phenytoin and carbamazepine by an absence of anticonvulsant activity in the maximal electroshock seizure test in mice and rats[1,2]. In contrast, it has been reported that LEV protects against seizures induced by 6 Hz electrical stimulation of mice via corneal electrodes, which is a phenytoin-resistant seizure model[17]. Furthermore, phenytoin, carbamazepine[18] and, recently, lamotrigine[2] are known to be inactive against focal seizures induced by systemic administration of pilocarpine, while LEV affords potent protection in this model[2]. Together with these results, the findings of the present study suggest that the antiepileptic action of LEV is unlikely to be mediated via inhibition of neuronal Na^+ channels.

In view of the commonly accepted association between absence epilepsy and the T-type Ca^{2+} current (in thalamic neurones), one might have anticipated that LEV, which is active in several rat models of absence epilepsy, would reduce the T-type current in hippocampal neurones, too. Specifically, LEV suppresses spontaneous spike-and-wave discharges in both GAERS rats[19], and in rats administered a subconvulsant dose of PTZ[20]. Also, the displacement of LEV from its stereo-specific binding site in rat brain membranes by the anti-absence drug ethosuximide at concentrations close to anti-convulsant plasma levels, reported twice[5,21], and by the T-type current antagonist amiloride[21] would further favour a possible interaction of LEV with the neuronal T-type current. In addition, the antagonism by LEV of a non-$GABA_A$ receptor-associated epileptiform effect of bicuculline in rat hippocampus was mimicked by the non-specific calcium antagonist flunarizine, known to preferentially block the T-type current, while the prototypic L-type calcium channel blocker nifedipine was without effect[22].

Table 4: Kinetic parameters of T-type current in pyramidal CA1 neurones of rat hippocampal slices, recorded over 40 minutes perfusion in either control ACSF, or levetiracetam (LEV) 32 μM or LEV 100 μM. TTP is the 'time to peak', defined in the text; τ_{inact} is the time constant of the exponential decay of T-type currents; τ_{rec} is the time constant of the recovery from inactivation, arising from the exponential fit of the inter-pulse dependence of T-type current amplitude (see Materials and methods section). The values are given as mean $\pm$ SEM, for the number of neurones indicated between parentheses. No difference between the groups is significant.

	Time of recording (min)	Activation TTP (ms)		Inactivation τ_{inact} (ms)		Recovery from inactivation τ_{rec} (ms)
		at -50 mV	at -10 mV	at -50 mV	at -10 mV	
Control	0	37.3 ± 5.3 (6)	15.7 ± 1.0 (6)	29.7 ± 2.7 (6)	11.0 ± 1.3 (6)	424.8 ± 14.0 (6)
	40	31.2 ± 3.0 (6)	14.1 ± 0.8 (6)	22.2 ± 2.0 (6)	11.4 ± 1.0 (6)	423.3 ± 31.6 (6)
LEV 32 μM	0	34.2 ± 4.0 (10)	14.1 ± 1.2 (10)	31.8 ± 5.8 (10)	11.2 ± 1.1 (10)	396.5 ± 15.0 (6)
	40	32.5 ± 3.1 (10)	14.2 ± 1.2 (10)	26.2 ± 3.2 (10)	10.3 ± 1.0 (10)	424.4 ± 18.3 (6)
LEV 100 μM	0	33.4 ± 6.4 (8)	14.2 ± 1.4 (8)	22.6 ± 1.7 (8)	9.8 ± 1.1 (8)	436.2 ± 11.0 (6)
	40	34.6 ± 6.7 (8)	14.2 ± 1.6 (8)	24.4 ± 2.3 (8)	9.9 ± 1.2 (8)	456.1 ± 12.0 (6)

In spite of all these indirect suggestions, the results of the present study (Fig. 4 and Tables 3 and 4) dismiss any direct effect of LEV on the T-type current in rat hippocampal neurones. Obviously, these results cannot exclude, however, a putative effect of LEV on the T-type current in thalamic neurones, on which the activity of the ethosuximide was originally shown[12]. However, our results support the view that an anti-absence activity *in vivo* is not compulsorily associated with an inhibition of the T-type Ca^{2+} current in all rat brain neurones, since LEV, a drug active in rodent models of absence-type seizures, did not affect the T-type current in rat hippocampal neurones. In fact, the anti-absence effects are in no way exclusive to T-type Ca^{2+} channel blockers and the very hypothesis that the therapeutic anti-absence action of ethosuximide arises from a reduction of the T-type current was questioned[23].

The current understanding of the antiepileptic mechanisms of LEV (whose antiepileptic properties have been revealed by serendipitous screening) is only incipient. The displacement of LEV from its specific binding site in rat brain by the GABA-related convulsants pentylenetetrazole and bemegride[5] suggested possible interactions between the LEV-binding site and the $GABA_A$ complex. Subsequently, LEV was reported to increase the activity of the GABA-degrading enzyme GABA aminotransferase (GABA-T) in several regions of the rat brain, including the striatum, where it also decreased the activity of the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD)[24]. A reduced GABAergic activity in the striatum is known to be anticonvulsant[25], since a disinhibited striatal output enhances the inhibition in the substantia nigra pars reticulata, a brain region which receives a strong GABAergic input from the striatum and controls seizure propagation[26]. Since Löscher *et al.*[24] recorded, indeed, a decreased spontaneous firing of (presumably) GABAergic neurons in the substantia nigra pars reticulata following LEV, these authors hypothesized that it might contribute to the anticonvulsant action of the drug. However, a wealth of data, starting with the absence of any affinity of LEV for the GABA receptors[5], prevent ascribing to LEV any conventional GABAergic facilitation. Indeed, the fact that LEV induced (only at the relatively high dose 170 mg kg^{-1} i.p.) in regions of rat brain both increases and decreases in GABA-T and GAD activities, while not altering the activities of these enzymes *in vitro*, led Löscher *et al.*[24] to conclude that the enzyme alterations they found were but indirect consequences of post-synaptic changes. Moreover, GABA turnover was normalized 60 minutes after LEV, casting serious doubts on the anticonvulsant relevance for the antiepileptic action of LEV of the alterations in GABA turnover, since the time of the peak effect of LEV was previously reported to be 60 minutes in both mice and rats[1]. Furthermore, LEV (up to 300 mg kg^{-1} i.p.; single/multiple doses) had no effect on the concentrations of GABA, glutamate and glutamine, and on the activities of GAD and GABA-T in mice brains[27]. Also, LEV had no effect on GABA transport and metabolism in rat astrocyte culture[28]. Accordingly, it was concluded that it is unlikely that the action of LEV would be mediated via the GABAergic system[27,28].

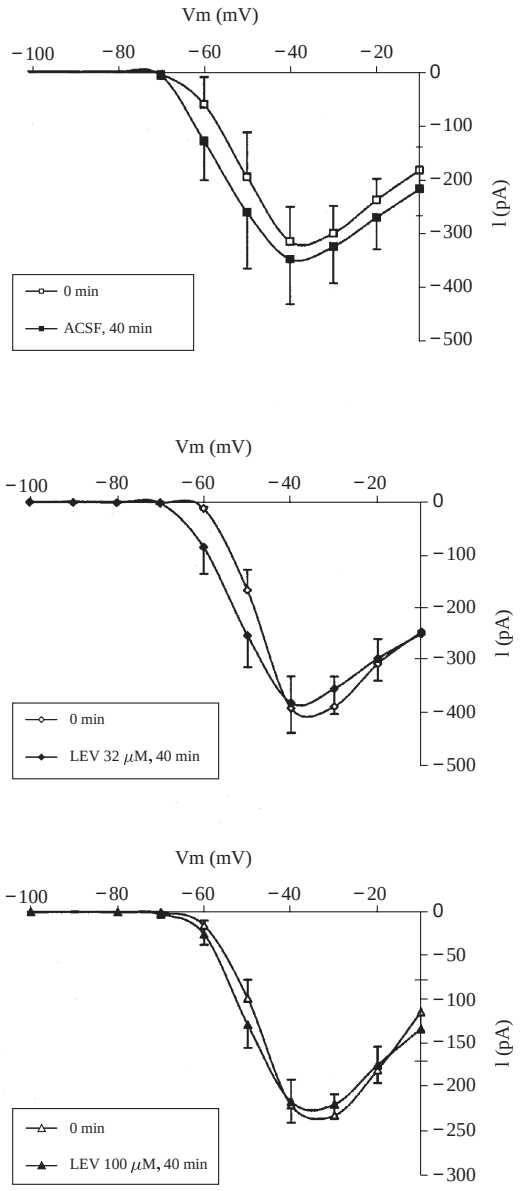

Fig. 4: Lack of effect of LEV on the amplitude of the low-voltage gated (T-type) Ca^{2+} currents in pyramidal CA1 neurones from rat hippocampal slices, elicited and isolated as described in Fig. 3. The graphs represent (mean ± SEM) the current–voltage dependencies before and after 40 minutes perfusion of either ACSF (upper graph, for $n = 6$ neurones), or LEV 32 μM (middle graph, for $n = 10$ neurones), or LEV 100 μM (lower graph, for $n = 8$ neurones). No statistically significant difference was found between the three groups of neurones.

While these studies, along with our results presented above, disclaim for LEV any conventional mechanism currently accepted for the established AEDs, very recent data suggest that LEV might control pathologic neuronal excitability via multiple modulatory actions. To date, these include an inhibition of high-voltage-activated Ca^{2+}currents in pyramidal neurons from rat hippocampal slices[29] and a suppression of the inhibitory effects of several negative allosteric modulators, including the Zn^{2+}ions and β-carbolines, on both GABA-gated currents in cultured rat hippocampal and cerebellar granule neurons and glycine-gated currents in spinal neurons[30]. Furthermore, an inhibition of the AMPA-gated current in cultured rat hippocampal neurons has also been reported, but only at relatively high concentrations[31]. Obviously, it is too early to conclude on the anti-seizure relevance of these effects and there is little doubt that further cellular effects of LEV might be unraveled in the forthcoming years.

CONCLUSION

This study reveals the absence of effect of LEV on neuronal voltage-activated Na^+ current and low-voltage-activated Ca^{2+} current, i.e. on two major mechanisms accepted for established AEDs. Along with previous reports, this suggests that LEV exerts its antiepileptic action via non-conventional mechanisms, the exact nature of which can only be identified by future studies. Beyond its mechanistic interest, the outcome of this study is of potential relevance for orienting antiepileptic polytherapy.

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