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Specific petit mal anticonvulsants reduce calcium currents in thalamic neurons

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Low-threshold calcium current (LTCC) in thalamic neurons is important in generation of normal thalamocortical rhythms, and may be involved in the genesis of abnormal activities such as spike-wave discharges that characterize petit mal epilepsy. Ethosuximide and dimethadione, anticonvulsants effective in petit mal, reduced the LTCC when applied to thalamic neurons at clinically relevant concentrations. Therapeutic concentrations of phenytoin and carbamazepine, drugs ineffective in the control of petit mal, had minimal effects on calcium conductances. Reduction in LTCC may be an important mechanism of action by which specific petit mal anticonvulsants depress spike-wave activity.

Petit mal epilepsy is a childhood-onset seizure disorder of unknown etiology characterized behaviorally by brief staring spells and occasional minor motor activity, and electrically by generalized 3 Hz spike-wave discharges in the electroencephalogram (EEG). The results of experiments in a well-studied animal model of petit mal suggest that thalamic relay neurons play a critical role in generation of the abnormal thalamocortical rhythmicity that underlies spike-wave discharges [7], a conclusion supported by EEG recordings from human thalamus in patients with petit mal epilepsy [19]. A prominent intrinsic membrane event, the low-threshold calcium spike (LTCS), plays a major role in the oscillatory behavior of thalamic relay neurons and in the development of normal thalamocortical rhythms such as sleep spindles [10, 18]. Generation of spike-wave discharges is closely linked to normal spindle generating mechanisms [7]. Taken together, these data suggest that LTCSs may contribute to the generation of spike-wave discharges in petit mal epilepsy. Reductions in LTCC by specific petit mal anticonvulsants would be consistent with such an hypothesis.

Patch-clamp techniques [8] were used to assess anticonvulsant actions on isolated calcium currents of thalamic neurons from guinea pigs (ages 1 day to adult) or rats (ages 1–15 days). Neurons from the ventrobasal complex of these animals were acutely dissociated using the methods described by Kay and Wong [11], and the effects

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of ethosuximide (ES) and dimethadione (DMD), the active metabolite of trimethadione, on the LTCC underlying the LTCS were examined. These drugs were chosen because of their selective effects in clinical petit mal epilepsy [1, 4, 12]; previous studies have not established their cellular mechanism of action [5, 20].

Drugs were either applied by localized perfusion, or by bath application (the latter method was used in Figs. 1 and 2). In all experiments, we used drug concentrations that were in the same range as free serum levels reported at therapeutic anticonvulsant doses in man (ES 300–700 μM; DMD 5–9 mM; phenytoin 4–8 μM; carbamazepine 4–12 μM; and valproic acid 20–200 μM) (reviewed in refs. 3 and 15). Whole-cell voltage-clamp electrode solutions contained (in mM): Tris-phosphate 110 (dibasic), Tris-base 28, EGTA 11, $MgCl_2$ 2, $CaCl_2$ 0.5, ATP 2, pH 7.3. The bath solution contained (in mM): NaCl 155, KCl 3, $MgCl_2$ 1, $CaCl_2$ 3, HEPES-Na^+ 10, and TTX 0.5 μM, pH 7.4. Patch experiments were conducted at room temperature (22–24° C). Leak and capacitance currents were subtracted from the evoked whole cell calcium currents.

Control recordings and drug effects were comparable between the two species. At least two calcium currents could be distinguished in all neurons on the basis of differences in time- and voltage-dependence of activation and inactivation. Both of these currents were completely blocked by cadmium (500 μM, not shown). The LTCC in thalamic neurons was very similar to the T or type I current described by others [2, 6, 17]. It was quickly inactivated by depolarization and was activated at low threshold when depolarizing steps to potentials between -70 and -30 mV were applied from a holding potential of -100 to -110 mV (e.g. Fig. 1A). The high-threshold calcium current (HTCC) was similar to the L or type II current described in other cells [6, 17], in that it was activated by depolarizations to -30 mV or more positive potentials, and was very slowly or non-inactivating during the time course of sustained depolarizing commands (e.g. Fig. 2A, B). Only the LTCC had activation and inactivation characteristics that were similar to those reported for the LTCS of thalamic neurons in vitro [10]. The LTCC was very stable, and could be evoked at frequent intervals over long periods of time with little variability in amplitude. The HTCC tended to run down with time if the patch pipette did not contain ATP, as has been previously noted (e.g. ref. 21). A third component of calcium current was found in some neurons, similar to the N-type current described by others [6]. Because it was small in amplitude (relative to the LTCC and HTCC), difficult to isolate, and present in only a small proportion of the population, the effects of drugs on the N-current were not analyzed [17]. This N component of current could be distinguished from T by a higher threshold, slower inactivation kinetics, and different steady-state inactivation. The T-type current could be resolved in isolation by steps to -50 mV from -110 mV, and L-type could be measured as the sustained component (remaining activated at the end of the 200 ms voltage step) evoked by steps to -10 mV from -110 mV. This later component was largely unaffected by changing the holding potential to -40 mV, where the N and T components would be mostly inactivated [6].

ES reversibly reduced the amplitude of the LTCC (65/84 neurons) (Fig. 1A, illus-

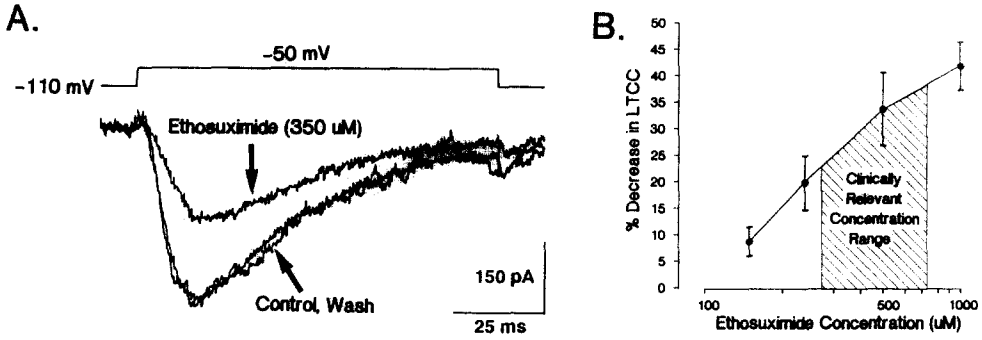

Fig. 1. ES-induced reductions of low-threshold calcium currents, measured in an acutely dissociated thalamic neuron with whole-cell voltage-clamp under ionic conditions in which calcium currents were isolated. A: application of 350 μM ES resulted in a reversible reduction of LTCC. Control, ES-reduced, and recovery traces are superimposed. B: bath application of increasing concentrations of ES (125, 250, 500 and 1000 μM) to 5 ES-responsive thalamic neurons induced a dose-dependent reduction in LTCC. Points are mean $\pm$ 1 S.E.M. Recovery was to 88 $\pm$ 8% for this population following ES washout.

trated by steps to -50 mV from a holding potential of -110 mV, for the LTCC). Similar reductions occurred throughout the voltage activation range of the LTCC (not shown). ES concentrations of 500–700 μM produced a maximal reduction in LTCC in ES-responsive cells. Increases in ES concentration to supraclinical levels (up to 7 mM) did not produce further decrements in LTCC. Within single cells, applications of several different concentrations of ES induced dose-dependent reductions of LTCC (Fig. 1B). In ES-unresponsive cells, which consistuted 24% of the population, no response to ES at any concentration was found. DMD also reversibly reduced the LTCC and HTCC in dissociated thalamic neurons ($n=17$, Fig. 2A). At concentrations comparable to clinical free serum levels [3], DMD produced a maximal reduction in the L- and HTCC of up to 60% and 40%, respectively. Succinimide is the unsubstituted ring base of ES, and is inactive in the control of petit mal [5, 20]. In 6 neurons we compared effects of ES and succinimide in identical concentrations (500–1000 μM). Succinimide did not reduce the L- or HTCC, whereas ES reduced the LTCC (Fig. 2B) and in some cases the HTCC in these cells.

Actions of ES and DMD were compared with those of carbamazepine (CBZ) and phenytoin (PT), anticonvulsants used in partial and generalized seizures, but ineffective in petit mal, and with valproic acid (VPA), a drug effective in both varieties of epilepsy. VPA had only small effects on calcium currents (less than 10% reductions) at clinically relevant concentrations ($n=8$, not shown). PT has been reported to selectively reduce LTCC in cultured hippocampal neurons at a concentration of 100 μM [21], however effective non-toxic clinical free serum levels of PT are 4–8 μM [15]. We found that 100 μm PT selectively reduced the LTCC by 40–50%, whereas 4–8 μM reduced the LTCC by only 5–10% ($n=9$). CBZ did not affect LTCC in clinically relevant concentrations (4–12 μM, $n=6$, not shown).

These are the first described actions of selective petit mal agents that seem to be consistent with their specific anticonvulsant effects. ES and DMD could certainly

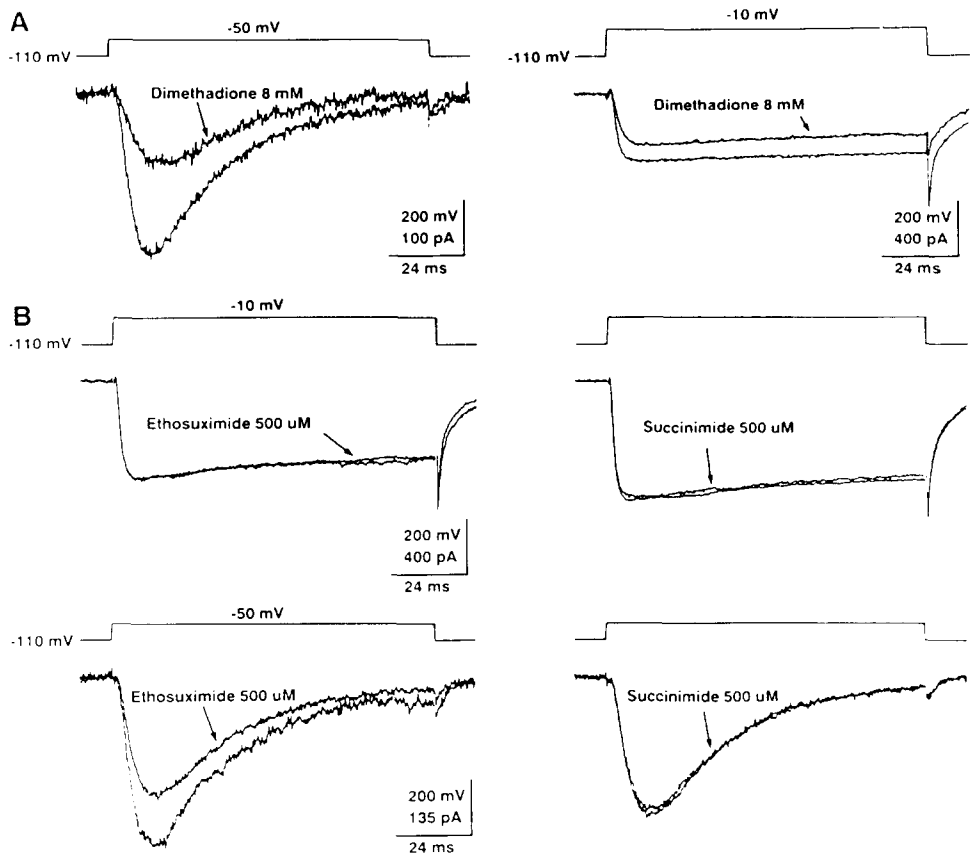

Fig. 2. Effects of dimethadione (DMD), ES and succinimide on calcium currents of thalamic neurons. A: DMD (8 mM) reduces the LTCC (right traces) and the HTCC (left traces) elicited by steps to −50 and −10 mV from −110 mV, by 56% and 28%, respectively. This effect was fully reversible. B: comparison of effects of ES and succinimide on LTCC and HTCC in the same neuron. Left traces: lower, superimposed sweeps of control and ES (500 μM) show that ES reduces the LTCC elicited by voltage steps from −110 to −50 mV by 30%; upper, voltage steps to −10 mV from −110 mV elicited a HTCC, which was unaffected by ES (500 μM). Right traces: succinimide (500 μM) had little or no effect on HTCC (upper) or LTCC (lower), following washout of ES in the same neuron.

have other actions on thalamic neurons and at other sites in the brain. However, the effect on LTCC shown here may be a primary one since the LTCC has a major role in development of rhythmic thalamic activity. One implication of these findings is that the thalamic LTCS has a potentially important role in generation of the spike-wave discharges that underlie petit mal seizures. Drugs ineffective in petit mal, such as PT and CBZ, had little or no effect on LTCC in these neurons at therapeutic concentrations. Failure of VPA to reduce LTCC in thalamic neurons may indicate that this agent exerts its actions on petit mal seizures through different mechanisms. VPA has a broader spectrum of clinical and electrophysiological actions [14, 16], perhaps mediated by metabolites not tested in our experiments [13]. A knowledge of the ac-

tions of specific anticonvulsants such as ES and DMD, may provide data potentially useful in understanding mechanisms important in the pathogenesis of petit mal epilepsy. Our findings also lead to the prediction that other agents which produce selective reductions in LTCC will be effective in therapy of this seizure disorder.

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