Voltage-Dependent Calcium Channels as Targets for Convulsant and Anticonvulsant Alkyl-Substituted Thiobutyrolactones

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ABSTRACT

Alkyl-substituted thiobutyrolactones increase or decrease γ-aminobutyric acidA responses at or near the picrotoxin site, but they are structurally similar to ethosuximide, which prompted us to determine the actions of thiobutyrolactones on voltage-dependent Ca\(^{2+}\) currents. We measured Ca\(^{2+}\) currents in cultured neonatal rat dorsal root ganglion neurons in the absence and presence of the anticonvulsant α-ethyl,α-methyl-γ-thiobutyrolactone (α-EMTBL) and the convulsant β-ethyl,β-methyl-γ-thiobutyrolactone (β-EMTBL). Low-voltage-activated (T-type) currents were reduced in a concentration-dependent manner, with a maximal reduction of 26% and 30% by α-EMTBL and β-EMTBL, respectively. α-EMTBL reduced high-voltage-activated currents in a concentration- and voltage-dependent manner: maximal responses were 7% when evoked from −80 mV, with more rapid current inactivation; 29% when evoked from −40 mV, with little effect on current inactivation. β-EMTBL increased high-voltage-activated currents ≥20% at 10 to 300 μM, but reduced currents at higher concentrations; the latter action was similar to that of α-EMTBL in its magnitude and voltage dependence. Block of N-type channels with ω-conotoxin GVIA (10 μM) reduced the effect of α-EMTBL and eliminated its voltage dependence. The L-type current component was also reduced by α-EMTBL, with little effect on P- or Q-type current components. The related compound, α-ethyl,α-methyl-γ-butyrolactone, had no effect on Ca\(^{2+}\) currents. We conclude that thiobutyrolactones affect voltage-dependent Ca\(^{2+}\) currents in a concentration- and voltage-dependent manner, with greater potency on low-voltage-activated channels. Both the ring structure and the position of its alkyl substitutions determine the identity of the targeted Ca\(^{2+}\) channel subtypes and the manner of regulation.

The mechanisms of action of antiseizure drugs have been studied intensively, relying on observed drug effects in vitro to make inferences about actions in vivo (Rogawski and Porter, 1990; Macdonald and Meldrum, 1995). For the most part, these drugs act on neuronal ion channels or on the neurotransmitters that regulate their activity. For example, phenytoin, carbamazepine and lamotrigine increase inactivation of voltage-dependent Na\(^+\) channels (McLean and Macdonald, 1983, 1986; Quandt, 1988; Cheung et al., 1992), and barbiturates and benzodiazepines increase GABA\(_A\)-mediated inhibition (e.g., Twyman et al., 1989; Macdonald and Meldrum, 1995). The role of Ca\(^{2+}\) channel blockade as an antiseizure mechanism is less clear, however. The Ca\(^{2+}\) channel blocksome antiseizure activity (Binnie, 1989; Meyer, et al., 1990), but the effects of the latter are not substantial. Barbiturates, at concentrations similar to those achieved during treatment of status epilepticus, profoundly block high-voltage-activated Ca\(^{2+}\) currents (Gross and Macdonald, 1988a,b). The best case for the antiseizure effect of Ca\(^{2+}\) channel blockade is ethosuximide, which blocks the low-voltage-activated T-type channel, likely responsible for its efficacy in primary generalized (absence) epilepsy (Coulter et al., 1989).

Although newer pharmaceuticals are being developed with increasingly specific and selective actions, some affect more than one ion channel or neurotransmitter system; it may be difficult, therefore, to determine which action is most desirable for clinical efficacy (or which produces undesirable toxicity) and whether more than one therapeutic action may be advantageous. The alkyl-substituted butyro- and thiobutyrolactones are a novel group of compounds with either anticon-
vulsant or convulsant activity, dependent on the location and size of their alkyl groups. Compounds with small α-substitutions are anticonvulsant, whereas β-substituted compounds are convulsant (Kluck et al., 1982a,b; Holland et al., 1990).

Both groups act at or near the picrotoxin site of the GABA_A receptor complex (Weissman et al., 1984; Levine et al., 1985; Canney et al., 1991; Xu et al., 1995), exhibiting either picrotoxin agonist (convulsant) or inverse agonist (anticonvulsant) properties. These compounds do not appear to gate the GABA_A receptor directly, but the actions of some are complex, with differing quantitative effects in the presence of varying GABA concentrations (Yoon et al., 1993).

The structural similarities of the butyrolactone analogs to succinimides, and to ethosuximide in particular, suggested that these compounds may regulate voltage-dependent Ca^{2+} currents as well (fig. 1). If this were to be the case, then butyrolactone analogs may afford the opportunity to make inferences about the relative merits of GABA or Ca^{2+} channel effects vis-à-vis convulsant or anticonvulsant action. We therefore sought to characterize the actions of a selected group of alkyl-substituted butyrolactones on voltage-dependent Ca^{2+} currents in cultured neurons. The effects of the anticonvulsant compound α-EMTBL were compared with those of the convulsant β-EMTBL and with α-EMGBL, an anticonvulsant compound that blocks the action of α-EMTBL on GABA responses in cultured hippocampal neurons (Holland et al., 1990). Our studies suggest that GABA_A receptor blockade is the major action of the convulsant compounds but that blockade of Ca^{2+} channels, particularly T-type channels, may participate in the anticonvulsant actions of the α-substituted thiobutyrolactones.

**Methods**

**Cell Culture**

Primary DRG cultures were prepared from day 7 to 10 neonatal Sprague-Dawley rats (Harlan) as described previously (Gross et al., 1990). The animals were sacrificed after CO_2 narcosis by decapitation. The DRGs were dissected away from the spinal cord and placed in ice-cold Hanks’ buffer (pH 7.4 with HEPES; Sigma Chemical Company, St. Louis, MO). After enzymatic digestion with trypsin (1 mg/ml; Sigma) and mechanical trituration, the cells were spun down and resuspended in plating medium (MEM containing bicarbonate (GIBCO, Grand Island, NY), 50 ng/ml newborn growth factor (Collaborative Biomedical Research, Bedford, MA), 5% equine serum and 5% fetal bovine serum (Hyclone Laboratories, Logan UT)). Cells were then plated on 35-mm dishes with collagen (Sigma) as substrate. The medium was changed within several hours and replaced with MEM containing 10% equine serum, without fetal bovine serum. Ara-C (1–5 μM; Sigma) was used, if needed, to inhibit the growth of non-neuronal cells within the first week of longer term cultures. The cultures were maintained at 37°C under a 95% air-5% CO_2 atmosphere and fed on a twice-weekly basis with 50% exchanges of growth medium. These cultures were used for experiments as early as day 2, and for up to 10 to 12 weeks. The vast majority of experiments were performed on neurons in culture for 2 to 4 days.

**Electrophysiology**

**Preparation of solutions.** Butyrolactones were dissolved in external solution (below) on the day of the experiment. Nifedipine and ω-conotoxin GVIA (both from Sigma) and ω-agatoxin IVA (Peptides International, Louisville KY) were made fresh on the day of the experiment. Nifedipine was dissolved in dimethyl sulfoxide (Sigma) at a concentration of 10 mM and was diluted in external solution to a final concentration of 10 μM. ω-Conotoxin GVIA and ω-agatoxin IVA were stored frozen in water at a concentration of 10 mM and were used for experiments within 2 to 3 months. On the day of the experiment, the stock solutions were diluted in external solution.

**Whole-cell patch-clamp recordings.** Whole-cell voltage-clamp recordings were obtained with the whole-cell variation of the patch-clamp technique. Cells were bathed in a solution containing (in mM): CaCl_2, 5.0; choline Cl, 67; MgCl_2, 0.8; TEA, 100; glucose, 5.6; KCl, 5.3; HEPES, 10 (pH 7.3–7.4, 310–330 mOsm; all reagents from Sigma). Glass recording patch pipettes were fashioned from Fisher-brand microhematocrit tubes with a Sutter Instruments Brown-Flaming P-87 pipette puller. These electrodes had resistances of 1.5 to 3.0 megohm when filled with a recordingsolution consisting of the following (in mM): KMeSO_3, 140; HEPES, 10; ethyleneglycol-bis(β-aminoethyl ether)-N,N,N',N-tetraacetic acid, 10; ATP-Mg_2^+, 5; GTP-Na, 0.1 (all reagents from Sigma). The pH (7.3–7.4) was adjusted with 1 N CaOH after the addition of ATP. The osmolality was 10 to 15% less than that of the bath solution, 280 to 300 mOsm.

Recordings were made at room temperature by the Axopatch 200 patch-clamp amplifier (Axon Instruments, Foster City, CA). Pipette and whole-cell capacitance and series resistance were corrected by compensation circuitry on the amplifier. Series resistance was estimated by cancellation of the capacitance-charging current transient after patch rupture; typical values for the series resistance were 2.5 to 4 megohm. In most cases, series resistance compensation of 80 to 90% was possible without significant noise or oscillation. Voltage step commands were generated, and currents were digitized (5 kHz), stored and analyzed by a microcomputer (CompuAdd 325, 425; Zeos Pentium; or clones) with the program pClamp 5.6 and 6.0 (Axon Instruments). The current traces were filtered with a Bessel filter at 2 kHz (–3 dB). Data were accepted only if space-clamp was adequate, determined by well-controlled incremental current activation over a series of voltage steps (usually, –65 to +10 mV), and if tail currents deactivated rapidly. Furthermore, with 10-mV test pulses, we required settling of the capacitance-charging transient within 2 msec.

Leak current was determined by a P/P4 or P/P6 protocol. This current was digitally subtracted from the relevant inward current to obtain the calcium current.

Butyrolactones, nifedipine, ω-agatoxin IVA and ω-conotoxin GVIA were applied to the cell under study by pressure ejection (6–10 kPa) from separate blunt-tipped (internal diameter, 12–15 μm) glass micropipettes positioned ~30 μm from the cell. Applications of these compounds were 10 to 15 sec in duration, just before currents were evoked and the puffer pipettes were removed from the bath when not in use. In some experiments, drug applications were accomplished by a “U-tube” microperfusion system; the results were similar to those.
obtained with puffer application. Diluents had no effect on evoked currents. Control experiments showed that dimethyl sulfoxide (nimodipine) at concentrations up to 1% had no effect on Ca\textsuperscript{++} currents.

In all experiments, the culture was perfused continuously with bath (external) solution by a gravity-fed, vacuum-removed system operating at about 0.3 ml/min; thus, drug concentrations were minimized in the remainder of the culture dish.

Data Analysis

Data are expressed as means ± S.E.M., and statistical comparisons between group means were made with Student’s two-tailed t test.

Results

Effects on low-voltage-activated whole-cell Ca\textsuperscript{++} currents. The effects of thiobutyrolactones were tested first on low-voltage-activated (T-type) Ca\textsuperscript{++} currents because of their structural similarity to ethosuximide, an antiabsence seizure drug that reduces T-type currents in thalamic and sensory neurons (fig. 2). T-type currents, which require very negative V\textsubscript{h} to remove steady-state inactivation, can be studied in isolation by evoking currents at relatively negative V\textsubscript{c} (negative to −20 mV), at which little if any high-voltage-activated currents are evoked. For these experiments, currents were evoked from V\textsubscript{h} = 52 to 90 mV at a series of potentials, ranging from −65 to −5 mV (see figure). Peak current magnitudes were measured, and the effect of each compound was assessed on all currents uncontaminated with the more slowly inactivating high-voltage-activated current components.

The anticonvulsant compound α-EMTBL reduced T-type currents, with a mean peak current reduction of 26 ± 3% (n = 12 ± SEM; P ≤ .001). There was no apparent effect on the voltage dependence of current activation as evidenced in the current-voltage plots (although unequivocal determination of the potential at which the maximal T-type current occurred was difficult because of contamination with high-voltage-activated current components), nor any effect on current kinetics. The convulsant compound β-EMTBL had a similar action. Low-voltage-activated currents were reduced with a maximal mean reduction of 30 ± 1% (n = 4; P ≤ .001).

By contrast, the anticonvulsant butyrolactone α-EMGBL (500 μM) had no effect on T-type currents (see also below). These actions were compared with those of 500 μM ethosuximide applied to neurons from the same culture groups. Ethosuximide reduced T-type currents, but was less efficacious than either thiobutyrolactone, producing a current reduction of 16 ± 4% (n = 8; P ≤ .002).

Thiobutyrolactones reduced T-type currents in a concentration-dependent manner but with slightly differing potencies and efficacies. T-type currents were not significantly affected by 10 μM α-EMTBL. At greater concentrations, T-type currents were reduced 9 ± 2% (50 μM, n = 6), 18 ± 2% (100 μM, n = 8), 25 ± 3% (300 μM, n = 5) and 26 ± 3% (500 μM, n = 13). Higher concentrations did not have a greater effect. The concentration-response plot (fig. 3) was used to estimate by visual inspection the half-maximal concentration (EC\textsubscript{50} ≈ 75 μM).

β-EMTBL had similar concentration-response parameters, slightly more efficacious but slightly less potent. Currents were reduced 1.5% (50 μM, n = 2), 2 ± 1% (100 μM, n = 5), 7 ± 1% (300 μM, n = 4), 30 ± 5% (500 μM, n = 9) and 30 ± 1% (1000 μM, n = 4), yielding an apparent EC\textsubscript{50} ≈ 350 μM.

![Fig. 2. Reduction of low-voltage-activated (T-type) Ca\textsuperscript{++} currents by α-EMTBL, β-EMTBL and ethosuximide. (A) Currents were evoked in different cells from V\textsubscript{h} = −90 mV at the potentials shown in the absence (Control) or presence of α-EMTBL (500 μM) or β-EMTBL (1000 μM). Current traces are shown above peak current-voltage plots obtained from the same cells. (B) Currents evoked in the absence or presence of 500 μM ethosuximide (left) and the peak current-voltage plot from the same cell (right).](image-url)
Effects on high-voltage-activated whole-cell Ca\(^{2+}\) currents. The effects of \(\alpha\)-EMTBL and \(\beta\)-EMTBL were assessed next on high-threshold-activated current components. The thiobutyrolactones had complicated actions on these current components, with both concentration- and voltage-dependent effects. Across a wide concentration range (10–1000 \(\mu\)M), \(\alpha\)-EMTBL reduced high-voltage-activated Ca\(^{2+}\) current components in all neurons tested, but did so in a voltage-dependent manner (fig. 4). By contrast, \(\beta\)-EMTBL increased currents at concentrations \(\leq\) 300 \(\mu\)M, without a clear voltage dependence, and reduced currents at greater concentrations in a voltage-dependent manner similar to that of \(\alpha\)-EMTBL (fig. 5).

High-voltage-activated Ca\(^{2+}\) current components were smaller in the presence of \(\alpha\)-EMTBL, with maximal peak current reductions at a concentration of 500 \(\mu\)M. When currents were evoked from \(V_h = -80\) mV at +10 mV, there was no apparent effect of 10 \(\mu\)M \(\alpha\)-EMTBL. At higher concentra-

![Graph showing concentration-response relationship for \(\alpha\)-EMTBL and \(\beta\)-EMTBL for T-type currents. The peak current magnitude was measured for the maximal T-type current of each cell, evoked from \(V_h = -90\) mV at -20 or -15 mV; and the percent reduction was plotted for both \(\alpha\)-EMTBL and \(\beta\)-EMTBL over the stated ranges of concentrations. See text for details.](image)

![Figure 4. Effect of \(\alpha\)-EMTBL on high-voltage-activated Ca\(^{2+}\) currents. (A) Currents were evoked from either -80 mV (○) or -40 mV (●) at +10 mV in the absence or presence of 500 \(\mu\)M \(\alpha\)-EMTBL. Traces are shown above the peak current-voltage plot obtained from the same cell. (B) Concentration-response relationship plotted as percent reduction in peak current magnitude for currents evoked from \(V_h = -80\) mV (○) or -40 mV (●) at \(V_c = +10\) mV.](image)
higher concentrations, peak current magnitudes were reduced 2 ± 1% (50 μM, n = 8), 2 ± 1% (100 μM, n = 11), 4 ± 1% (300 μM, n = 9, P ≤ .001), 7 ± 1% (500 μM, n = 45, P ≤ .001) and 6 ± 1% (1000 μM, n = 4, P ≤ .005). Although the reduction in peak current was modest, currents inactivated more rapidly (see fig. 4).

When currents were evoked from Vh = −40 mV at +10 mV, the peak reductions were greater, 4 ± 1% (50 μM, P ≤ .01), 8 ± 1% (100 μM, P ≤ .001), 16 ± 2% (300 μM, P ≤ .001), 28 ± 1% (500 μM, P ≤ .001) and 30 ± 4% (1000 μM, P ≤ .001). When currents were evoked from these more positive potentials in the presence of α-EMTBL, the rate of current inactivation was slightly greater than control, but not nearly so rapid as when evoked from −80 mV. The apparent EC50 was ~300 μM.

In the presence of β-EMTBL, currents were also affected in a concentration- and voltage-dependent manner, but an additional effect was seen, enhancement of Ca++ currents at relatively low concentrations (fig. 5). As with α-EMTBL, there was no effect of 10 μM β-EMTBL. When currents were evoked from Vh = −80 mV at +10 mV, peak current magnitudes were increased 10 ± 4% (50 μM, n = 9, P ≤ .02), 13 ± 5% (100 μM, n = 7, P ≤ .05) and 17 ± 5% (300 μM, n = 4); at higher concentrations, peak current magnitudes were reduced 7 ± 2% (500 μM, n = 10, P ≤ .02) and 7 ± 2% (1000 μM, n = 6, P ≤ .005). When currents were evoked from Vh = −40 mV at +10 mV, currents were increased 10 ± 4% (50 μM, P ≤ .02), 10 ± 5% (100 μM, P ≤ .05) and 5 ± 9% (300 μM); at higher concentrations, peak current magnitudes were reduced 23 ± 4% (300 μM, P ≤ .001) and 29 ± 3% (1000 μM, P ≤ .001). The current enhancements seen with β-EMTBL were not voltage dependent, i.e., the percent increase was similar when currents were evoked from either −80 mV or −40 mV. Current reductions seen in the presence of β-EMTBL, by contrast, were voltage dependent: lesser peak current reductions and faster current inactivation were evident in currents evoked from Vh = −80 mV; greater peak current reductions and more normal current inactivation were evident in currents evoked from Vh = −40 mV. For current enhancement, the EC50 could not be determined unequivocally, because we could not determine, at any given concentration, whether β-EMTBL was acting solely to increase currents. Assuming that the maximal enhancement was 13% at 100 μM, and given that 10 μM had no effect, a rough estimate of EC50 is 50 μM. With the same limitation, the EC50 for current reduction was ~400 μM.

At the higher concentrations tested, current-voltage plots showed that the maximal high-voltage-activated currents occurred at similar potentials in the absence and presence of thiobutyrolactones and that E1/2 did not appear shifted (see also fig. 2).

We next examined whether α-EMGBL, an anticonvulsant compound that blocks the effects of α-EMTBL on GABA_A receptors, affected Ca++ currents in a similar manner. α-EMGBL was tested in the absence and presence of α-EMTBL, with both compounds applied at a concentration of 500 μM (fig. 6). When applied alone, α-EMGBL had no effect on high-voltage-activated Ca++ currents, evoked from Vh of −80 and −40 mV; this lack of effect was observed across the entire physiological range of current activation, as shown in the current-voltage plot (Vh = −90 mV). In the next series of experiments, α-EMGBL was applied first to verify its lack of effect. Next, α-EMGBL and α-EMTBL were applied simultaneously from another puffer pipette; compared with the effect of α-EMTBL alone, obtained at the end of the experiment, α-EMGBL failed to alter the action of α-EMTBL. In a series of control experiments, α-EMGBL was applied con-
Fig. 6. The effect on Ca\(^{2+}\) currents of α-EMGBL alone and in the presence of α-EMTBL. (A) The effect of 500 μM α-EMGBL (top traces, peak current-voltage plot) on high-voltage-activated currents, evoked from either −80 mV (○) or −40 mV (☆) at +10 mV, compared with the effect of 500 μM α-EMTBL (bottom traces). (B) Currents were evoked from −80 mV at +10 mV in the absence and presence of α-EMGBL (left) or in the presence of α-EMGBL and α-EMGBL plus α-EMTBL (middle). The effect of α-EMTBL in this cell is shown on the right for comparison.

Discussion

This study investigated the regulation of voltage-dependent Ca\(^{2+}\) currents in cultured DRG neurons by alkyl-substituted thiobutyrolactones. The results show for the first
time that these compounds affected both low-voltage- and high-voltage-activated Ca\(^{2+}\) currents in a concentration-dependent manner and in the presumed active range of 200 to 400 \(\mu M\) (Canney et al., 1991). A particularly novel result was that \(\beta\)-EMTBL only reduced low-voltage-activated (T-type) currents, but had a biphasic effect on high-voltage-activated currents, producing an increase at low concentrations (\(\leq 300 \mu M\)) and a decrease at higher concentrations. By contrast, \(\alpha\)-EMTBL only reduced low-voltage- and high-voltage-activated currents and \(\alpha\)-EMGL had no effect on Ca\(^{2+}\) currents. The predominant effects of the thiobutyrolactones were on the T-, N- and L-type current components, with little effect on the P- and Q-type current components. Furthermore, thiobutyrolactone-induced reductions in the N-type current component were voltage dependent.

**Butyrolactone structure and action on Ca\(^{2+}\) channels.** Thiobutyrolactone actions were concentration dependent and exhibited some selectivity for Ca\(^{2+}\) channel subtypes. These findings stand in contrast to the lack of effect of the \(\gamma\)-butyrolactones and suggest that Ca\(^{2+}\) channel subtypes represent specific targets for the thiobutyrolactones. The inability of \(\alpha\)-EMGL to affect low-voltage- or high-voltage-activated currents, or to block the actions of \(\alpha\)-EMTBL, for example, illustrates this difference in binding sites. (This last result highlights a clear difference in the effects of these compounds, at similar concentrations, on the GABA\(_\text{A}\) receptor, at which \(\alpha\)-EMGL acts as an "antagonist" to \(\alpha\)-EMTBL [Holland et al., 1990].) It is possible, however, that \(\alpha\)-EMGL may bind at the thiobutyrolactone site on Ca\(^{2+}\) channels, but with a markedly lower affinity or with less potency. Additional experiments will be required to distinguish between these possibilities, but the present results make clear that the identity of the ring heteroatom is of primary importance in determining actions on Ca\(^{2+}\) channels. Studies with additional analogs may allow firmer conclusions regarding the importance of the ring structure in targeting Ca\(^{2+}\) channels of different subtypes. Ethosuximide, for example, which differs from \(\alpha\)-EMTBL in its ring heteroatom and in the number of ring carbonyl groups, targets only T-type channels.

The present results allow some conclusions regarding the importance of the alkyl substitutions in targeting channel subtypes. Low-voltage-activated (T-type) currents were only reduced by thiobutyrolactones. The action of \(\beta\)-EMTBL on T-type currents was slightly more efficacious but less potent than for \(\alpha\)-EMTBL. Thus, for low-voltage-activated currents, the position of the alkyl substitution confers differing properties and imparts to...
β-substituted compounds an unusual characteristic, Ca$$^{++}$$
current enhancement.

The structure of a given compound may determine its
action on particular Ca$$^{++}$$ channel subtypes. Unlike many
Ca$$^{++}$$ channel blockers, which show preferences for either
single-channel subtypes, such as $\omega$-conotoxin GVIA for the
N-type channel (for example, Regan et al., 1991), or a relative
preference for either low-voltage- or high-voltage-activated
channels, such as Ni$$^{++}$$ or Cd$$^{++}$$, respectively (Fox et al.,
1987), the thiobutyrolactones affect both low-voltage-acti-
vated (T-type) and high-voltage-activated Ca$$^{++}$$ current com-
ponents. Of the latter, both N- and L-type current compo-
nents were affected, with little apparent effect on the P- and
Q-type current components. This last finding must remain
tentative, however, because DRG neurons express relatively
little P- and Q-type current. In experiments with 500 nM
$\omega$-agatoxin IVA, for example, the high-voltage-activated cur-
rents were reduced by only 10 to 15% by this toxin, similar to
the findings of others who used this cell type (Mintz et al.,
1992; Regan et al., 1991). Thus, it may have been difficult to
determine with certainty whether and to what extent these
current components are affected by thiobutyrolactones. Ad-
ditional experiments will be required, with either expressed
channels or cells, such as cerebellar Purkinje or granule cells,
which express a greater proportion of P- and Q-type chan-
nels.

**Voltage dependence of thiobutyrolactone action.** The
thiobutyrolactone effects on T- and L-type currents did not
appear to be voltage dependent as did the $\omega$-conotoxin GVIA-
sensitive effects on the N-type current component. The mecha-
nism of this voltage-dependent effect on N-type channels is
uncertain, although the present results suggest possible al-
ternative explanations. The finding that thiobutyrolactones,
applied at $V_h$ = −80 mV, had little effect on peak currents,
but increased the rate of apparent current inactivation at
+10 mV, suggests that the major effect may have been on
open channels. This hypothesis predicts that Ca$$^{++}$$ channels
needed to be in the open state for the thiobutyrolactones to
effect a reduction in current, either by an inhibitory effect on
channel activity or by blocking open channels. The finding
that peak currents were substantially reduced by thiobuty-
rolactones applied at $V_h$ = −40 mV, a potential at which
high-voltage-activated channels were not open, counters this
hypothesis and suggests, rather, that closed channels can be
affected by these compounds. Further, the fact that inactiva-
tion rates of currents evoked from −40 mV were not substi-
tially affected by thiobutyrolactones suggests that the open
state of high-voltage-activated channels may not be the only
channel conformation to be affected by the thiobutyrolac-
tones. Indeed, if “open channel block” were the only or pre-
dominant mechanism of action, a shift in the apparent $E_{Ca}$$^{+}$
would be expected, which is not evident in the present re-
results.

The present data could be explained if thiobutyrolactones
have voltage-dependent binding: little effect would be seen at
very negative $V_h$, with increasing current reduction at $V_c$ of
+10 mV (and thus more rapid current “inactivation”); at less
negative $V_h$, greater effects would be seen on peak current,
with little greater effect produced by increased depolariza-
tion. This hypothesis predicts that drug binding is relatively
fast and that maximal binding is achieved at potentials near
−40 mV. Our findings that thiobutyrolactone effects on high-
voltage-activated currents were relatively low in affinity and
that onset and reversal of effects was rapid support this view.
Alternatively, thiobutyrolactones may bind in a voltage-in-
dependent fashion, but may change the voltage-dependent
properties of the target channel, resulting in an increase in
steady-state inactivation and/or inactivation at depolarized
potentials. Clearly, additional experiments are needed, per-
haps with expressed N-type channels, for a fuller explana-
tion of the mechanism of action of these compounds and their
voltage dependence on this channel subtype.

**Anticonvulsant action and the block of Ca$$^{++}$$ chan-
nels.** Butyrolactone analogs afford an opportunity to place in
perspective the role of Ca$$^{++}$$ channel blockade as an anticon-
vulsant mechanism of action. Some antiseizure drugs cur-
cently in use have the ability to block Ca$$^{++}$$ currents, al-
though in most cases it is not clear whether this action is
either necessary or sufficient or even contributes to clinical
efficacy. Phenytoin, for example, reduces synaptosomal Ca$$^{++}$$
uptake, but does so at concentrations above the therapeutic
range (Sohn and Ferrendelli, 1976). Similarly, the barbitu-
rates markedly reduce high-voltage-activated Ca$$^{++}$$ cur-
rents, but at concentrations that are relevant only in the
solution of treatment of status epilepticus, when anesthetic concentra-
tions are achieved (Gross and Macdonald, 1988a,b). The
Ca$$^{++}$$ channel block is substantial at these concentrations,
however, and may account for the cardiovascular suppres-
sion common seen when patients are under barbiturate
anesthesia. The reduction of T-type currents by ethosuximide
is the best example of an antiseizure agent that acts on Ca$$^{++}$$
channels at therapeutic concentrations; to date, T-type cur-
rent reduction is probably the most likely mechanism for
ethosuximide’s efficacy in treating primary generalized (ab-
sence) seizures (Coulter et al., 1989). Valproic acid may also
reduce T-type currents, although whether this contributes to
its broad antiseizure efficacy is not certain (Kelly et al.,
1990).

In this context, the thiobutyrolactones have significant
effects on Ca$$^{++}$$ currents, at concentrations within the pre-
sumed active range. In particular, the reduction of T-type
currents by thiobutyrolactones occurs at lower concentra-
tions than an equivalent reduction of T-type currents by
ethosuximide or the thiobutyrolactone-induced reduction of
high-voltage-activated currents. These concentrations are
similar to the concentrations effective in regulating GABAA
receptor activity (Holland et al., 1990). The effects on high-
voltage-activated Ca$$^{++}$$ currents are more complicated. It is
worth noting that β-EMTBL, a convulsant compound, not
only blocks T-type Ca$$^{++}$$ currents but also increases high-
voltage-activated Ca$$^{++}$$ currents, at concentrations that
reduce GABAA-mediated inhibition. At high concentrations,
β-EMTBL blocks both low-voltage and high-voltage-acti-
vated Ca$$^{++}$$ currents, but also increases GABAA-mediated
inhibition (Holland et al., 1995). It would seem likely that, at
low concentrations, the block of GABAA inhibition by
β-EMTBL is by far the more significant clinical (convulsant)
effect than its potentially anticonvulsant reduction of T-type
Ca$$^{++}$$ currents. It is also possible that enhancement of high-
voltage-activated currents may be contributing to the convul-
sant actions of this compound. As for α-EMTBL, its anticon-
vulsant action is likely caused by enhancement of GABAA
receptor activity, but may be caused in part by Ca$$^{++}$$ current
reduction, particularly of T-type currents. It will be interest-
ing to determine whether α-EMTBL, like ethosuximide, is effective for control of absence seizures.

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References

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