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T-type calcium channels mediate the transition between tonic and phasic firing in thalamic neurons

(lateral geniculate nucleus/low-threshold spike/nickel/dihydropyridine)

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ABSTRACT **Thalamic neurons undergo a shift from tonic to phasic (burst) firing upon hyperpolarization. This state transition results from deinactivation of a regenerative depolarizing event referred to as the low-threshold spike. Isolated adult guinea pig thalamic (dorsal lateral geniculate) neurons exhibited low-threshold spikes that could be blocked by low concentrations of nickel but were unaffected by the dihydropyridine nimodipine. Whole-cell voltage-clamp recordings from these cells demonstrated a low-threshold, rapidly inactivating (T) Ca^{2+} current that manifested similar voltage dependency and time course as the low-threshold spike. Like low-threshold spikes, the T-type Ca^{2+} current was eliminated by nickel but was unaffected by nimodipine. In thalamic neurons, T-type Ca^{2+} channels underlie the low-threshold spike and, therefore, play a critical role in regulating the firing pattern of these cells.**

Thalamic neurons exhibit two distinct response modes to afferent excitation (1, 2). At normal resting potentials these cells respond with a linear depolarization that faithfully follows the input signal and may result in the continuous discharge of Na^+-dependent action potentials in a manner referred to as tonic firing. Upon hyperpolarization an abrupt transition to a nonlinear response state occurs in which firing is blocked for at least 50–100 msec between single or grouped spikes so that the cells fire in a phasic (burst) mode (3, 4). Tonic firing is the pattern most frequently recorded from thalamic neurons in awake animals, whereas phasic firing is typical of slow-wave sleep and may account for the accompanying spindling in the cortical electroencephalogram (5). The shift to phasic firing occurs as a result of deinactivation of a broad, low-amplitude regenerative response, referred to as the low-threshold spike (LTS). Recently, the stimulus for deinactivation of the LTS has been demonstrated to be a prolonged period of synaptically mediated inhibition (6, 7). The LTS is resistant to tetrodotoxin (TTX), a blocker of Na^+-dependent action potentials, but is sensitive to inorganic Ca^{2+} channel antagonists and is, therefore, presumably dependent upon Ca^{2+} entry (8). However, characterization of the Ca^{2+} channels underlying the LTS has been hampered by the lack of a preparation allowing voltage-clamp recording with adequate spatial voltage control. Recently, methods were developed for isolating intact neuron cell bodies from slices of the adult mammalian brain (9). On the basis of recordings from freshly isolated guinea pig thalamic neurons, we now present evidence that the LTS results from activation of T-type voltage-gated Ca^{2+} channels with properties similar to those in other neuronal and nonneuronal cell types (10).

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METHODS

These experiments were done by using isolated neuron cell bodies obtained by enzymatic dissociation of guinea pig dorsal lateral geniculate nucleus (LGNd) slices (Fig. 1). Cells were prepared by a modification of the method of Kay and Wong (9) from slices (0.3–0.5 × 1 × 3 mm) of the LGNd of 250- to 300-g adult guinea pigs. The slices were enzymatically digested for 1 hr at 32°C under 100% O_2 in 120 mM NaCl/5 mM KCl/2 mM $CaCl_2$/1 mM $MgCl_2$/25 mM D-glucose/20 mM Pipes containing trypsin (Sigma type III) at 1 mg/ml and collagenase (Sigma type 1A) at 1 mg/ml (pH 7.0; 306 mOsm/kg of H_2O) (11). The slices were transferred to enzyme-free Pipes/saline of the same composition and stirred at room temperature for 1–3 hr. Neurons were isolated as needed immediately before use by vigorous shaking for 3–5 sec in Hepes-buffered Dulbecco's modified Eagle's medium (DMEM) with high D-glucose (25 mM) (1–2 ml per slice). The suspension was plated onto a 35-mm polystyrene Petri dish, and the cells were allowed to settle for 5–10 min before the DMEM was aspirated and replaced with recording solution. Perikarya used for recording were comparable in size and shape to those of type 1 and type 2 (presumed geniculocortical relay) neurons described by Kriebel (12). Typically, 2–3 amputated processes extended ≈30 μm (never >60 μm) from the cell body. An occasional smaller cell, comparable to type-3 neurons (presumed local circuit), was also present, but these were not examined electrophysiologically.

Current-clamp recordings were made from isolated LGNd neurons with an Axoclamp 2A amplifier (Axon Instruments, Burlingame, CA) in bridge mode with 7–11 MΩ patch electrodes prepared from 1.5-mm o.d. borosilicate capillaries. The recording electrodes were filled with 150 mM potassium acetate/1 mM $MgCl_2$/1.1 mM EGTA/10 mM Hepes containing sufficient D-glucose to obtain an osmolality of 304–305 mOsm/kg of H_2O (pH 7.36–7.37). The bathing solution was 140 mM NaCl/5 mM KCl/10 mM $CaCl_2$/1 mM $MgCl_2$/10 mM Hepes containing D-glucose to bring the osmolality to 305–307 mOsm/kg of H_2O (pH 7.40). Cells had mean resting potentials of −55 mV (range, −52 to −59 mV) and mean input resistances of 1.4 GΩ (range, 0.3–2 GΩ; 30 representative cells). To study the effects of membrane potential changes, depolarizing or hyperpolarizing current was injected through the recording electrode.

Voltage-clamp recordings were obtained with an Axopatch 1B (500 MΩ headstage feedback resistor) and patch electrodes filled with 140 mM *N*-methylglucamine acetate/1 mM $MgCl_2$/10 mM Hepes/10 mM EGTA/0.1 mM guanylyl-

Abbreviations: LTS, low-threshold spike; TTX, tetrodotoxin; LGNd, dorsal lateral geniculate nucleus.

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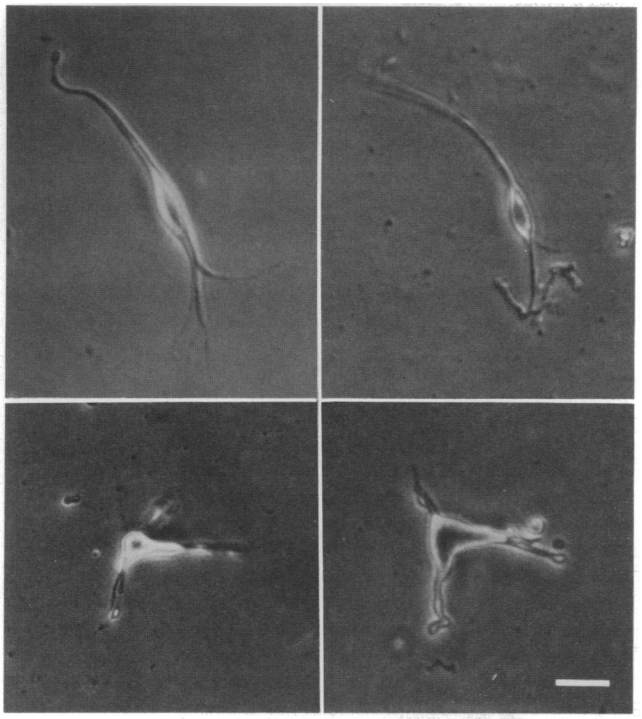

FIG. 1. Phase-contrast photomicrographs of enzymatically isolated LGNd neurons showing several typical cells. (Bar, 20 μM.)

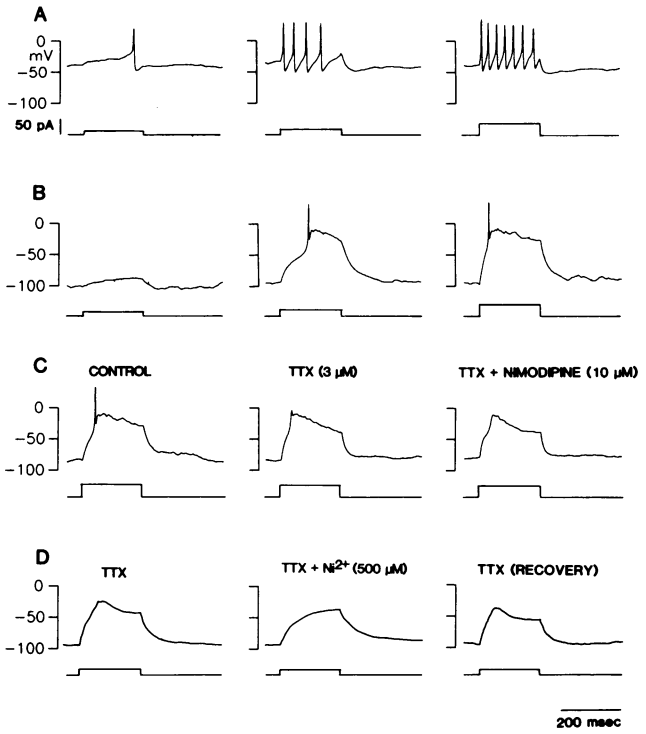

FIG. 2. Deinactivation of the LTS and the effects of Na^+ and Ca^{2+} channel blocking agents. (*A*) Depolarizing current pulses of increasing magnitude from an initial level of about −40 mV elicit tonic firing; the frequency of action potentials increases linearly with the magnitude of the injected current (1 spike per 8.5 pA). (*B*) In the same cell, depolarization from about −100 mV elicits an LTS and a single fast action potential; further depolarization does not increase the number of spikes generated (maximum injected current, 60 pA). (*C*) TTX (3 μM) blocks the fast spike without affecting the LTS. Nimodipine (10 μM) slightly decreases the amplitude of the voltage response, but the LTS persists (same cell as *A* and *B*). (*D*) In another cell, Ni^{2+} (0.5 mM) eliminates the LTS; 1 min after cessation of the superfusion, the LTS recovers. The bathing medium in this experiment contained 2 μM TTX.

imidodiphosphate tetralithium salt (GppNHp; Boehringer Mannheim) and sufficient D-glucose to bring the osmolality to 303–305 mOsm/kg of H_2O (pH 7.36–7.37). In a previous study, GppNHp slowed the rundown of Ca^{2+} currents in another cell type and appeared to retard rundown in LGNd neurons, although this effect was not studied systematically (13). The bathing solution was the same as that used for current-clamp recording, except that 2 μM TTX (Sigma) was added to block voltage-dependent Na^+ conductance.

Membrane potential values are uncorrected for liquid-junction potentials of −2 mV. Drugs and ions were applied by pressure ejection (<1 psi; 1 psi = 6.9 kPa) from blunt micropipettes (tip diameter, 1–2 μm) situated ≈70–100 μm from the cell surface. All experiments were done at room temperature, 23–25°C. Quantitative data are expressed as mean ± SD.

RESULTS

As illustrated by the recording shown in Fig. 2*A*, depolarization of LGNd neurons with injected current from a resting potential level of −50 mV elicited trains of spikes with a frequency proportional to the amplitude of injected current. In the same cells, depolarization from an initial level near −100 mV to potentials more positive than about −50 mV generated an all-or-none, triangular shaped voltage response or LTS (Fig. 2*B*). LTS duration was determined at one-half amplitude after subtraction of an appropriately scaled subthreshold voltage response. In a representative sample of 12 cells, the LTS ranged in duration from 50 to 80 msec and reached a peak of −23 to −11 mV. A fast spike can ride on the upstroke of the LTS, but firing is blocked during the remainder of the LTS, because the spike afterhyperpolarization does not reach a level sufficient to deinactivate voltage-dependent Na^+ channels. In isolated LGNd neurons, deinactivation of Na^+ channels requires hyperpolarization to at least −40 mV ($V_{1/2}$, −55 mV) (unpublished observations), whereas the afterhyperpolarization after the fast spike that rides on the crest of the LTS never reached potentials below −25 to −30 mV. Extracellular perfusion with TTX eliminated the fast spike, but the toxin had no effect on the underlying LTS (12 cells; Fig. 2*C*, center trace). The LTS was also insensitive to the dihydropyridine Ca^{2+} channel antagonist nimodipine (10 μM; 4 cells; Fig. 2*C*, right trace) whereas the inorganic Ca^{2+} channel blockers Ni^{2+} (0.5 mM; 3 cells) or Cd^{2+} (0.2 mM; 4 cells; data not shown) eliminated the LTS with a resultant linearization of the voltage response (Fig. 2*D*).

These observations support the conclusion that the LTS results from activation of voltage-gated Ca^{2+} channels. To identify the nature of these Ca^{2+} channels, we carried out whole-cell voltage-clamp recordings from isolated LGNd neurons that were internally perfused with *N*-methylglucamine to block K^+ currents. Depolarization from a holding level of −80 mV to potentials above −50 mV activated a rapidly decaying inward current, referred to as the T-type Ca^{2+} current [Fig. 3 A_1 and B_1 (●)]. Time-dependent inactivation of this current occurred in an exponential fashion with voltage-dependent time constants (e.g., at −20 mV, τ = 36 msec). As the cell was depolarized to potentials more positive than −10 mV, an additional sustained (L) Ca^{2+} current component becomes apparent (Fig. 3B_1 (○)]. When cells were depolarized from a holding potential of −40 mV, the T-type current was completely inactivated and only the L-type Ca^{2+} current was elicited (Fig. 3 A_2 and B_2). Ca^{2+} current components were examined in whole-cell voltage-clamp recordings from 44 isolated cell bodies. The mean maximum T- and

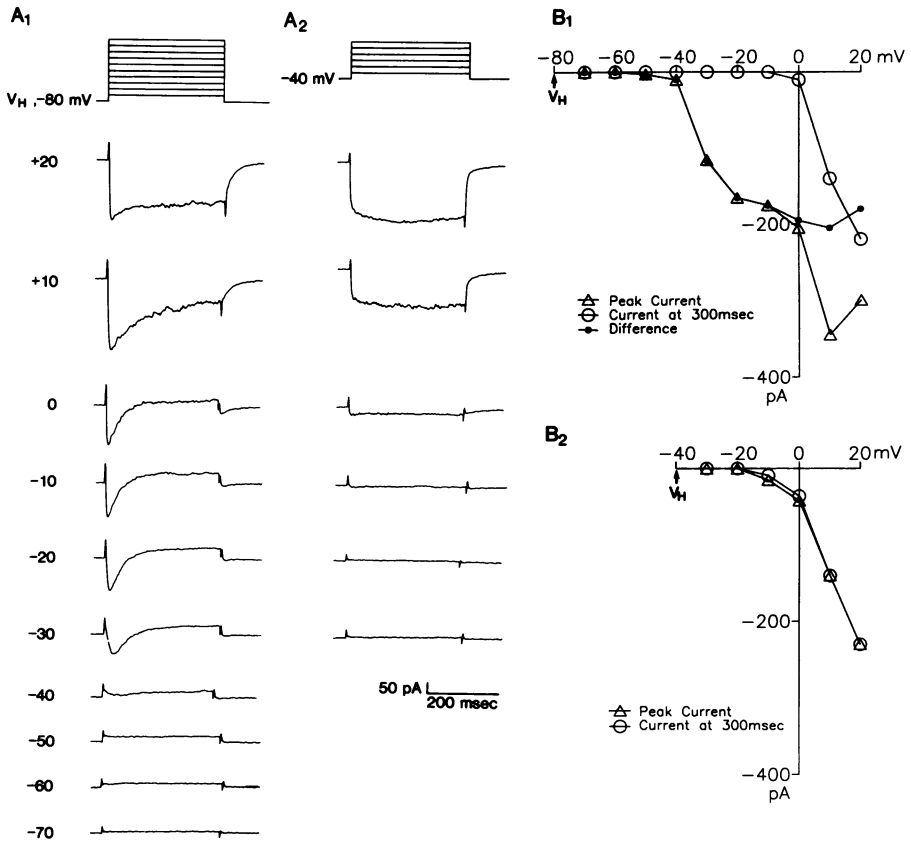

FIG. 3. Two types of Ca^{2+} currents in an isolated LGNd neuron. Whole-cell currents elicited upon depolarization from −80 mV and −40 mV are shown in A_1 and A_2, and plots of the leak-subtracted inward current versus step potential are displayed in B_1 and B_2, respectively. △, Peak inward current; ○, steady-state (L) current (at end of 300-msec voltage step); ●, difference between peak and steady-state current, representing magnitude of the inactivating (T) current. V_H, holding potential.

L-current amplitudes in these cells upon depolarization from −80 mV were 186 ± 136 pA (−40 to −10 mV) and 251 ± 120 pA (−10 to +20 mV), respectively, and the mean thresholds for activation of the two currents were −48 ± 7 and −12 ± 8 mV.

Fig. 4 illustrates recovery from steady-state inactivation for the T-type Ca^{2+} current. With conditioning pulses of variable duration to −80 mV from a holding potential of −30 mV, the amplitude of the T-type current recovered exponentially as a function of pulse duration with a time constant of 0.3 sec (Fig. 4 A_1 and A_2). The voltage dependency for inactivation is shown in Fig. 4 B_1 and B_2. One-half inactivation for the cell shown and two additional cells occurred at −70 ± 4 mV.

Fig. 5 shows the differential pharmacological sensitivities of the T- and L-type Ca^{2+} currents. The T-type current was essentially unaffected by the dihydropyridine Ca^{2+} channel blocker nimodipine (10 μM; Miles) (3 ± 5% block; four cells), whereas the L-type current was reduced by 61 ± 11% (five cells) (Fig. 5*A*). Conversely, 0.5 mM Ni^{2+} produced nearly complete blockade of the T-type current (83 ± 2%; four cells), but caused only 12 ± 7% (three cells) block of the L-type current (Fig. 5*B*). Other Ca^{2+} channel antagonists that failed to block either the T- or L-type Ca^{2+} currents in LGNd neurons included ω-conotoxin GVIA (10 μM; Peninsula Laboratories; five cells) (14, 15) and amiloride hydrochloride (0.25–5 mM; Sigma; nine cells) (16).

In the presence of TTX, depolarization under current-clamp conditions to −20 mV from potentials negative to −40 mV often elicited rapid (≈30-msec duration) depolarizing events that reached a peak at potentials that were more depolarized than the peak of the LTS (−6 to +5 mV; see, e.g., center trace of Fig. 2*C*). These events, which were blocked by nimodipine (10 μM; three cells) and Cd^{2+} (0.2 mM; two cells; data not shown) but not by Ni^{2+} (0.5 mM; two cells; data not shown), may represent high-threshold spikes as described by Jahnsen and Llinás (8).

DISCUSSION

There is now evidence for the existence of multiple Ca^{2+} channel species in a wide variety of excitable cells (10). In many of these cell types, rapidly inactivating, low-voltage activated (T) and slowly inactivating, high-voltage activated (L) Ca^{2+}-channels have been identified (17–28). In addition, some neurons express additional low-threshold, rapidly inactivating (N) Ca^{2+} channels that require strong depolarization for activation (29–31). The functional roles of each of these Ca^{2+} channel types have remained obscure. Whereas L-type Ca^{2+} channels have been postulated to carry large Ca^{2+} fluxes for transmembrane signaling and N-type channels may help regulate neurotransmitter release, T-type Ca^{2+} channels have been suggested to be involved in control of neuronal firing (32, 33). As yet, however, suitable preparations have not been available to evaluate this latter idea. Although Ca^{2+} channels are well known to exist in central neurons (32–38), the structural complexity of these cells in intact preparations along with the difficulty of adequately blocking opposing outward currents has precluded a sufficiently detailed analysis of the kinetic and voltage-dependent properties of Ca^{2+} currents to provide evidence for distinct Ca^{2+} current types. These problems can be obviated by the use of isolated neuronal perikarya that are suitable for whole-cell voltage-clamp recording. With this technique, intracellular electrolytes can be substantially replaced by impermeant ions carried in the patch pipette. In addition,

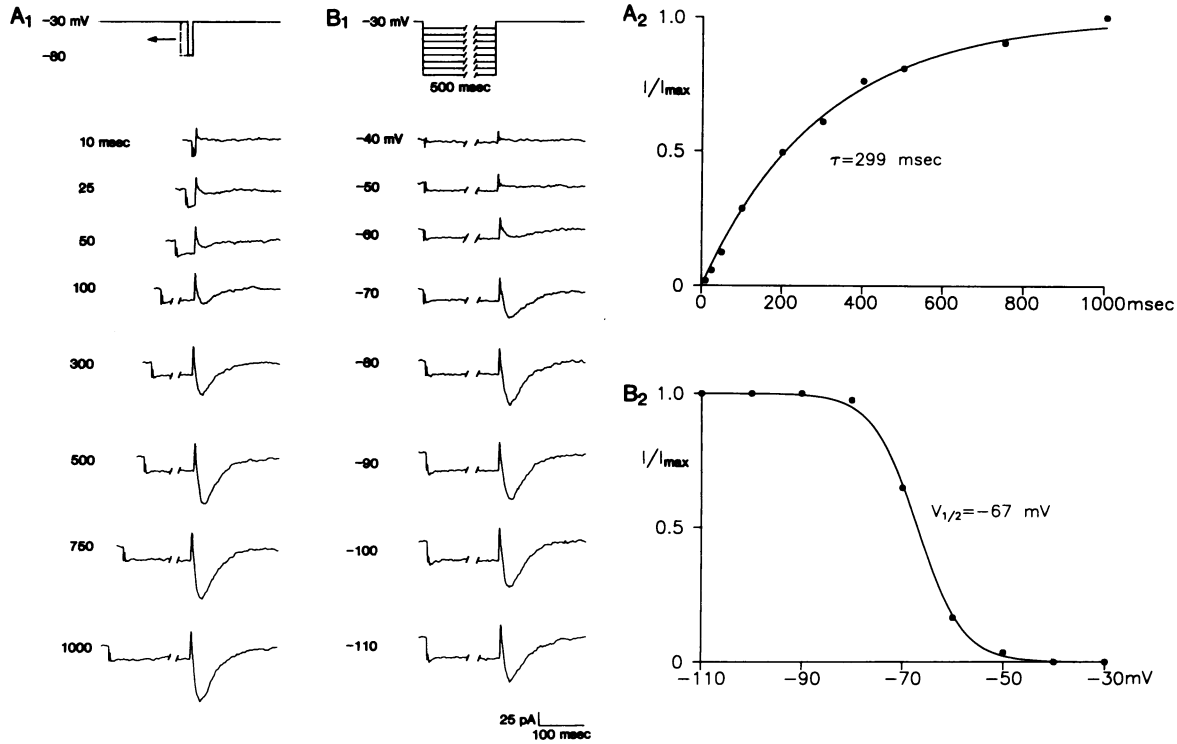

FIG. 4. Time- and voltage-dependent recovery from inactivation of the T-type Ca^{2+} current. (A_1) Progressively longer-duration conditioning voltage steps from −30 to −80 mV correspondingly increase the amplitude of current elicited at end of the step. Step duration is indicated at left of each trace. (A_2) Normalized peak amplitude plotted against step duration. The smooth curve is the best fit to the data according to an exponential function of the form $I/I_{max} = 1 - \exp(-t/\tau)$ where t is the step duration and $\tau = 0.3$ sec. (B_1) In the same cell, conditioning steps (500-msec duration) to increasingly hyperpolarized potentials progressively remove inactivation. Step potential is indicated at left of each trace. (B_2) Normalized peak amplitude plotted against the potential of the step. The data were fit according to a Boltzmann relation of the form $I/I_{max} = \{1 + \exp[(V - V_{1/2})/A]\}^{-1}$, where V is the step potential, $V_{1/2} = -67$ mV, and $A = 4.5$.

because cellular processes are all but lost during the dissociation procedure, near-ideal spatial voltage control is obtained (39).

In the present study, whole-cell voltage-clamp recordings from isolated LGNd neurons demonstrated two distinct Ca^{2+} currents that are similar to the T- and L-type Ca^{2+} currents in other excitable cells. T-type Ca^{2+} channels in sinoatrial cells (40), neuroblastoma cells (41), sensory neurons (30), and dorsal horn neurons (42) have previously been shown to be more sensitive to blockade by Ni^{2+} than L-type Ca^{2+} channels. We observed that the T-type Ca^{2+} current of thalamic neurons is also preferentially blocked by Ni^{2+} at comparable concentrations to those that block the T-type Ca^{2+} current in other cell types [refs. 30 and 42; however, neuroblastoma Ca^{2+} channels are blocked at lower concentrations (41)]. Some L-type Ca^{2+} channels, particularly in muscle cells, are exquisitely sensitive to dihydropyridine Ca^{2+} channel antagonists (43–45). However, L-type channels in neural cells have been found to be more resistant to dihydropyridine blockade (30), and this is also the situation in thalamic neurons, where relatively high concentrations of nimodipine produce only incomplete block of the current. Nevertheless, the pharmacological differences between T- and L-type Ca^{2+} channels provide a means to characterize the role of each Ca^{2+} current component. Because Ni^{2+} blocks the LTS, whereas nimodipine, which substantially inhibits the L-type current more than Ni^{2+}, does not, we conclude that T-type current is the main contributor to the LTS. This idea is supported by comparisons of the voltage dependency and time course of the T-type current and the LTS. Both the T current and the LTS require conditioning hyperpolarization for activation, both have thresholds near −50 mV, and both persist for ≈50–100 msec.

On the basis of indirect evidence, it has been suggested that the LTS originates in the soma (4, 46). Our present results conclusively demonstrate that T-type Ca^{2+} channels are present in the somatic (or proximal dendritic) membrane and, furthermore, that these channels are necessary to generate an LTS, although the data do not exclude the possibility that T channels are also located in distal dendrites (10, 47). Conversely, our data support the hypothesis that the high-threshold spike is mediated by L-type Ca^{2+} channels. While these channels have been implicated in the generation of dendritic spikes in cerebellar neurons (2), like T channels, they, too, are present in the somatic (or proximal dendritic) membrane of thalamic neurons.

Recordings in thalamic slices have demonstrated that trains of evoked, γ-aminobutyric acid (GABA)-mediated inhibitory postsynaptic potentials are a sufficient stimulus for triggering the LTS (6). The LTS is initiated as the cell repolarizes after the inhibitory postsynaptic potential train, without the intervention of any excitatory input. The voltage- and time-dependent characteristics of the T-type Ca^{2+} current are compatible with this concept. GABA-mediated inhibitory postsynaptic potentials drive the membrane potential to the reversal potential of GABA (−67 mV in thalamic slices, ref. 6; −70 mV in isolated LGNd neurons, unpublished observations). At this potential level, the T-type Ca^{2+}current is substantially deinactivated. Activation of the LTS occurs as the membrane potential returns to resting potential near the threshold of the T-type Ca^{2+} current. Moreover, slice recordings have shown that long-duration (>100 msec) inhibitory postsynaptic potential trains are necessary to elicit an LTS. The prolonged periods of inhibition are a consequence of the time needed to remove inactivation of the T-type Ca^{2+} current. Thus, the ability of thalamic neurons to transduce the synchronized inhibitory

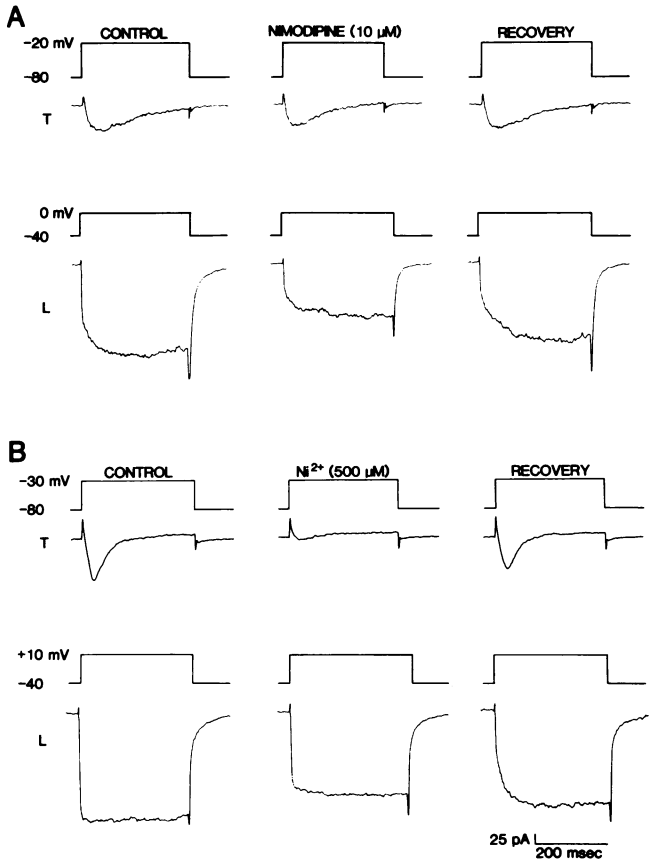

FIG. 5. Selective blockade of the T- and L-type Ca^{2+} currents. (*A*) Nimodipine (10 μM) has minimal effect on the inactivating (T) Ca^{2+} current elicited upon step depolarization from −80 to −20 mV, whereas in the same cell the drug produces a partial block of the sustained (L) Ca^{2+} current elicited upon depolarization from −40 to 0 mV. (*B*) Ni^{2+} (500 μM) causes nearly complete blockade of the T-type Ca^{2+} current, but the L-type Ca^{2+} current is reduced only moderately. Recovery records were obtained 1 min after cessation of the nimodipine or Ni^{2+} superfusion.

drive, such as occurs during slow-wave sleep (2), into coactivated phasic firing is a direct consequence of the voltage- and time-dependent properties of T-type Ca^{2+} channels.

Note. Coulter *et al.* (48) and Hernández-Cruz and Pape (49) have recently demonstrated the existence of T-type Ca^{2+} current in isolated neurons of the rat ventrobasilar thalamus and immature rat lateral geniculate nucleus, respectively.

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