Mechanism of Block of Thalamic T-Type Ca^{2+} Channels by Petit Mal Anticonvulsants

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Introduction

The low frequency spike-and-wave discharge which is the electroencephalographic signature of typical absence or petit mal seizures is generated by an underlying thalamocortical oscillatory interaction (Gloor and Fariello 1988). A low-threshold Ca^{2+} conductance, found in virtually all thalamic neurons, has been shown to be of primary importance in the generation of thalamocortical oscillatory behavior (Steriade and Llinás 1988). We have previously reported that the petit mal anticonvulsants ethosuximide (Zarontin, Parke Davis), dimethadione (the active metabolite of Tridione, Abbott), and α-methyl-α-phenyl succinimide (MPS; the active metabolite of methsuximide, Celontin, Parke Davis), in clinically relevant concentrations, all blocked the low-threshold (T-type) Ca^{2+} current (Coulter et al. 1989; Coulter et al. 1989; Coulter et al. 1989) which generates the low-threshold Ca^{2+} spike in thalamic relay neurons (Coulter et al. 1989). All three anticonvulsants blocked the current by a similar mechanism, without affecting the time course of activation or inactivation, and without affecting steady-state inactivation. The reduction of T current by these anticonvulsants was voltage-dependent, with maximal block occurring at threshold to elicit the current (approximately -65 mV). Two possible mechanisms could explain this block of T current. Since neither the voltage dependence of probability of opening nor the voltage and time dependence of inactivation of the T-type channels were affected by anticonvulsant exposure (as assessed through recordings of properties of block of the whole-cell current), we hypothesized that these anticonvulsants could be acting by either reducing the single channel conductance or by reducing the number of T-type channels available to be activated.

Methods and Results

In order to differentiate between these two possibilities, we employed ensemble fluctuation analysis, a form of noise analysis developed by Sigworth (Singworth 1980), and applied to Ca^{2+} currents by Bean and colleagues (Bean et al. 1984), to study the trace to trace variance in Ca^{2+} current records under control and drug-exposed conditions. These fluctuations are due to the opening and closing of single channels, and properties of these channels can be studied by analyzing this noise. Comparing sequential traces activated by identical depolarizing responses, one can quantify the fluctuations evident in the records by digitally subtracting the two traces (to remove the DC component of whole-cell current), squaring the resulting difference trace (to compute the variance), and dividing by two (to account for the combination of variance from two traces). Averaging many of these difference traces together gives an estimate of the mean change in variance with time as the whole-cell Ca^{2+} current activates and inactivates. By plotting this variance against the whole-cell Ca^{2+} current amplitude, a graph is generated which can be fitted by the function:

$$\sigma^2 = iI\text{-}I^2/N$$

where σ^2 is the variance, i is the single channel current, I is the mean current, and N is the number of channels (assuming that there is one non-zero conductance state for the channel and that the

Experimental Brain Research Series 20

channels are independent). This analysis provides information about the single-channel current and the number of channels involved in generation of the whole-cell current, since the variance and the mean current are known.

Whole-cell voltage-clamp techniques were used to assess anticonvulsant actions on Ca^{2+} currents of enzymatically isolated (Coulter et al. 1989) thalamic cells of young (1 - 15 day old) rat pups. Intracellular and extracellular solutions were designed to ionically and pharmacologically isolate Ca^{2+} currents (Coulter et al. 1989). An ATP reconstitution system was included in the electrode solution to improve cell viability (Forscher and Oxford (1985). Drugs were applied by a concentration clamp technique, which allowed solution changes in <<1 s. MPS was chosen as the anticonvulsant to test using ensemble fluctuation analysis, since the concentration-dependence of its actions on T current has been fully characterized (Coulter et al. 1989) and it has greater maximal efficacy than ethosuximide (100% vs 40%, (Coulter et al. 1989) and (Coulter et al. 1989)). All fluctuation analyses were performed on currents elicited by a command step to -40 mV from a holding potential of -100 mV. This step activates only T current since: (1) both the N and L type Ca^{2+} currents have been reported to have a more depolarized threshold than -40 mV (Carbone and Lux 1987; Coulter et al. 1989; Fox et al. 1987), and (2) the Ca^{2+} current activated by this step command steady-state inactivates as a single component (Coulter et al. 1989). This satisfies the fluctuation analysis requirement for activation of an independent population of T channels with a single open state (Singworth 1980).

Figure 1 illustrates the results of ensemble fluctuation analysis of MPS block of T current. Application of 750 *M* MPS resulted in a 37% block of T current (Fig. 1A) without affecting the time course of the current, as has been reported previously (Coulter et al. 1989). From analysis of the fluctuations during the rising phase of the current (between the arrows in Fig. 1A), it is evident that the variance of these fluctuations increases as the amplitude of the current increases, as expected, since these fluctuations are due to the opening and closing of individual T-type Ca^{2+} channels, the frequency of which increases as the whole-cell current increases (Fig. 1B). Plotting the mean variance vs the mean whole-cell current under control conditions, the curve can be fitted with the equation described above, using a single channel current (*i*) of 0.15 pA, and a number of channels estimate (*N*) of 4750 (Fig. 1C). Following drug application, the mean current was reduced by 37%. Assuming this reduction was accomplished by either blocking 37% of the channels (reducing the *N* from 4750 to 3000) or by reducing the single channel conductance 37% (reducing the *i* from 0.15 to 0.09 pA), the plot of mean current vs variance during drug exposure was fitted with these two models. It is evident that a model with a reduced number of T channels (solid line in Fig. 1D) fits the data much better than one with a reduction in the single channel current (dashed line in Fig. 1D). Thus, MPS appears to block T current by reducing the number of channels available to be activated.

Discussion

We have previously shown that the structurally similar petit mal anticonvulsants ethosuximide, MPS, and dimethadione all block T current in thalamic neurons in a similar voltage-dependent manner (Coulter et al. 1989; Coulter et al. 1989; Coulter et al. 1989). On the basis of the above fluctuation analysis data, and assuming that all three anticonvulsants are acting by molecular mechanisms similar to that of MPS, we conclude that the mechanism of block of T current by these anticonvulsants is by reducing the number of channels available to be activated without affecting the voltage-dependence of activation or inactivation or the single channel conductance. This type of block has been termed closed channel block.

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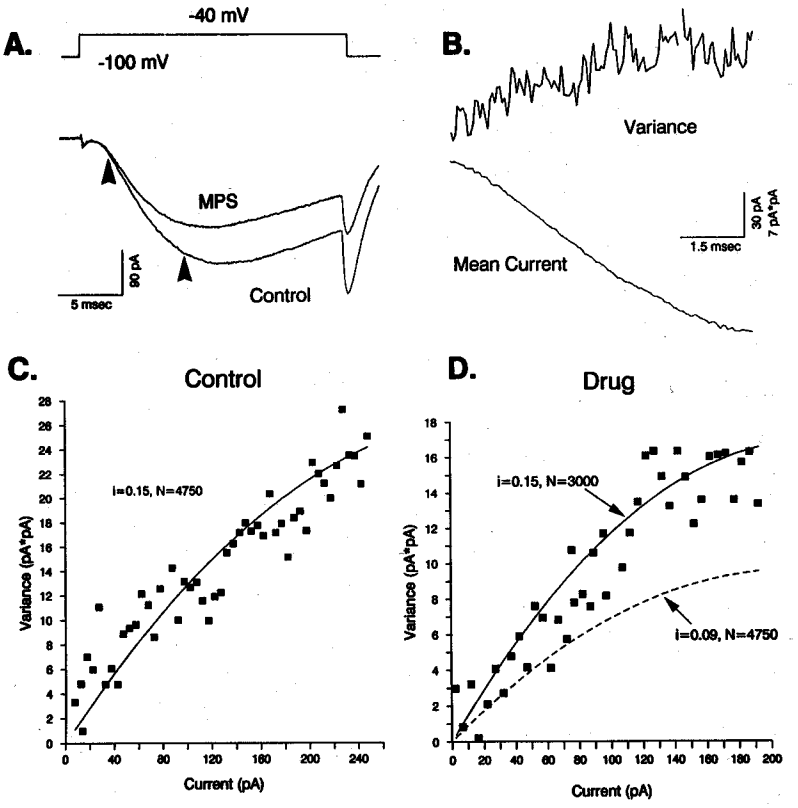

Fig. 1A-D. Ensemble fluctuation analysis of α-methyl-α-phenyl succinimide (MPS) block of T current in thalamic neurons. **A** Application of 750 *M* MPS resulted in a reduction of the T current of 37%. The T current was elicited by a step command to -40 mV from a holding potential of -100 mV (*top*). Ensemble fluctuation analysis was performed on the rising phase of the current (portion between the *arrows*). **B** Average variance (in pA^2, mean of 94 difference traces, *top sweep*) during the rising phase of the whole-cell Ca^{2+} current (in pA, *bottom trace*). Note that as the whole-cell Ca^{2+} current increases in amplitude, the fluctuation variance increases. **C** Plot of mean variance vs mean whole-cell current under control (wash from MPS) conditions. Background variance (due to leak and thermal noise) was subtracted from the curve by fitting the initial, linear portion of the trace by regression and using the y intercept (variance with 0 whole-cell Ca^{2+} current) as the background fluctuation variance of the cell at rest. The plot was then fitted to the ensemble fluctuation equation (see text) using a single channel current estimate (*i*) of 0.15 pA, and a channel number estimate (*N*) of 4750. The variance is the mean of 94 difference traces, averaged in 5 pA current bins in this plot. The mean whole-cell current is the average of 20 leak subtracted traces, 10 from immediately before the fluctuation analysis, and 10 from immediately subsequent to the analysis. **D** Plot of mean variance versus mean whole-cell Ca^{2+} current under MPS-exposed (750 μ*M*) conditions (calculated as in C). The plot was fitted assuming that the 37% reduction in whole-cell Ca^{2+} current was caused either by a 37% reduction in the single channel current (*i*, from 0.15 to 0.09 pA, *dashed line*), or in the number of channels (*N*, from 4750 to 3000, *solid line*). Note that the data is best fitted by assuming a reduced number of channels

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