Molecular Physiology of Low-Voltage-Activated T-type Calcium Channels

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Perez-Reyes, Edward. Molecular Physiology of Low-Voltage-Activated T-type Calcium Channels. Physiol Rev 83: 117–161, 2003; 10.1152/physrev.00018.2002.—T-type Ca\textsuperscript{2+} channels were originally called low-voltage-activated (LVA) channels because they can be activated by small depolarizations of the plasma membrane. In many neurons Ca\textsuperscript{2+} influx through LVA channels triggers low-threshold spikes, which in turn triggers a burst of action potentials mediated by Na\textsuperscript{+} channels. Burst firing is thought to play an important role in the synchronized activity of the thalamus observed in absence epilepsy, but may also underlie a wider range of thalamic dysrhythmias. In addition to a pacemaker role, Ca\textsuperscript{2+} entry via T-type channels can directly regulate intracellular Ca\textsuperscript{2+} concentrations, which is an important second messenger for a variety of cellular processes. Molecular cloning revealed the existence of three T-type channel genes. The deduced amino acid sequence shows a similar four-repeat structure to that found in high-voltage-activated (HVA) Ca\textsuperscript{2+} channels, and Na\textsuperscript{+} channels, indicating that they are evolutionarily related. Hence, the \( \alpha \)-subunits of T-type channels are now designated Ca\textsubscript{3}. Although mRNAs for all three Ca\textsubscript{3} subtypes are expressed in brain, they vary in terms of their peripheral expression, with Ca\textsubscript{3.2} showing the widest expression. The electrophysiological activities of recombinant Ca\textsubscript{3} channels are very similar to native T-type currents and can be...
differentiated from HVA channels by their activation at lower voltages, faster inactivation, slower deactivation, and smaller conductance of Ba\(^{2+}\). The Ca\(_{\text{v}3}\) subtypes can be differentiated by their kinetics and sensitivity to block by Ni\(^{2+}\). The goal of this review is to provide a comprehensive description of T-type currents, their distribution, regulation, pharmacology, and cloning.

I. INTRODUCTION

Rises in intracellular calcium trigger a variety of processes including muscle contraction, chemotaxis, gene expression, synaptic plasticity, and secretion of hormones and neurotransmitters. Although there are many channels and pumps involved in controlling intracellular Ca\(^{2+}\) levels, voltage-gated Ca\(^{2+}\) channels play a key role in this process (50). Calcium is not only an important second messenger, but its entry can also depolarize the plasma membrane, and thereby activate other voltage-gated ion channels. This property is especially important for neuronal T-type channels, which can generate low-threshold spikes that lead to burst firing and oscillatory behavior (175, 377). This activity is especially prominent in the thalamus, where it plays an important role in sensory gating, sleep, and arousal (277). The term thalamocortical dysrhythmias has been coined to describe pathological changes in these oscillations, and they have been implicated in a wide range of neurological disorders including absence epilepsy, the tremor associated with Parkinson’s disease, tinnitus, neuropsychiatric disorders, and neurogenic pain (186, 242).

The ability of neurons to fire low-threshold Ca\(^{2+}\) spikes suggested the existence of low-voltage-activated (LVA) Ca\(^{2+}\) channels. Since 1975 it has been recognized that there were at least two distinct types of Ca\(^{2+}\) channel (reviewed in Ref. 153). Hagiwara, Ozawa, and Sand, using two-microelectrode voltage-clamp recordings from starfish eggs, found channels that were activated after small depolarizations of the membrane, LVA, and other channels that required larger depolarizations of the membrane, high voltage activated (HVA) (154). LVA currents inactivated faster, more completely, and at more negative membrane potentials than HVA currents. Similarly, LVA and HVA currents were described in the marine pilie worm Neanthes (123). Of historical note, the first recordings of mammalian LVA channels were made by Moollenar and Spector in 1978 (290), who used the two-microelectrode voltage-clamp technique on the mouse neuroblastoma cell line N1E-115. However, these authors only observed one type of Ca\(^{2+}\) channel, which in 10 mM Ca\(^{2+}\) activated at −55 mV and peaked at −20 mV. Subsequent studies have shown that N1E-115 cells provide a convenient system to study the properties of native T-type currents (see sect. II). Using quinidine to block K\(^{+}\) currents, Fishman and Spector reported in 1981 (120) on the existence of two types of mammalian Ca\(^{2+}\) channel. One type activated at −50 mV, peaked at −20 mV, and inactivated rapidly (τ ~15–30 ms). The second type peaked at 0 mV and inactivated slowly (τ ~2,000 ms). The existence of LVA currents was firmly established by the work of many groups, including those headed by Kostyuk (115, 420), Carbone and Lux (62), Armstrong (12), Bean (32), Feltz (54), and Tsien (303, 306). Whole cell patch-clamp recordings showed that depolarizations in the −60 to −20 mV range elicited rapidly inactivating currents, while higher depolarizations elicited noninactivating currents. Plots of the current versus test potential (I-V) showed two components, with the LVA component appearing as a hump on the back of a larger HVA component. LVA currents also turned off, or deactivated, slower than HVA currents, producing slow tail currents (62). This property was studied in great detail in GH\(_3\) cells, where slowly deactivating (SD) tail currents were found to activate at lower thresholds than fast deactivating (FD) currents (12). Tsien and colleagues (306) extended the classification of dorsal root ganglion (DRG) channels and proposed that these channels be called T type for transient, L type for long lasting, and N type for neither T nor L type. LVA currents were also found to be resistant to rundown, unlike HVA currents that required cAMP, ATP, and Mg\(^{2+}\) in the pipette for stability (115). T- and L-type channels were also described in cardiac myocytes from guinea pig ventricle and dog atrium and could be distinguished by their voltage dependence, kinetics, single-channel currents, pharmacology, and conductance of Ca\(^{2+}\) and Ba\(^{2+}\) (32, 303). The observation that T-type currents inactivate at lower membrane potentials than L-type currents led to the development of an assay to separate these two components (32). The activity of both channels was recorded from a well-hyperpolarized potential such as −90 mV, then the activity of L-type channels was recorded at a potential where T-type channels are inactivated and L type are not (typically −50 mV), then the L-type currents are subtracted from the T- plus L-type currents to isolate the T-type current. Additional methods, such as tail current analysis, are required to isolate T-type currents in most other tissues due to the presence of HVA channels that also inactivate at −50 mV. Another possible contamination is voltage-gated Na\(^{+}\) currents that appear to conduct Ca\(^{2+}\) (I\(_{\text{Ca},\text{TTX}}\)); however, these can be differentiated by their sensitivity to tetrodotoxin (TTX) (162). Hallmark features of T-type channel electrophysiology are as follows: 1) they begin to open after small depolarizations of the plasma membrane (LVA); 2) their currents during a sustained pulse are transient; 3) they close slowly upon repolarization of the membrane, generating a SD tail cur-
rent; 4) they have a tiny, and equivalent, single-channel conductance of Ba^{2+} and Ca^{2+}; 5) they are relatively insensitive to dihydropyridines; and 6) their steady-state inactivation occurs over a similar voltage range as activation. In fact, T-type channels display a window current, i.e., there is a small range of voltages where T-type channels can open, but do not inactivate completely. This property may be particularly important in controlling intracellular Ca^{2+} levels (45). In conclusion, studies on native channels have provided a toolkit for separating Ca^{2+} channel subtypes, and these tools have proven useful in the characterization of both native and recombinant channels.

T-type channels are expressed throughout the body, including nervous tissue, heart, kidney, smooth muscle, sperm, and many endocrine organs. These channels have been implicated in variety of physiological processes including neuronal firing, hormone secretion, smooth muscle contraction, myoblast fusion, and fertilization. In general, the electrophysiological properties of T-type currents recorded from various cell types are similar, but differences have been noted in how they inactivate and in their pharmacology. This heterogeneity can be explained in part by the existence of three T-type channels that are encoded on separate genes (90, 228, 324).

Voltage-gated Ca^{2+} channels have been classified by their electrophysiological and pharmacological properties, and more recently by their amino acid sequence identity. There are at least 10 genes encoding α1-subunits of voltage-gated Ca^{2+} channels (Fig. 1). Alignment of their deduced amino acid sequences suggests that gene duplication and divergence of an ancestral Ca^{2+} channel gene gave rise to LVA and HVA subfamilies. Further duplication of the HVA gene gave rise to Ca,1 and Ca,2 subfamilies. This duplication must have occurred well over 500 million years ago, since the nematode Caenorhabditis elegans contains one member of each subclass. Eventually the Ca,1 subfamily evolved into four genes, while both the Ca,2 and Ca,3 subfamilies evolved into three. Cloning and expression studies have established that the Ca,3 family encodes LVA T-type channels (sect. iv), while the Ca,1 subfamily encodes L-type channels (although expression of Ca,1.4 has not been reported yet). These channels play a critical role in excitation-contraction coupling in cardiac, skeletal, and smooth muscle. In fact, the Ca,1.1 gene has evolved beyond a Ca^{2+} channel, and its physiological role in skeletal muscle is as a voltage sensor, coupling membrane depolarization directly to calcium release channels of the sarcoplasmic reticulum (SR) (340). In contrast, cardiac and smooth muscle contraction requires influx of extracellular Ca^{2+} through Ca,1.2 channels. In heart, this influx activates a larger release of Ca^{2+} from intracellular pools via a mechanism called calcium-induced calcium release (CICR) (37). Ca,1.2 channels are an important therapeutic target in the control of blood pressure and cardiac rhythm. The physiological roles of Ca,1.3 channels have been difficult to discern since pharmacological tools have not been described that can separate its activity from Ca,1.2, and because these channels appear to be coexpressed in a number of tissues. In contrast, the activity of Ca,2 family members can be selectively blocked by peptide toxins. Splice variation of the Ca,2.1 gene results in channels that differ in their sensitivity to the spider toxin ω-Aga-IVA and therefore encode both P- and Q-type channels (55). The Ca,2.2 gene encodes N-type channels, which are characterized by their irreversible and potent block by the snail toxin ω-conotoxin-GVIA. Studies with these toxins indicate that Ca,2.1 and Ca,2.2 channels mediate the Ca^{2+} influx into presynaptic terminals that trigger neurotransmitter release. In contrast to these channels, it has been difficult to determine which native Ca^{2+} current is carried by Ca,2.3 channels. Recombinant Ca,2.3 channels resemble T-type channels in their sensitivity to nickel and their voltage dependence of inactivation, leading to the early suggestion that they might encode T-type channels (376). However, Ca,2.3 channels require stronger depolarizations for channel opening, i.e., are HVA channels, and they deactivate 10-fold faster than T-type channels (228). Studies with SNX-482, a tarantula toxin that selectively blocks recombinant Ca,2.3 channels, suggests that these channels mediate R-type currents in some tissues (299).
Electrophysiological studies of recombinant channels show that Ca\textsubscript{v}3.1 (formerly \(\alpha_{1G}\)) and Ca\textsubscript{v}3.2 (\(\alpha_{1H}\)) have similar activation and inactivation kinetics, but can be differentiated by their recovery from inactivation and sensitivity to block by nickel (209, 230, 351). The kinetic properties of these \(\alpha_1\)-subunits closely resemble native T-type channels. In contrast, recombinant Ca\textsubscript{v}1 and Ca\textsubscript{v}2 channels require auxiliary subunits for normal gating behavior. This suggests that native T-type channels may be formed by a single \(\alpha_1\)-subunit. Ca\textsubscript{v}3.3 (\(\alpha_{1I}\)) channels also generate LVA currents; however, these currents activate and inactivate much more slowly than T-type channels. Native currents with these properties have only been described in a limited number of studies (19, 99, 177, 316, 398). Although there is no drug that is highly selective (>10-fold) for T-type over other ion channels, block of T-type channels appears to contribute to the therapeutic usefulness of antihypertensives, antiepileptics, anesthetics, and possibly antipsychotics. Therefore, T-type channels provide a novel target for drug development.

**A. Low-Threshold Calcium Spikes**

Low-threshold calcium spikes (LTS) have been described in slices and in isolated neurons from a variety of brain nuclei such as inferior olive (243, 244), thalamic relay (96, 183), medial pontine reticular formation (146), lateral habenula (437), septum (10), deep cerebellar nuclei (241), CA1-CA3 of the hippocampus (163, 307), association cortex (122), paraventricular (399) and preoptic nuclei of the hypothalamus (385), dorsal raphe (60), globus pallidus (296), and the subthalamic nucleus (40). Typically the spike is crowned by a burst of action potentials (Fig. 2). Addition of TTX to block voltage-gated Na\textsuperscript{+} channels abolishes the fast action potentials, effectively isolating the slowly activating and inactivating LTS. Numerous studies have shown that the LTS is mediated by a

**FIG. 2. Low-threshold Ca\textsuperscript{2+} spikes generate burst firing.** A: Many neurons can generate two distinct patterns of action potential firing in response to a depolarizing stimulus. Regular, or tonic, firing is elicited when the neuron is depolarized from a resting membrane potential near −55 mV. In contrast, when the membrane potential is below −70 mV, the same depolarizing stimulus triggers a high-frequency burst of action potentials. [From Huguenard JR. Low-voltage-activated (T-type) calcium-channel genes identified. Trends Neurosci 21: 451–452, 1998, with permission from Elsevier Science.] B: A representative example of currents that generate burst firing. Depolarization of the plasma membrane by hyperpolarization-activated current (\(I_h\)) leads to activation of T-type currents (\(I_{Ca}\)), and a second phase of depolarization called the low-threshold Ca\textsuperscript{2+} spike. Riding on top of the low-threshold Ca\textsuperscript{2+} spike are a burst of Na\textsuperscript{+} spikes mediated by fast voltage-gated Na\textsuperscript{+} channels. High-threshold Ca\textsuperscript{2+} and K\textsuperscript{+} currents can also be activated by the low-threshold calcium spikes. Ca\textsuperscript{2+} entry during the burst leads to activation of Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents, which in combination with voltage-gated K\textsuperscript{+} channels repolarize the membrane. (From Bal T and McCormick DA. Synchronized oscillations in the inferior olive are controlled by the hyperpolarization-activated cation current \(I_h\). J Neurophysiol 77: 3145–3156, 1997.) C: Thalamic neurons of transgenic mice lacking expression of Ca\textsubscript{v}3.1 do not fire bursts. Current-clamp recordings are from neurons held at −60, −70, or −80 mV, then depolarized or hyperpolarized by current injections of varying magnitudes as indicated below the set of traces. When the resting membrane potential is −60 mV, a depolarizing stimulus triggers tonic firing in both wild-type (+/+ ) and transgenic (−/− ) animals. When the membrane potential (\(V_m\)) is lowered to −70 mV, a hyperpolarizing stimulus triggers burst firing in neurons from wild-type, but not transgenic animals. When the resting membrane potential is −80 mV, a depolarizing stimulus only triggers burst firing in neurons from wild-type animals. (From Kim D, Song I, Keum S, Lee T, Jeong MJ, Kim SS, McEnery MW, and Shin HS. Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking \(\alpha_{1G}\) T-type Ca\textsuperscript{2+} channels. Neuron 31: 35–45, 2001, with permission from Elsevier Science.)
Ca\(^{2+}\) conductance; it is not observed in Ca\(^{2+}\)-free external solutions, and it can be blocked by divalent cations such as Co\(^{2+}\) and Ni\(^{2+}\). Definitive proof that thalamic LTS are mediated by T-type channels was provided by studies on transgenic mice, where knockout of the Ca.3.1 gene abolished these spikes and burst firing (Fig. 2C) (206).

With a few notable exceptions (7, 191), LTS cannot be triggered by depolarization of the neuron from the resting membrane potential, which is typically between −60 and −65 mV. However, LTS can be observed after a hyperpolarizing pulse is delivered. This process has been called “deinactivation” and is due to recovery of channels from inactivation. Table 1 lists a number of studies where the voltage and time dependence for recovery of these spikes has been examined in detail. LTS began to appear after the neuronal membrane was hyperpolarized below −69 mV, and full-amplitude spikes were observed when the membrane was below −73 mV. The rate of recovery was measured by varying the duration of a hyperpolarizing pulse, then testing for the presence of an LTS upon return to the resting membrane potential. These studies found that the LTS deinactivated relatively quickly, with half of it recovering in −100 ms and full recovery after 200 ms. Therefore, one physiological role of the LTS is as a pacemaker. Although increases in intracellular concentrations of Ca\(^{2+}\) act as a second messenger for a variety of processes, in this case the important property is that influx of the divalent cation leads to direct depolarization of the membrane. The amplitude of this depolarization was typically 25 mV (Table 1), which raised the membrane potential to approximately −40 mV. Since the threshold of many voltage-gated Na\(^{+}\) channels is around −55 mV, the LTS can trigger a burst of action potentials. These Na\(^{+}\)-dependent action potentials are brief (1–2 ms) and of high amplitude (Δ80 mV), and in some thalamic neurons of very high frequency (>400 Hz). High-threshold Ca\(^{2+}\) channels will also begin to open at potentials more positive than −40 mV (385). This will lead to more influx of Ca\(^{2+}\) and, if present, activation of Ca\(^{2+}\)-dependent K\(^{+}\) channels, which can lead to an afterhyperpolarization (AHP) (40, 244). Low-threshold channels can recover from inactivation during the AHP and could begin to fire again as the membrane potential returns to its resting level. Hyperpolarizations can also activate I\(_{\text{h}}\) channels, which allow the influx of both Na\(^{+}\) and K\(^{+}\), thereby depolarizing the cell and directly triggering another LTS (Fig. 2). In this scenario I\(_{\text{h}}\) are the primary pacemaker current (254). Clearly there are a variety of ionic conductances that mediate pacemaker activity, and it is an oversimplification to implicate a single channel as the mediator of a pacemaker current. This is especially true in the heart (61).

Another way that low-threshold channels are involved in generating oscillations includes reciprocal connections with an inhibitory interneuron. Due to its important role in controlling brain activity, the best studied of these circuits involves the GABAAergic neurons of the thalamic reticular nucleus that regulate the activity of thalamic relay neurons (377). Interestingly, the firing patterns of thalamic relay neurons vary with the state of consciousness (277, 378). In the awake state and during rapid-eye-movement sleep, they fire in tonic mode: the resting membrane potential is relatively depolarized, the LTS is inactivated, and excitatory inputs faithfully trigger action potentials. In deep sleep, or during thalamocortical dysrhythmias, these neurons fire in an oscillatory mode called burst firing; the resting membrane potential is hy-

<table>
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<tr>
<th>Brain Region</th>
<th>Resting Membrane Potential, mV</th>
<th>Threshold, mV</th>
<th>Amplitude, mV</th>
<th>Deinact V</th>
<th>Deinact V</th>
<th>Deinact Time</th>
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<td></td>
<td></td>
<td></td>
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<td>Midpt, mV</td>
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The properties of low-threshold Ca\(^{2+}\) spikes (LTS) were measured using current-clamp recordings. Threshold was defined by the voltage where a depolarizing pulse triggers a LTS. The amplitude of the LTS is shown and does not include the fast Na\(^{+}\)-dependent action potential. The voltage and time dependence for deinactivation (recovery) of the LTS spike is shown. Deinact., deinactivation; midpt, midpoint; CA1–CA3, hippocampal pyramidal neurons.
perpolared, allowing full-amplitude low-threshold spiking (Fig. 2A). Such firing is thought to underlie spike-wave discharges observed during absence seizures (176). Similar patterns of brain activity have been detected in a variety of neurological disorders by magnetoencephalography and have been termed thalamocortical dysrhythmias (242).

B. Neuronal

1. Isolated neurons

LVA T-type currents have been characterized using whole cell voltage-clamp recording of neurons isolated from a variety of brain regions. Very large T-type currents (>1 nA) have been recorded from cholinergic neurons of the basal forebrain (6), from relay neurons of the ventrobasal thalamus (222), and from sensory neurons of dorsal root ganglia (436). Prominent T-type currents have been found in neurons from many brain regions (Table 2). I-V relationships are typically measured by holding the cell at −90 mV, and then currents are elicited by depolarizing pulses that increase in 10-mV increments. T-type currents begin to activate when the membrane is depolarized above −60 mV. At threshold potentials, the currents activate relatively slowly, requiring many milliseconds to reach peak, and also inactivate relatively slowly. At higher potentials both activation and inactivation are faster. This produces a stereotypical pattern in the family of current traces where successive records cross each other, reminiscent of voltage-gated Na⁺ channels. In general, HVA channels do not produce this criss-crossing pattern because inactivation rates are much slower than activation rates (334). I-V curves commonly show two components, with the LVA currents peaking at −30 mV while the HVA currents peak around 0 mV. In many neurons, T-type currents can be measured in isolation with test pulses to −30 mV and below. However, at higher potentials both LVA and HVA currents are activated, complicating estimates of the LVA activation curve. Both I-V curves show a peak then decrease at more positive potentials, which is due to a reduction of the electrochemical gradient for calcium ions as the test potential approaches the theoretical reversal potential (~100 mV; see Fig. 7 for example). Therefore, the fraction of channels activated by a test pulse continues to increase beyond the peak of the I-V curve. This explains why estimates of the midpoint of channel activation (V₀.₅) derived by normalizing the current at each test potential to the maximum current observed (I/Iₘₐₓ) produces values that are more negative (~50 mV) than estimates that take into account changes in driving force (~41 mV). To circumvent this problem, activation curves have also been estimated by measuring the amplitude of slowly deactivating tail currents at a constant voltage. Most of these studies used a test pulse of constant duration (isochronal), which assumes that the rates of channel activation and inactivation are voltage independent. However, this is not the case, so this method can underestimate channel activation at negative potentials where channels open slowly. It can also underestimate activation if channels inactivate during the test pulse. This problem can be overcome by varying the duration of the activating pulse, so that the tail current is elicited at the peak of the current (288). So far this method has only been applied to recombinant channels. Another experimental variable that affects the position of the activation curve is the concentration and choice of charge carrier. Millimolar concentrations of positively charged divalent cations can bind to surface charges on both the channel and the membrane, thereby reducing the effective transmembrane voltage gradient (164). An effect called surface charge screening. Increasing external [Ca²⁺] from 2 to 10 mM shifts the apparent gating of T-type channels by ~10 mV (128).

A hallmark of T-type currents is that they are transient; currents reach a peak then decay. In solutions where K⁺ channels are blocked, this decay is due to channel inactivation. The inactivation rate is measured by fitting the current trace with an exponential function and is typically reported with a τ in milliseconds. Plots of inactivation rate versus test potential reveal two phases: inactivation is slow at ~60, gets faster between −50 and −30, then is constant above −30 mV. Activation rates have a similar voltage dependence, leading to the notion that channels must activate before inactivating, and that the inactivation process is essentially voltage-independent. Although most T-type currents inactivate relatively rapidly (<30 ms), there are also reports of slowly inactivating currents (Table 2). This variability was interpreted as evidence for multiple T-type channel genes (179). This hypothesis was supported by the cloning of T-type channels that differed in this property; Ca₃.₁ and 3.₂ display fast inactivation, while Ca₃.₃ displays slowly inactivating currents. Notably, Ca₃.₃ mRNA is expressed in the same brain regions as the slow currents (177, 228, 393). Excluding these cases, the average inactivation τ from 22 studies is 20 ms.

The voltage dependence of inactivation is also measured at “steady-state” by holding the membrane at varying potentials (prepulse), then assaying for available T-type channels with a test pulse to −30 mV (h∞). To approximate steady-state, many studies have used a prepulse duration of 1 s, which is considerably longer than the time required to inactivate open channels. However, increasing the prepulse duration to 10 s results in a ~10-mV shift in the apparent h∞ curve (53, 389). Despite the use of different preprepulses and concentrations of divalent cations, there is good agreement between the 34 studies shown in Table 2, and the average midpoint of the h∞ curve is −77 mV (Table 2). Somewhat surprisingly this
The divalent cation concentration used to measure the currents is shown. Properties of the current-voltage relationship are described in terms of the voltage threshold where currents become detectable, the voltage where they peak ($I$-$V$ peak), and the maximum amplitude observed ($I_{\text{max}}$). Methods used to calculate midpoints of the activation curves ($V_{1/2}$) are denoted with the following superscripts: C, chord conductance; T, tail current; $M$, $I_{\text{max}}$. The midpoint of the steady-state inactivation curve is reported ($h_{\infty}$). Kinetics of inactivation ($\tau_{\text{on}}$) can vary with test potentials; therefore, the values shown represent the voltage-independent rate, which was typically measured at $-10$ mV. Currents decayed in a biexponential manner; to simplify comparisons, a weighted tau was calculated according to the following equation: $\tau_{\text{w}} = A_1 \tau_1 + A_2 \tau_2$, where $A$ represents the fraction of current decaying with the respective time constant. The values shown for recovery from inactivation represent the voltage-independent tau of the slow component. Nickel responses represent either the $IC_{\text{in}}$ value obtained or the concentration tested and the percent block observed (in parentheses). LGN, lateral geniculate nucleus; MDN, magnocellular dorsal nucleus; L-M, lacunosum-molecular of hippocampus; DRG, dorsal root ganglion; inact., inactivation; recov., recovery.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Divalent, mM</th>
<th>Threshold, mV</th>
<th>$I$-$V$ Peak, mV</th>
<th>$I_{\text{max}}$, pA</th>
<th>$V_{1/2}$, mV</th>
<th>$h_{\infty}$, mV</th>
<th>$\tau_{\text{on}}$, ms</th>
<th>$\tau_{\text{recov}}$, ms</th>
<th>Nickel, mM</th>
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<tr>
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<td>-40</td>
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<td>-88</td>
<td>288</td>
<td>300</td>
<td>170</td>
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<tr>
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<td>-20</td>
<td>-38$^C$</td>
<td>-70</td>
<td>300</td>
<td>188</td>
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<td></td>
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<td>-30</td>
<td>-47</td>
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<tr>
<td>CA1 pyramidal</td>
<td>10 Ca</td>
<td>-60</td>
<td>-30</td>
<td>-150</td>
<td>-46$^C$</td>
<td>-79</td>
<td>15</td>
<td>430 (63%)</td>
<td>389</td>
</tr>
<tr>
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<td></td>
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<tr>
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<td>-52$^T$</td>
<td>-84</td>
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<td>251</td>
<td>85</td>
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<td>-30</td>
<td>-280</td>
<td>-50$^T$</td>
<td>-81</td>
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<td>200 (60%)</td>
</tr>
<tr>
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<td>-1,400</td>
<td>-96</td>
<td>15</td>
<td>353</td>
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<tr>
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<td>-45$^M$</td>
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<td>-10</td>
<td>-131</td>
<td>-49$^C$</td>
<td>-78</td>
<td>80</td>
<td>570</td>
<td>200 (54%)</td>
</tr>
<tr>
<td>Reticular</td>
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<td>-50</td>
<td>-128</td>
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<td>-35</td>
<td>-150</td>
<td>-62$^C$</td>
<td>-81</td>
<td>58$^W$</td>
<td>507</td>
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<td>-150</td>
<td>-46$^C$</td>
<td>-79</td>
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<td>215</td>
<td>389</td>
</tr>
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<td>-20</td>
<td>-400</td>
<td>-200</td>
<td>20</td>
<td>50 (50%)</td>
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<td>-64</td>
<td>-60</td>
<td>-250</td>
<td>-65</td>
<td></td>
<td>200</td>
<td></td>
<td>110</td>
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<td>Supraoptic</td>
<td>2 Ca</td>
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<td>-30</td>
<td>-400</td>
<td>-65</td>
<td></td>
<td>50 (84%)</td>
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<td>110</td>
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<td>-59</td>
<td>-33</td>
<td>-402</td>
<td>-67</td>
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<td>100 (67%)</td>
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<td>Midbrain and pons</td>
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<tr>
<td>Area postrema</td>
<td>5 Ca</td>
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<td>-10</td>
<td>-36</td>
<td>-72</td>
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<td>10 (100%)</td>
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<td>157</td>
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<tr>
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<td>-400</td>
<td>-50$^M$</td>
<td>-89</td>
<td></td>
<td>500 (50%)</td>
<td>101</td>
</tr>
<tr>
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<td></td>
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<tr>
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<td>-20</td>
<td>-400</td>
<td>-40$^C$</td>
<td>-79</td>
<td>23</td>
<td>3,300</td>
<td>110</td>
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<td></td>
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<tr>
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<td>-65</td>
<td>-35</td>
<td>-2,000</td>
<td>-82</td>
<td></td>
<td>100 (95%)</td>
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<td>436</td>
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<tr>
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<td>-25</td>
<td>-41$^C$</td>
<td>-60</td>
<td>25</td>
<td>330</td>
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<td>206</td>
</tr>
<tr>
<td>Nodose</td>
<td>5 Ca</td>
<td>-60</td>
<td>-20</td>
<td>-400</td>
<td>-48</td>
<td>30</td>
<td>400</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>Pelvic ganglion</td>
<td>10 Ca</td>
<td>-50</td>
<td>-25</td>
<td>-500</td>
<td>-38$^T$</td>
<td>-57</td>
<td>10</td>
<td></td>
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<tr>
<td>Oblactory</td>
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<td>-36</td>
<td>-50</td>
<td>-82</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Olfactory</td>
<td>3 Ca</td>
<td>-70</td>
<td>-40</td>
<td>-60</td>
<td>-45$^C$</td>
<td>-66</td>
<td>100 (71%)</td>
<td></td>
<td>200, 201</td>
</tr>
<tr>
<td>Retina</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Rod bipolar</td>
<td>10 Ca</td>
<td>-60</td>
<td>-30</td>
<td>-35</td>
<td>-30$^T$</td>
<td>-50</td>
<td>30</td>
<td>1,000 (80%)</td>
<td>316</td>
</tr>
<tr>
<td>Cone bipolar</td>
<td>10 Ca</td>
<td>-60</td>
<td>-29</td>
<td>-66</td>
<td>-35$^T$</td>
<td>-60</td>
<td>55</td>
<td>1,000 (85%)</td>
<td>316</td>
</tr>
<tr>
<td>Oligodendrocytes</td>
<td>20 Ba</td>
<td>-50</td>
<td>-25</td>
<td>-150</td>
<td>-63</td>
<td>10</td>
<td></td>
<td></td>
<td>47</td>
</tr>
</tbody>
</table>
value is considerably more negative than the apparent threshold of activation, indicating that channels can inactivate at potentials where they do not appear to open. Similar \( h_\text{m} \) values have been obtained with the recombinant Ca\(_{\text{v}}\)3 channels (see Table 8), where this closed-state inactivation has been studied in greater detail (127). Although most HVA channels inactivate at more positive potentials, R-type and recombinant Ca\(_{\text{v}}\)2.3 channels inactivate over a similar voltage range as T-type channels (334).

The time course of recovery from inactivation is an important property of T-type channels. LTS are often triggered after an inhibitory postsynaptic potential (IPSP), and this is due to fast recovery of T-type channels during the IPSP (deinactivation), followed by their opening as the membrane returns to its resting potential. Recovery is often measured by varying the time between (interpulse) an inactivating pulse and a test pulse. In most studies the interpulse voltage was \(-90\) mV, and similar results are obtained at more negative voltages. In contrast, recovery is slower at potentials where channels can also inactivate. There is considerable variability in the reported values for recovery, ranging from 100 to 3,300 ms (Table 2). Although most studies found that recovery followed a monoexponential time course, others have found evidence for a biexponential process. This discrepancy might be due to differences in the duration of the inactivating pulse, where long pulses allow channels to accumulate in a second inactivated state from which they recover slowly. As observed in sensory neurons (53), recombinant channels recover with a monoexponential time course from short pulses and with a slower, biexponential time course from long pulses, although this property is less pronounced for Ca\(_{\text{v}}\)3.3. Differences in recovery kinetics of T-type currents between different neurons can also be due to which Ca\(_{\text{v}}\)3 isoform they express (209). Of the three isoforms, Ca\(_{\text{v}}\)3.1 channels recover the fastest (\(~120\) ms from short pulses), and this time course is similar to that observed for recovery of both native T-type currents (\(~300\) ms; Table 2) and LTS (\(~150\) ms; Table 1).

Early studies found that LVA channels were more sensitive to block by 50–100 \( \mu\)M nickel than HVA channels (124, 152, 298). Subsequent studies found that many neuronal T-type channels required >10-fold higher concentrations for block, with many studies reporting IC\(_{50}\) values of \(~300\) \( \mu\)M (Table 2). Studies on recombinant channels provided an explanation for this diversity; only Ca\(_{\text{v}}\)3.2 channels are highly nickel sensitive (IC\(_{50}\) \(~10\) \( \mu\)M), while Ca\(_{\text{v}}\)3.1 and Ca\(_{\text{v}}\)3.2 are 20-fold less sensitive (230). Recombinant HVA channels also differ in their Ni\(^{2+}\) sensitivity, with Ca\(_{\text{v}}\)2.3 being relatively sensitive (455). Although the mechanisms of block of recombinant HVA and LVA channels are complex and subject to multiple interpretations, a consistent finding is that block is greatest at potentials near threshold. This voltage dependence would exaggerate block of LVA channels, making nickel appear more selective than it is. In summary, block by 10–50 \( \mu\)M Ni\(^{2+}\) can be used to implicate Ca\(_{\text{v}}\)3.2 channels, but additional evidence, such as a slowly deactivating tail current, is required to rule out Ca\(_{\text{v}}\)2.3 channels. Both these criteria, plus PCR, were recently applied to show the expression of Ca\(_{\text{v}}\)3.2 in sympathetic ganglion neurons (231).

The temperature sensitivity of T-type currents has been measured in thalamic neurons (85), DRG neurons (305), NIE-115 neuroblastoma cells (298), and GH\(_{\text{3}}\) cells (343). Heating from 22 to 37°C caused over twofold increases in current amplitudes and accelerated channel activation and inactivation. In contrast, heating did not affect the position of the I-V curve (305). The effects of temperature have been found to be nonlinear, making it difficult to calculate the effect of a 10°C increment (\(Q_{10}\)) in temperature (298, 343). In the linear range, \(Q_{10}\) values with an average of 2.5 have been found for effects on amplitude, activation kinetics, and inactivation kinetics.

The pH sensitivity of T-type currents has been studied in CA1 hippocampal neurons (405), ventrobasal thalamic neurons (365), and cardiac myocytes (83, 414). Acidification of the external solution decreased current amplitudes, whereas alkalization had the opposite effect. In contrast, T-type currents are relatively insensitive to changes in intracellular pH (405). The effect of external pH is in part mediated by changes in the single-channel conductance, which when measured with 110 mM Ca\(^{2+}\) increased from 3.5 pS at pH 6 to 10.8 pS at pH 9 (414). Small changes in pH have also been shown to shift the voltage dependence of activation and inactivation; changing pH from 7.3 to 6.9 shifted both of these parameters by +3 mV, while increasing the pH to 7.7 had the opposite effect (365). Larger shifts in the voltage dependence of activation (\(-15\) mV) have been observed using larger shifts in pH (from 7.5 to 9.8) (83). The observed pK\(_{a}\) values were \(-7\), indicating that T-type currents will be affected by modest changes in extracellular pH (365, 414).

2. Slices

LVA currents have been characterized in slices prepared from numerous brain regions (Table 3). The most striking difference between these data and that obtained with isolated neurons is that currents are much larger in slices. This difference has been attributed to loss of channels that were localized on dendrites (97). In fact, T-type channels appear to be preferentially localized to dendrites (77, 135, 195, 199, 328).

T-type currents recorded from slices also appear to activate and inactivate at more negative potentials than observed in isolated neurons. The average threshold in slices was \(-70\) mV, and peak currents were observed at \(-45\) mV (Table 3), while in isolated neurons threshold was \(-60\) and the peak occurred at \(-30\) mV (Table 2). This difference has been attributed to poor voltage clamp of
neurons in slices (97). Consistent with this suggestion, the voltage dependence of single T-type channels recorded from dendrites is similar to that observed in isolated neurons (256).

In summary, T-type currents are found in neurons throughout the brain, with particularly large currents found in thalamic, septal, and sensory neurons. Their activation near the resting membrane potential and their fast recovery from inactivation allow them to generate LTS. Their preferential localization in dendrites suggests T-type channels play an important role in synaptic integration.

C. Heart

Cardiac T-type currents have been the focus of many studies due to their possible role as a pacemaker current. These studies have established that T-type current densities are highest in conduction and pacemaker cells, and much reduced or nonexistent in ventricular myocytes. T-type currents are highest near birth and gradually decline but may reappear in pathological conditions. Currents are larger in lower species and have a wider distribution. For example T-type currents are prominent throughout the guinea pig and hamster heart but virtually absent in adult ventricular myocytes of rat, cat, and dog. Although Ca3.2 was cloned from a human heart cDNA library, T-type currents have not been detected in human myocytes (39, 235, 313).

Cardiac T-type currents were initially described in dog atrium (32) and guinea pig ventricle (283, 303). These studies showed that cardiac T-type currents resembled those recorded from neurons, but differed from L-type channels in terms of their kinetics (transient), pharmacology (dihydropyridine insensitive), conductance of divalent cations (Ca2+ = Ba2+), lack of regulation by β-adrenergic agonists, and smaller single-channel conductance of Ba2+. Subsequent work has established their presence in myocytes isolated from dog Purkinje (367), rabbit sinoatrial node (152), cat atrium and latent pacemaker cells (462), hamster ventricle (362), and rat atrium (446). In general, the currents were small, displaying a peak current density below −3 pA/pF. In contrast, prominent T-type currents (>−10 pA/pF) have been recorded from myocytes isolated from shark (271), chick (202), finch (48), and mollusks (452).

Calcium channels were originally classified by their inactivation kinetics: rapidly inactivating or transient channels were called T type, while slowly inactivating, or long-lasting channels were called L type (303, 306). It should be noted that this only holds for currents carried by Ba2+, as cardiac L-type currents carried by Ca2+ can inactivate at a similar rate as T-type currents. This is due to calcium-induced inactivation of the L-type channel, which appears to be mediated by calmodulin tethered to the carboxy terminus of the channel (111). This inhibition is triggered with every heartbeat as Ca2+ entry triggers a larger release of Ca2+ from internal stores (37). In contrast, Ca2+ currents through T-type channels inactivate a bit slower than Ba2+ currents (116, 410), which excludes a similar regulation by calmodulin. Another major difference between these channels is that L-type channels conduct Ba2+ threefold better than Ca2+, whereas T-type channels conduct both equally well (discussed further in sect. IIJ).

A proposed physiological role for cardiac T-type channels is as a pacemaker current during diastolic depolarization. These studies have been hampered by the lack of a selective blocker, relying heavily on the ability of 40 μM nickel to block T- but not L-type currents (152, 298). Current-clamp studies of spontaneous action potentials showed that nickel slowed the late phase of depolarization, and hence slowed the firing of rabbit sinoatrial nodal cells (SAN). Similar results have been obtained by a number of groups using rabbit SAN (98, 353) or cat latent pacemaker cells (462). Tetramethrin (0.1 μM) was re-

### Table 3. Properties of T-type currents in brain slices

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Divalent, mM</th>
<th>Threshold, mV</th>
<th>I-V Peak, mV</th>
<th>I_max, pA</th>
<th>V_0.5, mV</th>
<th>h, mV</th>
<th>τ_inact, ms</th>
<th>τ_recov, ms</th>
<th>Nickel, μM</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>0.5</td>
<td>−60</td>
<td>−33</td>
<td>−600</td>
<td>−51\textsuperscript{M}</td>
<td>−86</td>
<td>14</td>
<td>100 (19%)</td>
<td>292</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>1</td>
<td>−65</td>
<td>−53</td>
<td>−1,500</td>
<td>−61\textsuperscript{M}</td>
<td>−77</td>
<td>26</td>
<td>100 200 (53%)</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>LGN</td>
<td>2</td>
<td>−70</td>
<td>−45</td>
<td>−1,812</td>
<td>−61\textsuperscript{M}</td>
<td>−84</td>
<td>17</td>
<td></td>
<td>151</td>
<td></td>
</tr>
<tr>
<td>Laterodorsal, f\textsubscript{T-}</td>
<td>2 Ca</td>
<td>−80</td>
<td>−55</td>
<td>−670</td>
<td>−86</td>
<td>32</td>
<td></td>
<td></td>
<td>398</td>
<td></td>
</tr>
<tr>
<td>Laterodorsal, f\textsubscript{T-}</td>
<td>2 Ca</td>
<td>−80</td>
<td>−65</td>
<td>−630</td>
<td>−98</td>
<td>55, 69</td>
<td>25 (50%)</td>
<td>398</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothalamus MDN</td>
<td>2</td>
<td>−82</td>
<td>−50</td>
<td>−1,051</td>
<td>−70\textsuperscript{M}</td>
<td>−82</td>
<td>12</td>
<td>100 500 (100%)</td>
<td>302</td>
<td></td>
</tr>
<tr>
<td>Brain stem respiratory</td>
<td>1.5</td>
<td>−65</td>
<td>−40</td>
<td>−550</td>
<td>−50\textsuperscript{M}</td>
<td>−81</td>
<td>10</td>
<td>200 (50%)</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Dorsal horn</td>
<td>2 Ba</td>
<td>−54</td>
<td>−20</td>
<td>−1,000</td>
<td>−45\textsuperscript{M}</td>
<td>−86</td>
<td>14</td>
<td>200 (91%)</td>
<td>347</td>
<td></td>
</tr>
</tbody>
</table>

The voltage dependence of activation was estimated by normalizing the current observed at each test potential to the maximum (I_\_max), fit with a Boltzmann function, then reported as the midpoint voltage (V_0.5). A single concentration of nickel was tested, and the percent block is shown in parentheses. LGN, lateral geniculate nucleus; MDN, magnocellular dorsal nucleus.
ported to produce selective block as well, and to produce similar effects on pacemaker cycle (152); however, subsequent studies failed to confirm this selectivity (165).

Similarly, a number of studies have found lower sensitivity and selectivity for nickel (Table 4). Two possible explanations for these disparate results are 1) that heart expresses two isoforms of the T-type channel, Ca,3.1 and Ca,3.2, and these isoforms differ in their sensitivity to nickel, and 2) that isoform expression is age and species dependent. Cloned Ca,3.2 channels are blocked at 20-fold lower concentrations than Ca,3.1 (or Ca,3.3), displaying that native Ca,3.2 channels are as sensitive as the cloned channel (323). This suggests that nickel (<100 μM) can be used to implicate Ca,3.2 channel expression. Expression in atrium appears to be species specific: Ca,3.1 appears to predominate in rat and mice (91, 236), while 20% of the channels are inhibited with an IC₅₀ of 48 mM (230). There is a strong correlation between nickel sensitivity and Cav3.2 expression, suggesting that nickel selectivity (165).

Expression of T-type currents in developing heart has also been studied. T-type currents are readily detectable in neonatal hearts, increase slightly to a peak between postnatal days 4 and 8, then decline slowly to a steady-state (236, 246). In other studies T-type currents were only detected in neonatal rats, disappearing by postnatal day 21 (236). In contrast to L type, the biophysical properties of T-type currents are unchanged during neonatal development. Changes in nickel sensitivity have not been investigated but may be informative. No difference in T-type current density was detected in SAN myocytes from newborn (3–10 days old) and adult (41–48 days old) rabbits (329). Expression of Ca,3.2 mRNA is higher in fetal human heart than adult, whereas Ca,3.1 shows the opposite pattern (331).

### Table 4. Properties of T-type currents in peripheral tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Divalent, mM</th>
<th>Threshold, mV</th>
<th>E-I Peak, mV</th>
<th>pA/pF</th>
<th>Iₘₐₓ, pA</th>
<th>Vₐₓ, mV</th>
<th>hₐₓ, mV</th>
<th>τₑₒₓ, ms</th>
<th>τᵣₑₒₓ, ms</th>
<th>τᵣₑₒₓ, ms</th>
<th>Nickel, μM</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrium</td>
<td>5 Ca</td>
<td>−50</td>
<td>−30</td>
<td>−0.3</td>
<td>−24</td>
<td>20</td>
<td>197</td>
<td>100</td>
<td>444</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrium</td>
<td>5.4 Ca</td>
<td>−50</td>
<td>−30</td>
<td>−1.0</td>
<td>−75</td>
<td>−57</td>
<td>15</td>
<td>50</td>
<td>66 (90%)</td>
<td>23</td>
<td>323</td>
<td></td>
</tr>
<tr>
<td>Atrium</td>
<td>2 Ca</td>
<td>−50</td>
<td>−30</td>
<td>−2.6</td>
<td>−30</td>
<td>−24</td>
<td>13</td>
<td>1.3</td>
<td>160</td>
<td>116</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrium</td>
<td>1.8 Ca</td>
<td>−50</td>
<td>−30</td>
<td>−350</td>
<td>−24</td>
<td>−59</td>
<td>23</td>
<td>323</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrium</td>
<td>2.5 Ca</td>
<td>−60</td>
<td>−40</td>
<td>−15</td>
<td>−40</td>
<td>−59</td>
<td>10</td>
<td>116</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAN</td>
<td>2.5 Ca</td>
<td>−47</td>
<td>−10</td>
<td>−2.1</td>
<td>−88</td>
<td>−23</td>
<td>144</td>
<td>40 (100%)</td>
<td>152</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latent pacemaker</td>
<td>2.7 Ca</td>
<td>−50</td>
<td>−20</td>
<td>−3.3</td>
<td>−90</td>
<td>−31</td>
<td>40 (54%)</td>
<td>406</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purkinje</td>
<td>2 Ca</td>
<td>−60</td>
<td>−30</td>
<td>−1.7</td>
<td>−391</td>
<td>−48</td>
<td>10</td>
<td>100</td>
<td>100 (90%)</td>
<td>165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purkinje</td>
<td>5 Ca</td>
<td>−40</td>
<td>−25</td>
<td>−2.9</td>
<td>−700</td>
<td>−47</td>
<td>4</td>
<td>83 (67%)</td>
<td>50 (47%)</td>
<td>410</td>
<td>444</td>
<td></td>
</tr>
<tr>
<td>Ventricles</td>
<td>5.4 Ca</td>
<td>−50</td>
<td>−10</td>
<td>−100</td>
<td>−100</td>
<td>11</td>
<td>100</td>
<td>100 (100%)</td>
<td>129</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricles</td>
<td>20 Ba</td>
<td>−50</td>
<td>−30</td>
<td>−5.8</td>
<td>−858</td>
<td>−43</td>
<td>−77</td>
<td>13</td>
<td>302</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>1.8 Ca</td>
<td>−55</td>
<td>−15</td>
<td>−3.6</td>
<td>−400</td>
<td>−24</td>
<td>−41</td>
<td>16</td>
<td>224 (65%)</td>
<td>28</td>
<td>5,160</td>
<td></td>
</tr>
<tr>
<td>Spermatocytes</td>
<td>10 Ca</td>
<td>−60</td>
<td>−20</td>
<td>−6.5</td>
<td>−218</td>
<td>−47</td>
<td>4</td>
<td>7</td>
<td>1.6</td>
<td>118</td>
<td>200 (75%)</td>
<td>350</td>
</tr>
<tr>
<td>Adrenal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerulosa</td>
<td>20 Ba</td>
<td>−45</td>
<td>−30</td>
<td>−7</td>
<td>−56</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasciculata</td>
<td>10 Ca</td>
<td>−50</td>
<td>−15</td>
<td>−8.2</td>
<td>−272</td>
<td>−17</td>
<td>−50</td>
<td>18</td>
<td>17</td>
<td>400 (50%)</td>
<td>20</td>
<td>287</td>
</tr>
<tr>
<td>Pituitary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pars intermedia</td>
<td>10 Ca</td>
<td>−50</td>
<td>−15</td>
<td>−150</td>
<td>−5</td>
<td>1.8</td>
<td>4</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactotropes</td>
<td>5 Ca</td>
<td>−48</td>
<td>−12</td>
<td>−90</td>
<td>−28</td>
<td>−66</td>
<td>15</td>
<td>100 (80%)</td>
<td>240</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanotropes</td>
<td>11 Ba</td>
<td>−50</td>
<td>−20</td>
<td>−30</td>
<td>−30</td>
<td>−61</td>
<td>19</td>
<td>204</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gonadotropes</td>
<td>20 Ca</td>
<td>−40</td>
<td>−20</td>
<td>−75</td>
<td>−27</td>
<td>−61</td>
<td>15</td>
<td>3</td>
<td>202</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Cell</td>
<td>10 Ca</td>
<td>−40</td>
<td>−16</td>
<td>21</td>
<td>2.5</td>
<td>320</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Cell</td>
<td>15 Ba</td>
<td>−60</td>
<td>−30</td>
<td>−35</td>
<td>−14</td>
<td>100 (100%)</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Definitions are as in Table 2. Methods used to calculate activation curves are denoted with the following superscripts: C, chord conductance; T, tail currents; G, constant-field equation of Goldman-Hodgkin-Katz; M, Iₘₑₓ. In some cases recovery from inactivation was found to be biphasic, so the values represent the tau of the fast component, the fraction of channels that recover fast (in parenthesis), and the tau of the slow component.

SAN, sinoatrial node.
Cardiac hypertrophy appears to trigger a return to the neonatal pattern of gene expression (388), leading to reexpression of T-type channels in rat and cat ventricular myocytes (173, 268, 308). RNase protection assays indicate that Ca,3.1 transcripts are upregulated in a rat model where hypertrophy is induced by infarction (173). Although levels of Ca,3.2 transcripts were not examined, the sensitivity of reexpressed currents to nickel block suggests a contribution of Ca,3.2 channels as well (173, 268). T-type currents are also reexpressed in dedifferentiated rat ventricular myocytes (114). T-type currents have been found to be increased twofold in cardiomyopathic animals (362). In contrast, studies using 8-mo-old animals found that the sensitivity of reexpressed currents to nickel block shifted to more negative potentials in ventricular myocytes from cardiomyopathic animals (362). In contrast, studies using 1-day-old hamsters found that only inactivation was shifted (46). The relevance of these observations to human pathologies is unclear as T-type currents have not been detected in myocytes isolated from normal atrium (235) or diseased ventricle (39, 337).

Calcium influx through voltage-gated channels activates intracellular Ca release channels, leading to larger elevations in intracellular Ca and hence muscle contraction. The role of L-type channels in this process is well established. T-type channels are also capable of triggering this CICR, albeit much more weakly in both cardiac (370, 461) and skeletal muscle (133). T-type current-induced contraction developed more slowly, suggesting that these channels are not localized near SR stores as are L-type channels. This observation coupled with the lower expression of T-type channels in working myocytes indicates that they do not play a major role in excitation-contraction coupling. Consistent with this notion, mibebradil has little effect on cardiac inotropy at doses that lower blood pressure (356). In contrast, T-type channels may be localized near SR in atrial pacemaker cells of the cat (180). These authors suggested a novel pacemaking mechanism where Ca influx through the T-type channel triggers subsarcolemmal Ca sparks, which in turn activate Na/Ca exchange currents that depolarize the membrane to threshold.

A role for T-type channels in triggering atrial natriuretic peptide (ANP) secretion has been inferred from the effects of mibebradil (236, 434). Evidence that mibebradil acted on T-type channels was provided by the observation that L-type blockers were much less potent blockers, or stimulated ANP secretion.

### D. Kidney

Relatively little is known about the physiological roles of T-type channels in kidney. T-type currents have only been recorded from smooth muscle cells (SMC) isolated from rat interlobular and arcuate arteries (143). Heterogeneity in Ca currents was observed, with approximately one-third of the freshly isolated myocytes displaying only T-type currents (“T-rich”), one-third only L type, and the rest a mixture. Currents in T-rich cells were some of the largest ever recorded for cardiovascular smooth muscle (−156 pA in 2.5 mM Ca; Table 5), which allowed their recording in physiological Ca solutions. Consistent with their assignment as T-type channels, currents activated and inactivated at voltages 20 mV more negative than observed for L-type currents. In contrast to most T-type currents, these SMC currents activated (I-V peak −10 mV) and inactivated (h inact = −50 mV) at voltages that were slightly more positive, which might be characterized as mid-voltage activated. They also inactivated about twofold slower during a pulse (τ inact = 46 ms at −10

### Table 5. Properties of LVA currents in smooth muscle

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Divalent, mM</th>
<th>Threshold, mV</th>
<th>I-V Peak, mV</th>
<th>I max, pA</th>
<th>h inact, mV</th>
<th>τ inact, ms</th>
<th>Nickel, μM</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteries</td>
<td>Coronary</td>
<td>10 Ba</td>
<td>−60</td>
<td>−10</td>
<td>−125</td>
<td>−65</td>
<td>14</td>
<td>130, 131</td>
</tr>
<tr>
<td>Coronary</td>
<td>20 Ba</td>
<td>−40</td>
<td>−30</td>
<td>−250</td>
<td>−55</td>
<td>332</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic</td>
<td>20 Ca</td>
<td>−60</td>
<td>−30</td>
<td>−140</td>
<td>−80</td>
<td>20</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Aortic</td>
<td>1.5 Ca</td>
<td>−60</td>
<td>−30</td>
<td>&lt;−100</td>
<td>−65</td>
<td>339</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic</td>
<td>20 Ba</td>
<td>−40</td>
<td>−12</td>
<td>−30</td>
<td>−63</td>
<td>372</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesenteric</td>
<td>10 Ba</td>
<td>−40</td>
<td>−30</td>
<td>−20</td>
<td>−55</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit ear</td>
<td>110 Ba</td>
<td>−30</td>
<td>+10</td>
<td>−38</td>
<td>46</td>
<td>428</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat tail</td>
<td>20 Ba</td>
<td>−50</td>
<td>+10</td>
<td>−263</td>
<td>−50</td>
<td>13</td>
<td>246</td>
<td></td>
</tr>
<tr>
<td>Veins</td>
<td>Portal</td>
<td>5 Ca</td>
<td>−45</td>
<td>+10</td>
<td>−200</td>
<td>448</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organs</td>
<td>Lung</td>
<td>10 Ca</td>
<td>−50</td>
<td>−10</td>
<td>−200</td>
<td>453</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>1.8 Ca</td>
<td>−60</td>
<td>−30</td>
<td>−300</td>
<td>−70</td>
<td>382</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder</td>
<td>1.8 Ca</td>
<td>−56</td>
<td>−10</td>
<td>−200</td>
<td>−60</td>
<td>390</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>2 Ca</td>
<td>−60</td>
<td>−10</td>
<td>−385</td>
<td>−58</td>
<td>20</td>
<td>445</td>
<td></td>
</tr>
</tbody>
</table>

Definitions are as in Table 2.
nV). Atypical currents with similar properties have been reported for SMC isolated from rat portal vein (315) and from the terminal branches of guinea pig mesenteric arteries (291).

Quite surprisingly, the human tissue that expresses the most mRNA for Ca₃.2 is the kidney (90, 438). In contrast, both Ca₃.1 and Ca₃.3 are predominantly expressed in brain (228, 288, 289, 324). Skott and co-workers (11, 156) have studied the distribution Ca₃.1 and 3.2 in kidney and concluded that Ca₃.1 was in the tubules, while Ca₃.2 was in renal smooth muscle (11, 156). RT-PCR indicated that mRNAs for Ca₃.2, and to a lesser extent Ca₃.1, were expressed in rat (but not rabbit) glomerular afferent and efferent vessels, and vasa recta. The same study reported that K⁺-induced contraction of perfused rabbit afferent arterioles could be blocked by low concentrations of mibefradil (IC₅₀ ~ 10 nM) that should be selective for T-type channels. Nickel could also block this response; however, the concentrations required (1 mM) would be expected to block almost all Ca²⁺ channels. Similar results were obtained with rat isolated perfused hydronephrotic kidneys: either 1 μM mibefradil or 100 μM nickel (a relatively selective dose) dilated afferent and efferent arterioles constricted with ANG II (314). Selectivity of mibefradil for T-type channels was demonstrated by coadministration with the L-type blocker nifedipine. In these experiments 1 μM nifedipine caused a modest dilation of the efferent arteriole but did not prevent the more pronounced dilation induced by mibefradil.

Ca₃.1 was detected in dot blots of human fetal, but not adult, kidney (288). RNase protection assays of rat kidney regions indicated that Ca₃.1 was predominantly expressed in the inner medulla (11). RT-PCR of microdissected nephron segments indicated that Ca₃.1 was expressed in distal convoluted tubules, connecting tubules, and collecting ducts. This study also examined the distribution of Ca₃.1 protein using a polyclonal antibody raised against the first 22 amino acids of the deduced Ca₃.1 sequence (11). Immunoreactivity was detected in the apical domains of the distal convoluted tubules and in principal cells of connecting tubules and inner medullary collecting ducts. Preincubation of the antibody with peptide blocked the signal; however, Western blots were not presented to demonstrate the selectivity of the antibody. These results suggested that T-type channels, along with endothelial calcium channels (ECaC), might be involved in Ca²⁺ reabsorption.

Calcium channel blockers are widely used in the treatment of hypertension. It is generally accepted that they work by blocking L-type channels in vascular smooth muscle, leading to vasodilation. Recent studies suggest that part of their effect may be mediated by changes in renal hemodynamics (172). In intact dogs, both nifedipine and mibefradil increased renal blood flow, whereas only nifedipine affected glomerular filtration rate. Mibefradil has also been reported to decrease renin secretion in rats with renal artery clips, but had no effect on renin secretion from isolated juxtaglomerular cells (423). In conclusion, these studies suggest that T-type channels on afferent and efferent glomerular arterioles may be an important therapeutic target (89). A possible advantage to a T-selective antihypertensive drug is that it may also prevent glomerular damage (192).

E. Smooth Muscle

In addition to L-type currents, SMCs isolated from arteries, veins, and organs have also been reported to have LVA currents. With the exception of the kidney results noted above, cardiovascular SMCs have tiny LVA currents. Therefore, most studies have had to use very high concentrations of charge carrier, which shifts gating to positive potentials, and currents have only been characterized in terms of their I-V relations and sensitivity to holding potential. These characteristics are not sufficient to establish an LVA current as T type, since HVA currents actually represent a spectrum of mid- to high-voltage-activated currents. HVA currents also inactivate over a wide range of potentials, with some R-type currents inactivating over the same voltage as T-type channels. These concerns take on additional weight with the finding that some vascular SMCs express non-L-type HVA currents (291) such as P-type currents (155). Native P-type currents can be mid-voltage activated (33). In addition, a nickel- and mibefradil-sensitive LVA current has been described in colonic myocytes that gates similar to T-type channels but has different permeability properties (214). LVA channels can be classified as T type by their slow tail currents, which surprisingly have not been reported, and by their single-channel properties, which have been reported (34, 130, 450). Because T-type channels inactivate at the resting membrane potentials of most SMCs (~75 to ~60 mV, reviewed in Ref. 168), it has been hard to discern their physiological role. Perhaps they contribute to basal intracellular Ca²⁺ concentrations via their window current, or after transient hyperpolarizations induced by spontaneous transient outward currents (STOCs).

LVA currents have been found in the following arterial SMCs: coronary (130, 281, 332), aortic (3), mesenteric (150, 311, 372), rabbit ear (34), rat tail (327, 428), and middle cerebral arterioles (167). RT-PCR detected the expression of both Ca₃.1 and Ca₃.2 in rat mesenteric arterioles (150). Single-channel studies have established the presence of 7.5-pS T-type channels in freshly isolated guinea pig coronary SMCs (130). This study also reported that whole cell LVA currents could only be observed in half of the cells. In contrast, LVA currents were never detected in freshly isolated coronary SMCs from rabbits.
LVA currents were found if the cells were cultured, and these currents appear to be T type based on their voltage dependence and 1:1 conductance of Ca\(^{2+}\) and Ba\(^{2+}\) (332). Similar results have been obtained with aortic SMCs; T-type currents are expressed in proliferating cultures, but not in confluent cultures (3, 339) nor in freshly isolated cells (220). In fact, T-type currents were only detected in cells that were in G\(_0\) or G\(_1\) phases (220), leading to the hypothesis that they might play a role in proliferating SMCs (338). Consistent with this notion is the finding that mibebradil could reduce neointima formation following balloon injury to rat carotid arteries (355). However, it should be noted that T-type currents were not observed in SMCs prepared from injured rat aorta, although a transient downregulation of L-type channels was documented (333).

LVA currents have also been detected in SMCs isolated from veins, such as saphenous (450), portal (246), and azygous (282). T-type single-channel currents were recorded in saphenous vein SMCs (450). Isradipine (PN 200–110) was found to block T-type currents of rat portal vein with an IC\(_{50}\) of 0.5 µM, while L-type currents were blocked at 1 nM (246). The sensitivity of these T-type currents was greater than observed in other preparations (see sect. VA), suggesting that Ca\(_{3}\) isoforms may differ in their pharmacology. Current-clamp recordings revealed that portal vein SMCs could fire action potentials that resembled those observed with cardiac myocytes (246). Isradipine (10 nM) inhibited the plateau phase with no effect on the rising phase, suggesting that T-type channels might play a pacemaker role in these cells.

LVA currents have been described in SMCs from bronchi (185, 448), ileum (373), colon (445), bladder (382), and uterus (453). The results with pregnant human uterine SMCs are notable because the T-type currents (−3 pA/pF in 1.8 mM Ca\(^{2+}\)) were larger than the L-type currents (453). These currents activated and inactivated (\(h_{\text{act}} = −70\) mV) at potentials typical of most T-type currents (Table 5). Current-clamp recordings indicated that a depolarizing pulse could trigger an action potential; however, the threshold (−20 mV) was higher than observed with neuronal LTS. Sizable (−200 pA) T-type currents were also described for porcine bronchial smooth muscle, being detected in 30% of the cells, but absent from tracheal SMCs (448). LVA currents (−1.4 pA/pF) were found to disappear when bladder SMCs were cultured (382). Concomitant with this loss was a shift in the threshold for action potential generation from −25 to −15 mV, suggestive of a role for T-type channels.

F. Skeletal Muscle

Newborn rodent skeletal muscle was also used in early studies to establish the existence of T-type channels as separate from L-type channels (30, 81). Developmental studies of mice and rats have shown that the T-type current is only found in embryonic and newborn muscle and disappears by 3 wk of age (29, 38, 142). Concomitantly, the slow L-type current increases. Studies on the muscular dysgenesis (mdg) mouse clearly established that the T- and L-type channels were encoded on separate genes (30, 67, 212), and it is the L-type channel (Ca\(_{1.1}\)) that plays a critical role in excitation-contraction coupling (395). Recent studies have shown that the T-type current is important for myoblast fusion (45). In particular, it was shown that T-type channels could generate sufficient window currents to alter intracellular Ca\(^{2+}\) concentrations, and trigger fusion. This hypothesis was further supported by pharmacological experiments using low concentrations of Ni\(^{2+}\) and amiloride, and by antisense oligonucleotides directed against Ca\(_{3.2}\) (45). The selective expression of Ca\(_{3.2}\) in skeletal muscle fibers has also been demonstrated using single-cell PCR (38). The electrophysiological properties of these currents (Table 4) and Ni\(^{2+}\) sensitivity closely resemble recombinant Ca\(_{3.2}\) currents, although differences have been noted in kinetics of activation, inactivation, and recovery from inactivation (38). Early studies also noted kinetic differences as a function of development (142), suggesting that these differences are not due to rapid events such as phosphorylation, but rather slower events such as gene transcription of auxiliary subunits (see sect. nE).

G. Sperm

Calcium channels appear to play a role in mediating the egg-induced acrosome reaction that precedes fusion (for review, see Ref. 94). T-type channels have been implicated in this process, although other Ca\(^{2+}\) conductances may be involved (134, 435). One reason for this uncertainty is that it is virtually impossible to patch clamp mature sperm (335). Therefore, electrophysiological studies have used spermatogenic cells, which are primary spermatocytes in the pachytyne stage. The only voltage-dependent Ca\(^{2+}\) channels in these cells are T type, and their biophysical (350) and pharmacological properties (15) have been well characterized. PCR results indicate that Ca\(_{3.2}\) is the predominant isoform expressed in human testis (182, 375). Consistent with this result is the high sensitivity (IC\(_{50} = 34\) µM) with which nickel blocks the spermatocytic T-type current (15), although it should be noted that Sertoli cells also express nickel-sensitive T-type currents (225). Similar concentrations of nickel also block the acrosome reaction (121, 375). Similarly, the following compounds have been found to block spermatocyte T-type channels and the acrosome reaction at similar concentrations: isradipine (PN 200–110), nifedipine, pimozide, amiloride, mibebradil, W-7, and trifluoper-
azine (13, 15, 121, 247, 350, 375). Spermatocytic T-type channels appear to be regulated by tyrosine and calmodulin-dependent protein kinases (14, 247).

H. Endocrine Tissues

1. Adrenal

T-type channels have been implicated in the secretion of aldosterone (76) and cortisol (109). Calcium currents, and their regulation by secretagogues, have been extensively studied in cells isolated from bovine adrenal zona glomerulosa and fasciculata. Glomerulosa cells express a mixture of T- and L-type channels (82), whereas fasciculata cells predominantly express T-type channels (22, 287). Although these cells can be considered nonexcitable due to their lack of Na+ channels, they are still capable of generating Ca2+-dependent action potentials (22). In contrast, adrenal chromaffin cells express HVA Ca2+ and Na+ currents but no LVA currents (16).

Serum K+ is regulated by secretion of aldosterone from adrenal glomerulosa cells. Glomerulosa cells are excellent K+ sensors and depolarize after small increases in serum K+, which in turn leads to the activation of T-type channels, Ca2+ influx, and stimulation of aldosterone synthesis and release. For example, increasing K+ from 2 to 5 mM causes the resting membrane potential of isolated cells to depolarize from −97 to −78 mV (76). Aldosterone secretion is also stimulated by ANG II, an effect mediated by stimulation of T-type channel activity (discussed in sect. III).

Cortisol secretion from adrenal zona fasciculata cells is regulated by ACTH, which depolarizes these cells by inhibiting a K+ conductance (286). Depolarization would activate T-type channels, leading to a rise in intracellular Ca2+, and ultimately cortisol synthesis and secretion. Cortisol secretion can be inhibited by a variety of blockers including mibebradil, and this block occurs over the same concentration range as their block of the T-type channels (109, 141, 345). ACTH may also regulate the expression of T-type channels (22).

Adrenal T-type channels are encoded by Cav3.2. In situ hybridization of rat and bovine glands detected high levels of Cav3.2 expression in the cortex, with only trace levels of Cav3.1 and Cav3.3 (358). The biophysical properties and sensitivity to Ni2+ block of both glomerulosa and fasciculata cells are also consistent with their being encoded by Cav3.2 (287, 358).

2. Pituitary

LVA Ca2+ currents have been recorded from cells of the anterior and intermediate lobes of the pituitary gland including (84, 392, 439) lactotropes (240), corticotropes (261), gonadotropes (379), and thyrotropes (204). These cells generate spontaneous action potentials and Ca2+ spikes, leading to secretion of hormone (see Ref. 406 and references therein). Hormonal regulation of these LVA currents supports the idea that these currents are involved in secretion (see sect. III). The voltage- and time-dependent properties of these currents are similar, but not identical to other native T-type currents (Tables 2 and 4). Notably the peak of the I-V curve is slightly depolarized. In addition, one study reported that the LVA current was fivefold larger in Ba2+ than Ca2+ (379), a property typically ascribed to HVA currents. As suggested previously, not all LVA currents are carried by T-type channels, and other criteria must be applied (19, 33, 376). One such discriminating property is their tail deactivation kinetics, and slowly deactivating T-type currents are clearly present in pituitary cells (84). Similarly, these channels can be distinguished at the single-channel level by their conductance for Ba2+, and again, pituitary T-type currents have been clearly identified (203). In situ hybridization studies suggest all three Cav3 isoforms are expressed, although Cav3.2 was the predominant isoform detected (393). Consistent with this result, T-type currents were reported to be nickel sensitive, with 80% of the current blocked by 100 μM NiCl2 (240). Currents recorded from GH3 cells are not nickel sensitive (IC50 = 777 μM), suggesting that this tumor-derived cell line most likely expresses Cav3,1 channels (160). In conclusion, T-type channels appear to play a pacemaker role in anterior pituitary, although they may also be involved in stimulus-secretion coupling (258).

3. Pancreas

T-type channels appear to play a similar pacemaker role in insulin secretion from pancreatic β-cells of the islets of Langerhans. Increases in plasma glucose and its metabolism in β-cells leads to increases in cellular ATP and inhibition of KATP channels (300). Closure of KATP channels initiates the pacemaker cycle by depolarizing the β-cell membrane to about −55 mV. Riding on top of this slow plateau depolarization are rapid calcium-dependent spikes (reviewed in Ref. 352). Insulin secretion can be blocked by a wide variety of agents, indicating that these spikes activate L-, P-, and R-type currents (238, 258, 418).

T-type currents in pancreatic β-cells have been characterized at the whole cell (Table 4; Refs. 25, 320) and single-channel level (17, 348). Expression is species dependent, with little or no expression in rodents, but readily detectable in humans (25, 426). Therefore, it is notable that LVA currents were found in β-cells from diabetes-prone NOD mice (426). The expression of LVA currents in mouse cells has been reported to be stimulated by a 6-h treatment with cytokines (25 U/ml of interleukin-1β plus 300 U/ml of interferon-γ; Ref. 427). However, the I-V curve for the cytokine-induced current peaked between +10 and +20, while under similar recording conditions this group found that bona fide, slowly
deactivating, T-type currents peaked between −20 and −10 mV (426). Human β-cells express both LVA and HVA currents, and in 20% of the cells tested, the LVA current was dominant (25). Voltage-clamp recordings indicated that 100 μM Ni²⁺ could block the LVA current, while current-clamp recordings indicated that it slowed action potential frequency. Similar studies using the rat insulinoma cell line INS-1 found that low concentrations of nickel could completely block T-type currents (IC₅₀ = 30 μM) and partially block insulin secretion (42). Nickel-sensitive T-type currents (IC₅₀ = 3 μM) have also been recorded from the mouse insulinoma cell line NIT-1 (426). Li and co-workers (463) also cloned a splice variant of Cav3.1 from an INS-1 cDNA library. The high sensitivity to nickel block suggests that Ca₃.2 channels are also expressed, and this conclusion is supported by Northern analysis (438).

I. Cell Lines

T-type currents have been reported in a number of cell lines such as 3T3 fibroblasts (73) and many lines of tumor origin, such as neoplastic B lymphocytes (128), the related mouse neuroblastoma-derived lines N1E-115 (298, 368), NG108–15 (196, 334), 140–3 (144), N18 (397), and ND7–23 (213); human neuroblastoma lines IMR-32 (65), SK-N-MC (18); rat pituitary lines GH3 (160, 270); human neuroblastoma lines IMR-32 (65) and N1E-115 (298, 196, 334), 140–3 (144), N18 (397), and ND7–23 (213); human neuroblastoma lines IMR-32 (65) and SK-N-MC (18); rat pituitary lines GH3 (160, 270); human TT cells (also called h-MTC) and rat 6–23 (clone 6) cells from medullary thyroid tumors (43, 44); human Y79 retinoblastoma cells (24); AT-1 from mouse atrium (351); rat smooth muscle lines A7r5 and A10 (272); and the retinoblastoma cells (24); AT-1 from mouse atrium (351); rat smooth muscle lines A7r5 and A10 (272); and the pancreatic insulinoma lines INS-1 (rat; Ref. 42), HIT-T15 (hamster; Ref. 259), and NIT-1 (mouse; Ref. 426). Many of these cell lines were reported to express almost exclusively T-type currents, allowing characterization of these channels in the absence of contaminating HVA currents (Table 6). The study of T-type currents in cell lines has advanced our understanding of gating, permeation, and pharmacology (73, 160, 368). For example, Chen and Hess (73) developed models of gating using recordings from 3T3 fibroblasts. They also showed that the T-type currents of NG108–15 cells had different kinetics, leading them to predict the existence of multiple channel isoforms. A similar conclusion was reached from studies on TT cells, which also recovered from inactivation much more slowly than observed previously (44). Differences in the pharmacology of T-type channels also suggested the existence of distinct channel isoforms (160). The cloning of multiple Ca₃ isoforms that differ in their kinetics, pharmacology, and recovery from inactivation supports this hypothesis (209, 228, 404).

J. Single-Channel Recordings

Single-channel studies provided conclusive evidence that T-type channels were distinct from HVA Ca²⁺ channels (63, 303, 306). T-type channels were distinguished by their smaller currents, insensitivity to dihydropyridines, and voltage dependence of activation and inactivation. In addition to providing criteria for resolving T-type currents, single-channel studies have provided insights into both hormonal regulation of activity and gating.

With isotonic Ba²⁺ as the charge carrier (110 mM), the single-channel current at a test potential of 0 mV is −0.3 pA for T-type channels (Table 7) and −1.5 pA for L-type channels. Single-channel conductance is defined as the slope of the line relating single-channel amplitudes versus test potential and is commonly reported in pico-

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**TABLE 6. Properties of T-type currents in cell lines**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Divalent, mM</th>
<th>Threshold, mV</th>
<th>I–V Peak, mV</th>
<th>I_max, pA</th>
<th>V₄₀, mV</th>
<th>h_m, mV</th>
<th>τ_max, ms</th>
<th>τ_deact, ms</th>
<th>τ_recov, ms</th>
<th>Nickel, μM</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>10 Ca</td>
<td>−40</td>
<td>−15</td>
<td>−211</td>
<td>−27⁰</td>
<td>−51</td>
<td>16</td>
<td>2</td>
<td>1,900</td>
<td>5 (57%)</td>
<td>44</td>
</tr>
<tr>
<td>IMR-32</td>
<td>10 Ca</td>
<td>−50</td>
<td>−20</td>
<td>−80</td>
<td>−32⁰</td>
<td>−40</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td>65</td>
</tr>
<tr>
<td>Y79</td>
<td>20 Ba</td>
<td>−50</td>
<td>−20</td>
<td>−87</td>
<td>−32⁰</td>
<td>−40</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-cell 6–23</td>
<td>10 Ca</td>
<td>−50</td>
<td>−15</td>
<td>−500</td>
<td>−31⁰</td>
<td>−57</td>
<td>20</td>
<td>2</td>
<td>1,260</td>
<td>5 (22%)</td>
<td>43</td>
</tr>
<tr>
<td>GH3</td>
<td>10 Ca</td>
<td>−50</td>
<td>−20</td>
<td>−600</td>
<td>−33⁰</td>
<td>−71</td>
<td>20</td>
<td>5</td>
<td>200 (80%)</td>
<td>777</td>
<td>160</td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAb-7B</td>
<td>2.5 Ca</td>
<td>−65</td>
<td>−35</td>
<td>−80</td>
<td>-82</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>128</td>
</tr>
<tr>
<td>N1E-115</td>
<td>50 Ba</td>
<td>−50</td>
<td>−20</td>
<td>−300</td>
<td>−53</td>
<td>20</td>
<td>2</td>
<td>300</td>
<td></td>
<td>47</td>
<td>208</td>
</tr>
<tr>
<td>NG108-15</td>
<td>10 Ba</td>
<td>−60</td>
<td>−15</td>
<td>−140</td>
<td>−27⁰</td>
<td>−53</td>
<td>20</td>
<td>2</td>
<td>845</td>
<td>100 (60%)</td>
<td>334</td>
</tr>
<tr>
<td>ND7-23</td>
<td>10 Ba</td>
<td>−50</td>
<td>−30</td>
<td>−119</td>
<td>−67</td>
<td>18</td>
<td>32</td>
<td>845</td>
<td></td>
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<td>3T3</td>
<td>20 Ba</td>
<td>−50</td>
<td>−20</td>
<td>−500</td>
<td>−56</td>
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<td>2</td>
<td>100</td>
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<td></td>
<td>73</td>
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<tr>
<td>AT-1</td>
<td>2.5</td>
<td>−60</td>
<td>−35</td>
<td>−500</td>
<td>−48⁰</td>
<td>−64</td>
<td>14</td>
<td>2.5</td>
<td>150 (73%)</td>
<td>351</td>
<td>1,200</td>
</tr>
</tbody>
</table>

Definitions are as in Table 2. Methods used to calculate activation curves are denoted with the following superscripts: C, chord conductance; T, tail currents; G, constant-field equation of Goldman-Hodgkin-Katz; M, I/I_{max}.
seimens (pS). When recorded in Ba$^{2+}$, the conductance of T-type channels is 7–8 pS (Table 7), which is smaller than that observed for either N-type (13–15 pS) or brain L-type channels (25–28 pS) (118, 125, 417). Somewhat surprisingly, all three families have a similar Ca$^{2+}$ conductance. For example, the L-type channel conductance drops threefold in Ca$^{2+}$ (149, 367). Recombinant Ca$_{2.1}$ and Ca$_{2.2}$ channels also preferentially conduct Ba$^{2+}$ (>2-fold), whereas Ca$_{2.3}$ conducts both ions equally well (56). In contrast, native T-type channels conduct Ca$^{2+}$ and Ba$^{2+}$ (and Sr$^{2+}$) equally well (64, 104, 367, 368). The conductance plots differ in their relative position, with T-type channels gating at more negative ranges, and extrapolating to a reversal potential of approximately +40 mV, while HVA currents appear to reverse at +60 mV (34, 52, 118, 124, 194, 203, 213, 367, 417).

Estimates of permeability based on reversal potentials yield the opposite rank order, with Ca$^{2+}$ being more permeable than Ba$^{2+}$ (128). Similar results have been obtained using recombinant T-type (363) and native L-type channels (161) and suggest that Ca$^{2+}$ binds with high affinity in the pore and permeates when displaced by a second Ca$^{2+}$. A very interesting property of all voltage-gated Ca$^{2+}$ channels is that they become highly permeable to Na$^+$ in the absence of Ca$^{2+}$. Apparently Na$^+$ are not capable of displacing the bound Ca$^{2+}$, and hence do not permeate. This block is unidirectional, since monovalent cations can displace Ca$^{2+}$ bound in the pore when they approach from the intracellular side of the channel (255, 363). The selectivity sequence for monovalent cations through T-type channels is Li$^+$ ≈ Na$^+$ > K$^+$ ≈ Rb$^+$ > Cs$^+$ (128, 255). In fact, T-type channels begin to pass outward K$^+$ currents at physiological concentrations of Ca$^{2+}$ and voltage (+28 mV; Ref. 128). HVA channels can also pass outward currents, but this requires depolarization of the membrane beyond +70 mV (161). Our understanding of Ca$^{2+}$ channel permeation is far from complete, and subject to multiple interpretations (275, 442).

Single-channel currents have also been studied at Ca$^{2+}$ concentrations closer to the physiological levels, displaying a single-channel conductance of −2 pS in 5 mM (64) and 4.6 pS in 10 mM Ca$^{2+}$ (21). Whole cell studies using varying concentrations of Ca$^{2+}$ indicate that currents increase sharply over the 1–10 mM range, then saturate at higher concentrations. These studies have allowed estimates of the affinity of the open channel for Ca$^{2+}$. The average $K_a$ value from seven studies is 4.9 mM, with considerable variability (range 0.3–10 mM) (4, 22, 54, 152, 160, 389, 429).

The transitions between open and closed states have been studied in detail in DRG neurons (64), cardiac myocytes (104), fibroblasts (73), and N1E-115 neuroblastoma cells (368). T-type channels respond to depolarizing pulses by opening in bursts, that is, channels open and close many times, become silent for a while, and at some voltages may burst again. The fast opening and closings

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**Table 7. Single-channel properties**

<table>
<thead>
<tr>
<th>Source</th>
<th>Divalent, mM</th>
<th>Current at 0 mV, pA</th>
<th>Conductance, pS</th>
<th>Selectivity</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat DRG</td>
<td>40 Ca</td>
<td>−0.2</td>
<td>5.2</td>
<td>Ca = Ba</td>
<td>64</td>
</tr>
<tr>
<td>Chick DRG</td>
<td>110 Ba</td>
<td>−0.4</td>
<td>8</td>
<td>Ba &gt; Sr</td>
<td>217</td>
</tr>
<tr>
<td>Mouse DRG</td>
<td>60 Ba</td>
<td>−0.25</td>
<td>7.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal CA3 pyramidal</td>
<td>95 Ba</td>
<td>−0.3</td>
<td>7.8</td>
<td></td>
<td>118</td>
</tr>
<tr>
<td>Septum and diagonal band</td>
<td>100 Ba</td>
<td>−0.2</td>
<td>7.8</td>
<td></td>
<td>147</td>
</tr>
<tr>
<td>Cerebellar Purkinje</td>
<td>110 Ba</td>
<td>−0.3</td>
<td>9.0</td>
<td></td>
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<td>Pituitary adenohypophysis</td>
<td>110 Ba</td>
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<tr>
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<td>−0.3</td>
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<td>Retinal ganglion</td>
<td>96 Ba</td>
<td>−0.36</td>
<td>8</td>
<td>Ba = Ca</td>
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<tr>
<td>Cardiac ventricle</td>
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<td>−0.33</td>
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<td>Cardiac Purkinje</td>
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<td>DRG</td>
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<td>10 Ca</td>
<td>−0.16</td>
<td>4.7</td>
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<tr>
<td>Coronary artery SMC</td>
<td>10 Ba</td>
<td>−0.2</td>
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Single-channel properties were measured from cell-attached patches using the indicated concentration of divalent cation as charge carrier. The slope conductance of high-voltage-activated channels is similar to T-type channels when recorded in Ca$^{2+}$ solutions but can be distinguished by their current amplitudes at a test potential of 0 mV. DRG, dorsal root ganglion; SMC, smooth muscle cell.

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can be modeled as transitions between a closed state C (C_4 in Fig. 3) and an open state O. The transition rates out of the open state determine the mean open time, which is typically 0.5–2 ms (Table 7). Multiple bursts are seen during depolarizing pulses just above the threshold for channel opening. At more depolarized potentials a single burst is observed, indicating that channels entered an inactivated state. Analysis of the time a channel spends in closed states reveals a bimodal distribution; closings within a burst are short (~1 ms), whereas the time between bursts is longer (~10 ms). Channels can also open partially, leading to a subconductance state that has a smaller current (27, 64, 104). In contrast to other ion channels (66), the gating behavior of this subconductance state was found to be similar to the main conductance state (64).

A distinguishing kinetic feature of T-type channels is that whole cell current traces recorded during an I-V protocol produce a criss-crossing pattern, where successive traces cross each other. This is largely due to slow activation kinetics at threshold potentials (~60 to ~40 mV). At the single-channel level, T-type channels show a long latency to the first opening, especially at threshold potentials, where 40 ms may pass before the first opening. This delay presumably reflects slow transitions between closed states and can lead to a sigmoidal rise in the whole cell current. This rate is voltage dependent, such that channels open faster at more depolarized potentials (104).

In most sweeps (~70%) the channel does not open at all, resulting in a blank sweep (73, 104). This suggests that channels might inactivate without opening. Such closed state inactivation has been observed in other channels and is particularly prominent in Ca_{v2} channels (321). Closed state inactivation also provides an explanation for the whole cell observation that steady-state inactivation can be observed at potentials more negative than channel opening. This inactivation is slow and voltage dependent (364). Inactivation from open (or the proximal closed state C_4) has similar kinetics and limits the channel to one burst per sweep. Whole cell recordings suggest that this rate is relatively voltage independent at potentials above ~20 mV. Several models of T-type channel gating have been proposed that can simulate the observed activity. One reason these models differ is that they are based on disparate results. Unresolved questions include the following: Does the voltage dependence of the mean open time explain the slowly deactivating tail currents observed at the whole cell level (64)? One study found that open times were shorter at more negative test potentials (64), another found no difference (104), while two found that they increased (217, 368). To complicate matters more, two studies have found evidence for a second type of opening of longer duration (27, 147). Discrepancy in mean open time measurements has been attributed to the low signal-to-noise ratio of channel openings (73). A related question is: Does the burst duration vary with test potential? Most studies report the burst duration at a single voltage, although one study found that it increased from 5 ms at ~40 mV to 14 ms at ~10 mV (104). These models might also differ because of T-type channel heterogeneity, as evidenced by the distinct gating behavior of the three recombinant Ca_{v3} channels (228). Recently, a model has been proposed that can simulate the gating behavior of both recombinant Ca_{v3.1} and Ca_{v3.3} channels, although different rate constants for the transitions are required (127, 364). The model assumes that depolarization leads to sequential movement of each of the four S4 voltage sensors (see sect. nB), culminating in a final closed state that precedes the open state. The open state can either deactivate (return to C_4) or inactivate (I_0). Closed state inactivation is allosterically coupled to S4 movement and becomes prominent after three S4 sensors have moved (Fig. 3). Movement of the fourth voltage sensor does not affect the inactivation rate, so open channels inactivate at the same rate as closed channels in C3 and C4 states. The speed of voltage sensor movement and subsequent transitions among closed states appears to be slower in Ca_{v3.3} than Ca_{v3.1}, leading to slower activation kinetics. The channels also differ in their equilibrium between C_4 and O states, with Ca_{v3.3} favoring C_4 while Ca_{v3.1} favors O. Therefore, Ca_{v3.1} channels inactivate more from open states, whereas Ca_{v3.3} channels inactivate more from closed states. The model accounts for most, but not all, of the observed properties of T-type channels; in particular, it does not attempt to explain multiple open states or ultraslow (>1 s) inactivation (127). In summary, voltage-gated Ca^{2+} channels can be distinguished by their single-channel properties, with T-
III. REGULATION

Among the first criteria used to distinguish LVA from HVA channels was their resistance to rundown in solutions that lack cAMP, ATP, and Mg\(^{2+}\), suggesting that LVA channels were not phosphorylated by cAMP-dependent protein kinase (115). Consistent with this hypothesis, hormones that stimulate adenylyl cyclase, such as the \(\beta\)-adrenergic agonist isoproterenol, had no effect on T-type currents recorded from either canine atrial myocytes (32), rabbit sinoatrial nodal cells (152), canine Purkinje cells (166, 410), guinea pig ventricular myocytes (415), rabbit ear artery (35), or guinea pig hippocampal CA3 neurons (117). Stimulation of L-type currents via cAMP-dependent pathways provided a positive control for most of these studies. Although some studies have reported a small stimulation of T-type currents by isoproterenol (9, 283), this may be due to either incomplete separation of T- from L-type currents (as evidenced by BAY K 8644 stimulation) or due to secondary regulation induced by changes in internal Ca\(^{2+}\) concentrations (411). In contrast to the apparent lack of protein kinase A (PKA) regulation, hormonal regulation of T-type currents has been reported in many systems. Both inhibition and stimulation have been reported, and in some cases the same agonist did both. For example, the GABA\(_A\) agonist baclofen has been reported to cause stimulation at 2 \(\mu\)M but inhibition at 100 \(\mu\)M (361).

A. Hormonal Inhibition

Many hormones and neurotransmitters have been reported to inhibit T-type currents, including dopamine, serotonin, somatostatin, opioids, ANP, and ANG II. Although most of these studies were performed on isolated neurons, regulation has also been noted in secretory cells of the pituitary and adrenal.

Dopamine inhibition of T-type currents has been described in DRG neurons (260), adrenal glomerulosa cells (312), pituitary melanotrophs (204), lactotrophs (240), and cells from the pars intermedia (309). Chick DRG currents were shown to be inhibited 60% by either 10 \(\mu\)M dopamine or norepinephrine (260). Inhibition occurred with no apparent effect on kinetics. Recovery upon washout was not observed in whole cell recordings. Similar inhibition was observed at the single-channel level, although in these experiments washout was observed. Single-channel current amplitude was not affected, suggesting that inhibition was due to a reduction in the probability of opening (\(P_o\)).

Dopamine (10 nM) inhibited T-type currents of rat anterior pituitary lactotrophs by 25–40% (239, 240). Dopamine shifted the \(h_\infty\) curve from −63 to −77 mV (5 Ca) and accelerated current decay during the pulse. It had no effect on voltage dependence of activation. Dopamine’s effects were reversible, mimicked by the dopamine agonist (D\(_2\) class) bromocryptine (10 nM), and prevented by the D\(_2\) antagonist sulpiride (100 nM). Inhibitory regulation could be abolished by omitting GTP from the intracellular solution or by pretreatment with pertussis toxin. Inhibitory modulation could also be disrupted by inclusion of antibodies that specifically recognize the \(\alpha\)-subunit of G\(_\alpha\), while antibodies against G\(_i\) subunits had no effect (239). Parallel studies showed that D\(_2\) receptors coupled to potassium channels via a different G protein, G\(_{i\alpha}\).

Dopamine inhibited rat adrenal glomerulosa T-type current by 33% (103). Inhibition was mediated by the D\(_1\) receptor class as evidenced by 1) inhibition with the D\(_1\) agonist SKF 82958; 2) block by the D\(_1\) antagonist SCH 23390; 3) but not by the D\(_2\) antagonist spiperone. Dopamine-mediated inhibition was blocked by agents that disrupt signaling by 1) G proteins [2 mM guanosine 5’-O-(2-thiodiphosphate) (GDP\(_S\))], 2) adenylyl cyclase (100 \(\mu\)M 2’,3’-dideoxyadenosine), and 3) protein kinase activity (10 \(\mu\)M H-89). Consistent with an action through D\(_1\) receptors, dopamine caused a stimulation of cAMP formation. Somewhat surprisingly, bath application of 8-bromo-cAMP had no effect on basal T-type currents but could block the effects of dopamine. Addition of 100 nM G\(_{\beta\gamma}\) subunits to the pipette solution did not affect T-type currents. However, G\(_{\beta\gamma}\) inhibition was observed after addition of 1 nM 8-bromo-cAMP to the bath. These results suggested that G\(_{\beta\gamma}\) subunits might bind directly to the T-type channel.

Somatostatin (10 nM) was found to inhibit T-type currents by 50% in rat somatotrophs (72). Somatostatin caused the currents to decay faster during a sustained pulse and shifted the midpoint of the steady-state inactivation curve (\(h_\infty\)) curve from −52 to −63 mV (charge carrier was 5 mM Ca\(^{2+}\)). This regulation was abolished by pertussis toxin. Neurotensin (200 nM) and substance P (200 nM) inhibited by 25% the T-type currents from rat cholinergic neurons (263). The \(\mu\)-opioid agonist Tyr-D-Ala-Gly-Met-Phe-Gly-ol (DAGO) was found to inhibit DRG currents by 50% (360). Enkephalin was found to inhibit T-type currents 20–40% in the neuroblastoma cell line NG108–15 (196). In these cells there was no effect of either the phorbol ester 4\(\beta\)-phorbol 12-myristate 13-acetate (PMA), arachidonic acid (10 \(\mu\)M), or forskolin. Bradykinin (0.1 \(\mu\)M) inhibited 57% of the T-type current from ND7–23 cells, a cell line derived from DRG (213). This study also showed that (−)-baclofen (1 \(\mu\)M) could inhibit currents by 56%, whereas guanosine 5’-O-(3-thiotriphosphate) (GTP\(_\gamma\)S) inhibited by 20%. Pertussis toxin treatment did not block baclofen inhibition. Baclofen (20 \(\mu\)M)
has also been reported to inhibit the T-type currents in hippocampal neurons by 53% (126).

Muscarinic inhibition of T-type currents from hen granulosa cells has also been reported (425). In contrast to most studies that report ~40% inhibition, this study reported 90% inhibition. Part of this difference may be due to their use of the perforated patch technique, which is expected to preserve the integrity of the intracellular milieu. The effect of carbachol was blocked by atropine. In contrast, muscarinic agonists have been reported to have no effect on LVA channels in cholinergic neurons (5).

In contrast, muscarinic agonists have been reported to have no effect on LVA channels in cholinergic neurons (5).

ANP (3 nM) inhibited T-type currents of bovine adrenal glomerulosa cells by 28% (274). Inhibition was dependent on the holding potential, with little block when membrane potential ($V_M$) was $-90$ mV, but near complete block when $V_M$ was $-45$ mV. This voltage dependence of block also caused a $-10$ mV shift of the $h_{\text{in}}$ curve to more negative potentials. In contrast, ANP did not affect the voltage dependence of activation as measured using tail currents (26). The effects of ANP were also studied at the single-channel level. Single T-type channels were identified by their 8.5-pS conductance. Inhibition was accompanied by a decrease in the number of active sweeps, with no change in the amplitude of single openings. The ability of bath-applied ANP to modulate channels recorded in the cell-attached mode indicated that ANP induced the production of a soluble second messenger.

ANG II and the AT$_{1}$ selective ligand CGP-42112 inhibited by 25% the T-type current in NG108–15 cells (58, 59). Inhibition was associated with a $-5$-mV shift in the I-V but no effect on the $h_{\text{in}}$ curve. Inclusion of 3 mM GDP$eta$S in the pipette blocked the ANG II effect. In contrast, 3 mM GTP$\gamma$S did not block the ANG II inhibition. This result is surprising since this concentration of GTP$\gamma$S should have maximally stimulated G protein signaling. Washout was not reported. A role for tyrosine phosphorylation in the signaling pathway was suggested by the ability of 50 $\mu$M sodium orthovanadate to disrupt ANG II modulation of the T-type current.

B. Hormonal Stimulation

T-type currents can also be stimulated acutely by a variety of hormones. One of the first studies showed that 10 $\mu$M serotonin could stimulate rat spinal motoneuron T-type currents by 66% in a slice preparation (36). Also in a slice preparation, dorsal horn neurons were found to be stimulated by substance P (1 $\mu$M), although some neurons were inhibited (347). Cholinergic agonists also stimulate T-type currents, and this was demonstrated at the single-channel level. Carbachol and muscarine doubled the number of openings of single T-type channels in adult guinea pig hippocampal CA3 neurons (117). This stimulation was blocked by 0.1 $\mu$M atropine. The modulation was likely due to an increase in the probability of opening of each channel, as there was no change in the single-channel conductance, which was 7.1 pS in a solution containing 95 mM Ba$^{2+}$. Under similar conditions, carbachol inhibited single L-type currents. Carbachol (50 $\mu$M) and serotonin (30 $\mu$M) were also shown to stimulate hippocampal T-type currents 54 and 61%, respectively (126). These effects were blocked by the appropriate receptor antagonists and were quickly reversible. Serotonin appeared to shift the I-V to the left (peak shifted from $-25$ to $-30$ mV), but this effect was not quantitated (126). Erythropoietin increased T-type currents 20% in the human neuroblastoma cell line SK-N-MC (18). It also stimulated increases in intracellular Ca$^{2+}$ that was dependent on external Ca$^{2+}$ but not internal stores. Norepinephrine acting via $\alpha$-adrenoceptors was reported to increase T-type currents in canine Purkinje cells by 69% (410).

Endothelin has been reported to stimulate T-type currents from both cardiac and smooth muscle myocytes (129, 181). In neonatal rat ventricular myocytes, endothelin-1 displayed an EC$_{50}$ of 1.3 nM and a maximal stimulation of 54% (129). Heat inactivation of the endothelin, or omission of GTP from the pipette, occluded the effects. The stimulation induced by 10 nM endothelin was blocked by 0.2 $\mu$M staurosporine or 20 $\mu$M H-7. Protein kinase C (PKC) agonists stimulated the currents directly, and this could be blocked by H-7 as well. In guinea pig smooth muscle myocytes, endothelin stimulated the activity of a 12-pS channel, that may or may not be carried through T-type channels (181). Endothelin has been reported to have no effect on T-type currents recorded from smooth muscle cells from rat renal resistance arteries (143).

Acetylcholine stimulated T-type currents in NIH 3T3 cells that had been transfected with recombinant m3 and m5 muscarinic receptors (322). Stimulation was modest (25%) and was accompanied by a leftward shift in the I-V relation. Parallel assays demonstrated that cAMP levels were increased. T-type currents were also stimulated by treatments designed to stimulate cAMP-mediated signaling, such as 500 $\mu$M 8-bromo-cAMP or 10 $\mu$M forskolin. Likewise, the PKA inhibitor 10 $\mu$M Rp$_{c}$-cAMP$\beta$-s cyclic monophosphothionate ($R_p$cAMPS) inhibited basal activity and occluded the response to acetylcholine. These results suggested that T-type channels might be directly phosphorylated by PKA. An apparent contradiction to this hypothesis was that T-type currents were not regulated in cells transfected with the m1 muscarinic receptor, although these cells showed robust increases in cAMP. The authors speculated that m1 receptors might simultaneously activate inhibitory PKC pathways that obscured the PKA-mediated stimulation. First they showed that the PKC activator phorbol 12,13-dibutyrate (PDBu; 0.5 $\mu$M) directly inhibited T-type currents as observed in other cells types. Next they showed that preincubation
with the PKC inhibitor calphostin C (0.5 μM) allowed m1-mediated stimulation of the T-type current. Basal T-type currents were not regulated by acetylcholine in cells transfected with either m2 or m4. However, inhibition of T-type currents, as well as inhibition of cAMP formation, could be observed in these cells after stimulation of adenylyl cyclase by 10 μM forskolin. These results indicate that hormonal regulation of T-type channels may depend on both basal phosphorylation levels as well as cross-talk between PKA and PKC pathways.

Barrett and colleagues (82) have extensively studied the stimulation of adrenal glomerulosa T-type Ca$^{2+}$ channels by ANG II. Initial studies established the presence of T-type channels in these cells through the use of tail current analysis (82). Slowly activating currents gated with the voltage dependence expected of T-type channels, while fast-deactivating currents behaved like HVA, L-type channels. ANG II increased the T-type tail currents by 50% (82). The effects of ANG II were also studied at the single-channel level (273). ANG II increased both the number of active sweeps and increased the number of openings per sweep, with no appreciable change in the amplitude of single openings. The ability of bath-applied ANG II to modulate channels recorded in the cell-attached mode indicated that ANG II induced the production of a soluble second messenger. The effects of ANG II were blocked by the ANG II receptor antagonist saralasin (1 μM). ANG II also shifted the voltage dependence of activation by 10 mV to more negative potentials, with no change in the $h_{in}$ curve. Pretreatment of the cells with pertussis toxin abolished these effects, indicating that ANG II receptors couple through G proteins, possibly G$_i$ (251). The final step in this signal transduction pathway appears to be phosphorylation of the T-type channel by calmodulin-dependent protein kinase II (CaMKII; see below) (27, 252). However, not all studies have found an ANG II stimulation of adrenal glomerulosa currents, with reports of either no effect (250) or frank inhibition (103, 344).

C. Guanine Nucleotides

As noted above, T-type currents are regulated by G protein-coupled receptors. Therefore, this regulation should require the presence of GTP and should be modified by activators (GTPγS) and inhibitors (GDPβS) of G proteins. Accordingly, no hormonal regulation was observed when GTP was omitted from the patch pipette (129, 239) or when it was replaced with GDPβS (59, 103).

The effects of GTPγS are difficult to interpret because it can activate all G proteins. With the use of photoactivation of caged GTPγS in DRG neurons, low concentrations were observed to stimulate T-type currents by 54%, while higher concentrations inhibited them up to 87% (361). Inhibition was accompanied by an acceleration of inactivation, such that the $t$ decreased from 22 to 18 ms. Neither cholera toxin pretreatment nor photorelease of caged GDPβS had any effect. Pertussis toxin pretreatment blocked GTPγS inhibition, but not stimulation. GTPγS has also been shown to inhibit T-type currents (40%) of rat nodose ganglion neurons (148). This effect was blocked by pretreatment with pertussis toxin.

Somewhat surprisingly, G proteins do not appear to mediate the regulation of T-type currents by serotonin and nociceptin. Serotonin inhibition (25%) of T-type currents has been observed in Xenopus sensory neurons (383, 384). Inhibition was not observed when the T-type channels were recorded in the cell-attached patch configuration, indicating that its effects were membrane delimited. Inhibitory regulation was not blocked by pertussis toxin pretreatment or inclusion in the pipette of GDPβS, GTPγS, or 5′-guanylyl-imidodiphosphate (GMP-PNP). The ability of these manipulations to block the regulation of HVA currents in the same neurons provided a convenient positive control. It was concluded that G proteins are not involved in the serotonin inhibition of T-type channels. A similar conclusion was reached in a study on the nociceptin inhibition of DRG T-type currents (1). Nociceptin inhibited currents with an IC$_{50}$ of 0.1 μM, and in 22 of 77 neurons it blocked 100% of the current at 1 μM. Inclusion in the pipette of GTPγS, GDPβS, or AlF$_3$ did not alter response, leading the authors to conclude that a G protein is not involved. It should be noted that nociceptin had no effect on T-type currents from small nociceptive trigeminal ganglion neurons (51). The endogenous cannabinoid anandamide also blocks T-type currents independently of G proteins and receptors (71).

The ability of pertussis toxin treatment to block regulation indicates that G$_i$/G$_o$ proteins are involved. Support for this hypothesis comes from studies that used specific antibodies in the patch pipette to block regulation (239, 251). Although the $\beta\gamma$-subunits of G proteins have been suggested to interact directly with T-type channels (103), more studies are required to establish this point.

D. Protein Kinases

With the notable exception provided by studies on fibroblasts (322), T-type currents appear not to be regulated by PKA. In contrast, T-type currents appear to be inhibited by members of the PKC family and stimulated by tyrosine kinases and CaMKII.

The effects of PKC have been evaluated by activating the kinase directly with compounds such as diacylglycerol 1-oleoyl-2-acetylglycerol (OAG), or various phorbol esters: PMA, 12-O-tetradecanoylphorbol-13-acetate (TPA), or PDBu. Although many studies have found no effect of these compounds (310), both stimulation and inhibition of
activity have been reported. Studies on rat DRG neurons found that 10 nM PMA inhibited the T-type current by 27%. Likewise, the inactive analog 4α-phorbol had no effect. Of note, the PMA effect was not observed at room temperature, requiring preincubation at 29°C or higher (359). Similarly, 100 nM TPA was found to inhibit the T-type current by 26% in ventricular myocytes and Purkinje cells. A phorbol ester analog that does not activate PKC had no effect, while the protein kinase inhibitor H-8 prevented TPA inhibition (411). In contrast, 50 μM H-7 was not able to block the inhibition of hippocampal T-type currents by 10 μM TPA or 5 μM OAG (407). In addition, the TPA and OAG effects occurred faster than would be expected (200 ms), leading Toselli and Lux (407) to conclude that the compounds were blocking the channel directly. PKC may in part mediate the inhibitory effects of arachidonic acid (459).

PKC-mediated stimulation of T-type currents has also been reported (129). Currents in rat neonatal ventricular myocytes were stimulated 30% by either 0.2 μM PDBu or PMA. The inactive analog 4α-PDBu had no effect. Stimulation was blocked by inclusion of 20 μM H-7 in the pipette.

The ability of CaMKII to stimulate adrenal T-type currents has been studied at both the whole cell and single-channel level, and recently with recombinant channels. Inclusion of Ca-CaM in the pipette shifted the voltage dependence of activation to more negative potentials (252). The CaMKII inhibitor KN-62 or the synthetic peptide inhibitor that corresponds to residues 290–309 blocked this effect. Stimulation was also demonstrated at the single-channel level in both cell-attached and excised patches (27). In cell-attached patches, manipulation of intracellular Ca^{2+} led to an increase in channel activity that could be blocked by KN-62, but not the inactive analog KN-04. Channel activity could also be stimulated when excised inside-out patches were exposed to calmodulin and appropriate concentrations of Ca^{2+}. This stimulation could be blocked with the autacamide 2-related inhibitory peptide (AIP). Stimulation could also be mimicked by the direct addition of a constitutively active mutant of CaMKII. Heat-inactivated kinase had no effect. As observed previously with ANG II (273), this regulation appeared to be due to an increase in active channels. These studies clearly establish that native bovine glemorulosa T-type channels are regulated by CaMKII. Adrenal cortical T-type channels are encoded by α_{1H} or Ca_{3.2} (358). Recent studies have shown that calcium/calmodulin can stimulate the activity of cloned human Ca_{3.2} channels when coexpressed with CaMKIIγC in HEK-293 cells (443). Stimulation could be blocked by CaMKII inhibitors added to either the bath (3 μM KN-62) or to the intracellular pipette used for whole cell recording (AIP, 2 μM). Replacement of pipette ATP with the nonhydrolyzable analog 5′-adenylyl-imidodiphosphate (AMP-PNP) also blocked regulation. As observed with native channels, stimulation was accompanied by an 11-mV hyperpolarizing shift in the voltage dependence of activation, with no shift in the h_{∞} curve. In contrast, the activity of human Ca_{3.1} was not regulated under similar conditions. These studies strongly suggest that the modulatory phosphorylation site(s) reside directly on the α_{1}-subunit of T-type channels and that these sites are subtype specific, in this case only found on Ca_{3.2}. Although CaMKII regulation of T-type currents has not been reported in other systems, it might have mediated the 30% stimulation observed in cardiac myocytes (411).

E. Voltage

An intriguing property of many HVA Ca^{2+} currents is that their activity can be increased, or “facilitated,” by strong depolarizing pulses. These prepulses can induce channel conformations that are 1) more susceptible to phosphorylation, 2) have a lower affinity for divalent cations in the pore, or 3) have a lower affinity for G protein βγ-subunits. The prepulse can also directly induce high activity gating modes (mode 2) of cardiac L-type channels. Both native (below) and recombinant (140) T-type currents have also been reported to be facilitated by prepulses.

The T-type currents of guinea pig coronary smooth muscle myocytes were stimulated twofold by 200-ms prepulses to voltages above −30 mV (131). Studies where the prepulse potential was varied indicated that saturation occurs at −10 mV. Use of longer prepulses (10 s) shifted this voltage dependency to the left, such that saturation occurred at −60 mV. Changing the duration of the prepulse showed that the peak effect occurred between 160 and 320 ms, which then decayed and was gone by 5 s. This potentiation was greater when the interpulse voltage was −100 compared with −80 mV, and at 36 versus 24°C. Potentiated currents inactivated faster (τ_{inact} 5.6 vs. 14.5 ms).

Facilitation has also been observed in bone marrow cells, where T-type currents were stimulated twofold by a 750-ms prepulse to +150 mV (330). Compared with the results obtained in smooth muscle, stronger depolarizations were required; stimulation was not observed with prepulses lower than −30 mV, and saturated at +100 mV. Changing the duration of prepulse (+10 mV) pulse) showed that half-maximal stimulation occurred at ~250 ms, and saturated at 1,000 ms. Changing the interval between the prepulse and the test pulse showed that potentiation peaked after 1 s and decayed in a biexponential manner: 9.4 and 43.5 s. Potentiation was not affected by omission of ATP or GTP or bath application of non-specific protein kinase inhibitors (e.g., 100 μM H-7). Potentiated currents decayed slightly faster (τ_{inact} decreased
from 17 to 13 ms; currents isolated by subtraction). These results suggest that voltage directly affects channel activity without the involvement of second messenger signals. The opposite conclusion was reached in studies of bullfrog atrial cells (8). Amphibian channels are stimulated by β-adrenergic agonists, and a prepulse enhances this stimulation. These effects were enhanced by GTPγS and ATPγS, but blocked by either GDPβS or pertussis toxin treatment. From these studies it was concluded that G proteins are involved and that phosphorylation of G proteins may play a role as well.

Facilitation of T-type currents from mouse spermatogenic cells has also been reported (14). In this case, prepulses could only stimulate activity 50% above control. Prepulses to voltages greater than −30 mV were required to observe stimulation, and saturated around +60 mV. Short prepulses (>10 ms) were capable of stimulating currents, and this effect wore off very slowly. Membrane-permeable inhibitors of tyrosine kinases (tyrophostins A47 and A25) stimulated basal T-type currents but had no effect on facilitated currents. This suggests that tyrosine kinases may tonically inhibit activity in this system and that a prepulse somehow reverses their activity. Consistent with this hypothesis, inhibitors of tyrosine kinase phosphatases inhibited basal T-type currents and prevented voltage-induced potentiation.

In summary, T-type channels can be both stimulated and inhibited by hormones and neurotransmitters that are coupled to the Gq or Gi/Go families. Regulation appears to be mediated by phosphorylation, with inhibition mediated by PKC and stimulation by CaMKII. Additional studies are required to establish the location of the modulatory phosphorylation site(s), whether G protein subunits are capable of interacting directly with the channel, and the mechanisms by which voltage regulates channel activity.

IV. MOLECULAR CLONING OF T-TYPE CHANNELS

A. Cloning

Cloning of high voltage-gated Ca2+ channels began with purification and microsequencing of the skeletal muscle dihydropyridine receptor (396). Low-stringency screening of cDNA libraries with probes derived from the α1-subunit of skeletal muscle (α1S, or Ca1.1) led to the discovery of cardiac (α1C, Ca1.2) and brain (α1D, Ca1.3) isoforms of the L-type family (Ca1.1; reviewed in Ref. 326). The more distantly related members of the Ca2.1 family were cloned during the “brain era” (Fig. 4A) using even lower stringency hybridization (374). PCR provided an alternative approach, and primers based on highly conserved regions (IIS5 and IVS5) were used successfully to isolate a fragment of Ca2.3 (357). Despite the efforts of many groups, these approaches were not successful in isolating cDNAs encoding T-type channels.

Progress in the sequencing of human, yeast, and C. elegans genomes provided a novel library that could be screened with a computer, or in silico (Fig. 4B). Screening with the highly conserved P loop, or K+ channel signature sequence, allowed Ketchum et al. (205) to identify a novel K+ channel sequence from Saccharomyces cerevisiae. We decided to try a similar approach using S5 segments. This led to the identification of two Ca2+ channel-like proteins that were predicted from the C. elegans genome (325). These proteins were homologous to the skeletal muscle Ca2+ channel, and this similarity was noted in the GenBank entry. We reasoned that there might be other sequences in the GenBank that were similarly labeled and used a text-based search (Fig. 4B) to find sequences that were “similar to a calcium channel.” This led to the identification of the human EST clone H06096 (324). Although this clone is 1.4 kb long, only a part of the 5’-sequence was in the database. This fragment had a very low sequence identity to the S1 segment of other Ca2+ channels, precluding its direct identification by homology search. The full sequence of H06096 showed that it might encode a protein with many of the hallmarks of voltage-gated channels, notably the S4 voltage sensor sequence was well conserved, and the pore loop sequence was highly homologous to other Ca2+ channels. Screening of the GenBank with the H06096 sequence and the program BLAST identified C54d2.5 as a C. elegans homolog. Similar to in vitro cloning where one “walks” through a cloning project using probes to the ends, we screened the GenBank with the 3’-end of C54d2. This led to the identification of the EST clone H19230. In vitro screening of a rat brain cDNA library with H06096 allowed the cloning of Ca3.1 (324), whereas use of this clone to screen a human heart library led to the cloning of Ca3.2 (90). Screening of a rat brain library with H19230 under low-stringency conditions allowed us to identify not only rat versions of Ca3.1 and Ca3.2, but also Ca3.3 (228). A similar approach was used by Monteil et al. (288) to identify C54d2.5 and the H06096 and H19230 ESTs, which then allowed them to clone the full-length human Ca3.1. Similar PCR approaches were also used to clone a human Ca3.2 cDNA (438) and mouse and rat versions of Ca3.1 (211, 463). PCR approaches based on the C. elegans sequence C27f2.5 led to the cloning of a distantly related channel gene whose function remains unknown (227).

Sequencing of the human genome also played an important role in the cloning of T-type channels. The Ca3.3 gene is located on chromosome 22, which was the first chromosome to be completely sequenced (105). BLAST search with rat Ca3.1 identified the coding regions of Ca3.3, which then allowed the design of PCR primers to either clone its cDNA (228, 289) or to examine
the splicing patterns of its gene (285). Proper identification of the 5′- and 3′-ends requires traditional cDNA cloning or specially designed PCR protocols. Recent studies indicate that the human Ca v3.3 mRNA contains an additional exon that encodes 214 additional amino acids (140). Studies comparing the full-length to truncated Cav3.3 channels revealed differences in channel expression and their ability to be modulated by a strong prepulse (140).

Genetic mutations in various Ca2+ channel subunit genes have been associated with ataxic and epileptic phenotypes (187). As a first step in exploring such a link, we mapped the human and mouse genes for Ca v3.1, CACNA1G (324), and Ca v3.2, CACNA1H (90). The genes were mapped using the Cambridge 4 radiation hybrid panel and fluorescent in situ hybridization. The locations of the human genes are shown in Table 8. Mutations in human Cav3 genes have yet to be described.

All three T-type channel genes are alternatively spliced, producing variants that differ in their intracellular loops (68, 284, 285, 463). Splicing produces Ca v3.1 variants that differ in at least two locations: 1) the II-III loop, where two variants are produced by insertion/deletion of exon 16; and 2) the III-IV linker, where three (or actually four, Ref. 229) variants are produced by a combination of insertion/deletion of exon 26 and use of an alternate 5′-splice site in exon 25 (288). These variants differ in their electrophysiological properties (68) and may account for the differences noted in the gating of T-type channels between lateral geniculate (LGN) relay neurons and interneurons (318). Splice variants of Cav3.3 have been detected by PCR that differ in the I-II loop and carboxy terminus (285). Similarly, PCR has identified splice variation in the III-IV linker of Ca v3.2 in both rat (231) and human (182).

Ca v3 cDNAs have been cloned from rat, mouse, and human sources (Table 8). Although all three have been cloned from rat and human, cloning from mouse is still ongoing. Comparisons of their nucleotide sequences reveal a high level of conservation between species. Although species differences in the predicted amino acid sequence have been noted, some of these changes can be ascribed to either frameshifts in the reading, cloning artifacts, or differences in splicing. For example, it is likely that a cloning artifact caused the deletion of three amino acids from III54 in a rat cDNA of Ca v3.3 (279).
TABLE 8. Comparison of the three cloned T-type channels

<table>
<thead>
<tr>
<th>Tissue expression</th>
<th>Ca_{\alpha,1}</th>
<th>Ca_{\alpha,2}</th>
<th>Ca_{\alpha,3}</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue expression</td>
<td>Brain, ovary, placenta, heart</td>
<td>Kidney, liver, adrenal cortex, brain, heart</td>
<td>Brain</td>
<td>90, 228, 288, 289, 324</td>
</tr>
<tr>
<td>Electrophysiology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threshold, mV</td>
<td>-70</td>
<td>-70</td>
<td>-70</td>
<td>209</td>
</tr>
<tr>
<td>Peak of I-V, mV</td>
<td>-30</td>
<td>-30</td>
<td>-30</td>
<td>209</td>
</tr>
<tr>
<td>Activation kinetics, ms</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>209</td>
</tr>
<tr>
<td>Inactivation kinetics, ms</td>
<td>11</td>
<td>16</td>
<td>69</td>
<td>209</td>
</tr>
<tr>
<td>h_{\alpha}, mV</td>
<td>3.0</td>
<td>2.2</td>
<td>1.1</td>
<td>209</td>
</tr>
<tr>
<td>Deactivation kinetics, ms</td>
<td>-72</td>
<td>-72</td>
<td>-72</td>
<td>209</td>
</tr>
<tr>
<td>Recovery from inactivation, ms</td>
<td>117</td>
<td>395</td>
<td>352</td>
<td>209</td>
</tr>
<tr>
<td>Conductance, pS; Ba</td>
<td>7.5</td>
<td>9</td>
<td>11</td>
<td>228, 324, 438</td>
</tr>
<tr>
<td>Pharmacology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nickel (IC_{50}, M)</td>
<td>250</td>
<td>12</td>
<td>216</td>
<td>230</td>
</tr>
<tr>
<td>Mibebradil (IC_{50}, M) in 10 Ba^{2+}</td>
<td>1.2</td>
<td>1.1</td>
<td>1.5</td>
<td>207</td>
</tr>
<tr>
<td>Mibebradil (IC_{50}, M) in 2 Ca^{2+}</td>
<td>0.27</td>
<td>0.14</td>
<td>0.27</td>
<td>207</td>
</tr>
</tbody>
</table>

* Accession numbers are for a representative GenBank entry followed by the SWISS-PROT entry. † Results for conductance were obtained in 110 mM Ba^{2+}, while all other electrophysiological measurements were made in 1.25 mM Ca^{2+}. Activation and inactivation kinetics were measured with exponential fits to the currents elicited by test potentials to -10 mV. Deactivation kinetics were measured at -90 mV. Pharmacology was measured in 10 mM BaCl_{2} or as noted, in 2 mM CaCl_{2} solutions.

B. Structure

Conservation of amino acid sequence (Figs. 1 and 5) and predicted secondary structure (Fig. 6) indicates that T-type channels are evolutionarily related to the \( \alpha \)-subunits of K\(^+\), Na\(^+\), and HVA Ca\(^{2+}\) channels (184). Although little is known about the structure of Na\(^+\) and Ca\(^{2+}\) channels, recent advances with K\(^{+}\) channels provide a framework to interpret their structures. The most significant advance was the determination of the KcsA K\(^{+}\) channel structure by X-ray crystallography (102). This channel contains two transmembrane segments separated by a pore loop (Fig. 6). The second transmembrane segment forms the barrel walls of the pore, while the selectivity filter is formed by the pore loops that dip partially into the barrel. In addition to this fundamental motif, voltage-gated channels also contain four additional transmembrane segments, with one (S4) acting as a voltage sensor. In K\(^{+}\) channels, four proteins assemble to form one channel, while in Na\(^+\) and Ca\(^{2+}\) channels a single protein contains four of these modules, or repeats. Each transmembrane segment can be identified by a number to indicate the repeat it is in (traditionally a Roman numeral) followed by its position within a repeat (Fig. 6). For example, the third transmembrane segment of the third repeat can be abbreviated IIIIS3. Among voltage-gated ion channels, the most highly conserved sequence belongs to the S4 segments, where every third residue is positively charged. This region is thought to move outward when the plasma membrane is depolarized. Little is known about how this movement is transduced into channel opening. The pore loops are also well conserved, especially within the members of a particular ion channel class. In voltage-gated Ca\(^{2+}\) channels, the pore loops all contain the following signature sequence: T-x-E/D-x-W. In HVA channels, all four repeats have a glutamate residue in the pore, leading to the abbreviation EEEE. In repeats III and IV of Ca_{\alpha,3} channels, the glutamate has been replaced by an aspartic acid residue, or EEDD. The importance of these residues in Ca\(^{2+}\) permeation has been extensively established in HVA channels (449), and recently with Ca_{\alpha,3.1} (391).

Site-directed mutagenesis studies on the S6 region of Ca_{\alpha,3.1} suggest that these segments play a similar structural role in these channels as in KcsA (206). This provides a theoretical basis for the use of the KcsA crystal structure to guide studies of drug binding pockets, which in the case of Ca_{\alpha,1.2} appear to be located within the channel walls on the cytoplasmic side of the pore loops (174).

As shown in Figure 6, most of the channel is thought to be intracellular. The extracellular loops are predicted to be quite short, with the notable exception of the loop connecting IS5 to the pore loop (98–103 amino acids). This extracellular loop contains six cysteine residues that are conserved in all three Ca_{\alpha,3} channels and a number of possible sites for glycosylation. Neuraminidase treatment of cardiac myocytes stimulates T-type (but not L-type)
currents threefold, suggesting that glycosylation of the channel inhibits its activity (116, 451). Despite the fact that the sequences of intracellular loops differ considerably across Na\(^+\) and Ca\(^{2+}\) channels, they have a similar overall size: long loops connect repeats 1–2 and 2–3 (range 207–375 amino acids for Ca\(_3\) channels), while repeats 3–4 are connected by short loops (55–62 amino acids). Similarly, the amino termini are usually short (<100 amino acids), whereas the carboxy termini are quite long (433–493 amino acids). The sequence of these loops is poorly conserved, with the exception of sequences close to the end of the S6 segments (Fig. 5). In HVA channels these loops are important for binding auxiliary subunits such as the β-subunit, binding to regulatory proteins such as calmodulin or the G protein βγ-complex, and are sites for protein phosphorylation (424). Although the roles of these loops in T-type channels are presently unknown, it is likely they contain important regulatory sites as well.

C. Distribution

The expression patterns of these genes in peripheral tissues and brain have been studied using Northern blots, RNA dot blots, and in situ hybridization (Table 8). Ca\(_{3.1}\) mRNA is primarily expressed in human brain, but also in ovary, placenta, and heart (288, 324). A wider distribution was noted in dot blots prepared from human fetal tissues, with strong expression noted in kidney and lung (288). Ca\(_{3.2}\) mRNA is primarily expressed in kidney and liver, but also in heart, brain, pancreas, placenta, lung, skeletal muscle, and adrenal cortex (90, 438). Ca\(_{3.3}\) mRNA is almost exclusively expressed in brain (228); the only peripheral tissues that showed expression on dot blots were adrenal (but see Ref. 358) and thyroid (289).

The distribution of all three Ca\(_3\) channel transcripts in rat brain has been studied in exquisite detail using in situ hybridization (393), and similar patterns of expression have been obtained using Northern blots of human brain mRNA (288, 289, 438). Their patterns of expression are complementary in many regions, with most brain regions expressing more than one isoform. In fact, some neurons may express all three genes as suggested by the labeling of olfactory granule cells and hippocampal pyramidal neurons. Many brain regions showed heavy expression of Ca\(_{3.1}\) mRNA, including thalamic relay nuclei, olfactory bulb, amygdala, cerebral cortex, hippocampus, hypothalamus, cerebellum, and brain stem. Two separate studies have confirmed this pattern of mRNA expression and extended the findings by showing a similar distribution of Ca\(_{3.1}\) protein (88, 197). Ca\(_{3.2}\) mRNA expression was detected in olfactory bulb, striatum, cerebral cortex, hippocampus, and reticular thalamic nucleus. Ca\(_{3.3}\) mRNA expression is high in olfactory bulb, striatum, cerebral cortex, hippocampus, reticular nucleus, lateral habenula, and cerebellum. DRG neurons express both Ca\(_{3.2}\) and Ca\(_{3.3}\) mRNA (393). One study found that this expression was restricted to small and medium-sized neurons (383), while a second study reported that Ca\(_{3.3}\) transcripts were equally abundant in large DRG neurons (454).

D. Electrophysiology of Recombinant Channels

Expression of recombinant Ca\(_3\) channels leads to the appearance of robust currents in a variety of heterologous expression systems. Currents mediated by the cloned α\(_3\)-subunit are nearly identical to those recorded from native channels: they both open and inactivate at potentials near the typical resting membrane potential, recover rapidly from inactivation, and close slowly producing prominent tail currents (Fig. 7).

The currents mediated by rat and human isoforms of the three Ca\(_3\) channels have been compared under a variety of experimental conditions (209, 218, 228, 279). In general these results agree quite well with each other, with the differences most likely due to recording conditions or sequence variability. For example, Ca\(_{3.1}\) and Ca\(_{3.2}\) currents activate (∼1.7-fold) faster with Ba\(^{2+}\) than Ca\(^{2+}\) (209, 211). Similarly, recording conditions such as choice of charge carrier or length of depolarizing pulses can affect estimates of both deactivation kinetics (211, 216, 430) and recovery from inactivation (430). Therefore, comparisons under one set of conditions are the most relevant; Table 8 presents the results obtained in 1.25 mM Ca\(^{2+}\) (209), whereas Figure 7 results were obtained in 5 mM Ca\(^{2+}\).

All three Ca\(_3\) clones form LVA channels. Depolarization of the membrane to −70 mV is sufficient to trigger channel opening, and the I–V curves for all three channels peak around −30 mV (Fig. 7B). Some studies have found that Ca\(_{3.3}\) currents activate at slightly more positive potentials than either Ca\(_{3.1}\) or Ca\(_{3.2}\) (127, 289). Part of this difference can be ascribed to the methods used to estimate the voltage dependence of activation. Of note, not all the recombinant channels within a given preparation gate at the same low potentials, with ∼40% of the channels requiring much stronger depolarizations (+10 to +50 mV) for opening (288, 438). Activation curves constructed using tail currents that detect both channels show a lower slope and higher midpoint of activation than other methods.

The kinetics of the currents are very voltage dependent between −70 and −20 mV, producing a typical crisscrossing pattern of traces (Fig. 7A; Ref. 334). Therefore, it is useful to compare the kinetics of the three channels at −10 mV or above (Table 8). Ca\(_{3.1}\) and Ca\(_{3.2}\) both activate relatively quickly at this potential (τ = 1–2 ms) and
FIG. 5. Alignment of the predicted amino acid sequence of human Ca v3.1, 3.2, and 3.3 channels. Residues conserved in all three sequences are shown in the consensus sequence (CON). The approximate location of the membrane-spanning regions is indicated above the sequence. β-Subunits bind to the I-II loop of HVA channels through a sequence termed the α-interaction domain (AID). The consensus sequence of the AID is shown above the homologous region in the Ca v3 channels. The residues are colored according to the structural properties of the individual amino acids: red, positively charged; green, negatively charged; blue, polar; yellow, nonpolar.
inactivate 10-fold slower ($\tau = 11-16$ ms). In stark contrast, Ca$_{v3.3}$ currents activate and inactivate very much slower. This difference is even greater when comparisons are made using Xenopus oocytes (228), and it remains a mystery why Ca$_{v3.3}$ channels behave so differently in amphibian eggs than in mammalian cells.
Recombinant Ca\textsubscript{v}3 channels, like their native counterparts, close slowly producing prominent tail currents. The voltage protocol used to measure tail currents includes a short pulse to open channels, followed by repolarization to a voltage where they close (Fig. 7C). Although all three Ca\textsubscript{v}3 channels show slow tail currents upon repolarization to \(-90\) mV, Ca\textsubscript{v}3.1 are the slowest (\(\tau = 3\) ms), while Ca\textsubscript{v}3.3 are the fastest (\(\tau = 1\) ms). In contrast, HVA channels close 10-fold faster.

Despite large differences in the rate at which they inactivate, the voltage dependence of steady-state inactivation of the three recombinant Ca\textsubscript{v}3 channels is remarkably similar. The midpoint of the \(h_{\infty}\) curves are \(-72\) mV (Table 8). Native T-type currents inactivate over a similar range. These studies indicate that T-type channels, as observed with other channels, can reach inactivated states without passing through open states (364). In fact, during a depolarizing pulse up to 30% of Ca\textsubscript{v}3.3, channels inactivate without opening (127).

Ca\textsubscript{v}3 channels recover rapidly from short-term inactivation. Ca\textsubscript{v}3.1 channels recover fastest, displaying a monoexponential recovery with a time constant of \(~100\) ms (Fig. 7, Table 8). In contrast, Ca\textsubscript{v}3.3 channels recover threefold more slowly, while Ca\textsubscript{v}3.2 channels recover...
steady-state can be estimated by multiplying the Boltzmann functions that describe activation and inactivation. This is commonly referred to as a window current and is operationally defined as the overlap region between the activation and steady-state inactivation curves (Fig. 7, G and H). All three Ca₃ channels are predicted to generate window currents supported by <1% of the channels. Recording conditions and methods of analysis have a dramatic effect on estimates of the window current, so these estimates must be interpreted with caution. For example, analysis of Ca₃.3 results obtained in Xenopus oocytes indicates that up to 2% of the channels can be open in the steady-state. Evidence for window currents have also been obtained in thalamocortical neurons, where they play an important role in signal amplification (441). Window currents also appear to be important in determining intracellular Ca²⁺ concentrations (18, 69, 264). Express-
sion of recombinant Ca3.1 or Ca3.2 was found to increase basal Ca2+ in HEK-293 cells, and this increase was blocked by appropriate concentrations of either mibebradil or nickel. Similarly, basal Ca2+ is increased after differentiation of the human prostatic cancer cell line LNCaP, and this correlates with the expression of Ca3.2 mRNA and currents (264). Hormonal regulation of the T-type window current provides cells with another mechanism to regulate intracellular Ca2+. In adrenal glomerulosa cells, ANG II increases T-type window currents by selectively shifting activation to more negative voltages (273, 443), an effect mediated by CaMKII phosphorylation (discussed in sect. mD).

Another distinguishing property of T-type channels is that they have a lower Ba2+ conductance than HVA channels. Therefore, studies of cloned T-type channels have focused on the amplitude of single-channel currents in isotonic barium solutions. All three recombinant channels were found to have small single-channel currents, corresponding to slope conductances in the 7–11 pS range (Table 8). In contrast, Ca1.2 channels display slope conductances of 20–30 pS, while Ca2 channels are in the 13–16 pS range. Measurement of the single-channel amplitude is complicated by the presence of subconductance activity (228). Failure to account for these subconductance openings would result in lower estimates, and this may explain why Ca3.2 was reported to have a conductance of 5.3 pS (90). Subconductance activity has also been observed with native T-type channels (64).

The effect of changing external pH has been studied with Ca3.1 (391) and Ca3.2 (95). In contrast to native channels, whose activity is affected by small deviations from pH 7.2, the recombinant channels are largely unaffected by pH changes in the 8.2 to 6.9 range. Further acidification of the external solution to pH 5.5 decreases the currents measured during standard test pulses. Mutation of the aspartate in the dmain III pore loop to glutamate (EEDD to EEED) shifted the apparent pKa of Ca3.1 from 6.0 to 7.0 (391). In addition, this mutation increased the sensitivity to Cd2+ block. Therefore, each domain contributes a negatively charged residue to form a high-affinity binding site for Ca2+ (and Cd2+) in Ca3 channels (EEDD) as shown previously for Ca1 and Ca2 (both EEDE) channels (75, 207, 319, 449). In addition to affecting permeation, shifting the pH from 8.2 to 5.5 produced a dramatic 40-mV shift in the voltage dependence of activation of Ca3.2, lowered its slope factor, and produced an 11-mV shift in the hrev curve (95). These results suggested that protonation of the channel affected the voltage dependence of transitions between deep closed states (R to C3 transitions in Fig. 3). Although it is unknown where on the channel the affected residues lie, they may very well be in the pore. Consistent with this hypothesis, mutations in the Ca3.1 pore also lowered its voltage dependence and shifted activation to more positive potentials (391).

Studies using mock action potentials suggest that most of the Ca2+ influx through T-type channels occurs after the peak voltage and corresponds to the tail current (218, 276, 334). This Ca2+ influx might play a role in spike repolarization and afterhyperpolarization mediated by apamin-sensitive Ca2+-dependent K+ channels (440). Due to differences in their rates of activation and inactivation, the three T-type channels respond quite differently to trains of mock action potentials (218). Both Ca3.1 and Ca3.2 channels inactivate rapidly during trains delivered at >20 Hz. In contrast, Ca3.3 continues to gate during 100-Hz train frequencies. These differences suggest that the three channels play distinct roles in determining neuronal firing patterns and signal integration. Ca3.1 and Ca3.2 channels are likely involved in short burst firing as seen in thalamocortical neurons, whereas Ca3.3 channels are likely involved in sustained burst firing as seen in thalamic reticular neurons (70).

E. Auxiliary Subunits?

Auxiliary subunits of HVA Ca2+ channels were first identified in purified preparations of skeletal muscle channels (see Ref. 326 for review). This channel complex was found to contain at least four subunits: α1 (Ca1.1, 1.2), α2δ (α2δ1), β (β1), and γ (γ1). Purified cardiac L-type and brain N-type channels have a similar α2δβγ structure, but with distinct α1- and β-subtypes. Additional members of each subunit class have been cloned (Fig. 4), such that there are now seven HVA α1-subunits (30, 234) and more than 10 β-subunits (reviewed in Ref. 171). The β-subunits play a critical role in channel function, controlling both channel expression at the plasma membrane, voltage dependence, and pharmacology. The α2δ- and γ-subunits also play a role in expression of functional channels. Mutations in Ca2+ channel genes, or calcium channelopathies, have been linked to a variety of disease phenotypes in both mice and humans (reviewed in Ref. 248).

Native T-type channels have yet to be purified, so their subunit structure is unknown. Two strategies have been used to infer its structure: to assay whether antisense oligonucleotides directed against HVA β-subunits can alter native T-type channel function, and to test whether the activity of the cloned T-type α1-subunit is affected by coexpression with cloned HVA subunits. Antisense depletion of β-subunits causes profound reductions in HVA channel density; however, this treatment has no effect on T-type channels recorded from the same neurons (226, 234). Similarly, coexpression of cloned β-subunits has little or no effect on cloned T-type channel activity (100). In contrast, coexpression with either α2δ1 or α2δ2 was found to double the size of Ca3.1-mediated currents (100, 326). Concomitant immunolocalization studies supported the contention that this rise in currents...
was due to increased expression of α1-α2δ1 complexes at the plasma membrane (100). Similar results were obtained by coexpression of α1-α2δ1 with mouse Ca,3,1 (169), although the effects of α2δ1 failed to reach statistical significance (224). Coexpression with α2δ1 causes no detectable changes in the biophysical properties of Ca,3,1 channels, while α2δ2 has been reported to have minor effects on inactivation (169). Mutations in the murine α2δ2 gene have been linked to an absence of epilepsy phenotype in the mouse strain ducky (23). Recordings from Purkinje neurons isolated from ducky cerebella showed half the P-type current density observed in controls. These results, in combination with coexpression studies, suggest that α2δ2 plays a greater role in HVA than LVA channel expression.

Identification of the mutation in the mouse strain stargazer, which also exhibits an absence epilepsy phenotype, led to the cloning of γ2 (233). In silico cloning has extended the γ-like family to eight members (78). The γ2 protein is only 25% identical to skeletal muscle γ and may play different physiological roles. Immunoprecipitation studies have shown that γ2 may be a subunit of both HVA Ca,2,2 Ca^{2+} channels and glutamate receptors of the AMPA class (74, 190, 306). Coexpression of γ2 inhibits the expression of Ca,2,1 and Ca,2,2 channels in the presence of α2δ1 and has minor effects on their biophysical properties (190, 346). In contrast, γ3 had little effect on Ca,3,3 expression, although it could slow the decay of the tail current (145). The related isoforms, γ2 and γ4, have been reported to have no effect on Ca,3,3 currents (145). In contrast, γ2, γ4, and γ6 have been reported to accelerate inactivation of Ca,3,1 currents and shift steady-state inactivation by −4 mV (210). To reiterate, the electrophysiological activity of T-type α1-subunits expressed alone is very similar to that observed with native channels. This is not the case for HVA channels, which require β-subunits for proper gating (223), and α2δ1- and γ-subunits for proper pharmacological activity (381). In conclusion, these studies indicate that HVA auxiliary subunits can interact with Ca,3 α1-subunits, but additional biochemical studies are required to establish this interaction in vivo.

V. PHARMACOLOGY

Cardiovascular L-type calcium channels are an established drug target in the control of blood pressure and cardiac rhythm. Selective Ca,1 blockers have been developed from over three drug classes, including dihydropyridines, phenylalkylamines, and benzothiazepines. In contrast, there are no highly selective drugs or peptide toxins (309) that selectively block Ca,3 channels. A comprehensive review of drugs tested on native T-type channels was recently published (158); therefore, this review focuses on drugs whose mechanism may involve block of T-type channels.

A. Antihypertensives

Marketing of mibefradil (Posicor, Roche) as the first selective T-type channel blocker stimulated considerable interest in the pharmacology and physiology of these channels (412). Mibefradil was approved for use in essential hypertension and stable angina pectoris until it was withdrawn from the market due to drug-drug interactions leading to irregular heart rhythms (219). Submicromolar concentrations of mibefradil, formerly Ro-40–5967, preferentially block T-type channels, while higher concentrations are required to block cardiovascular L-type channels (282). Its 10- to 30-fold selectivity for T- over L-type channels has been confirmed in a number of preparations, including recombinant channels expressed in mammalian cells (267, 323). The average IC_{50} from 11 studies for mibefradil block of native T-type channels was 1.5 μM (see Table 1 in Ref. 267). Mibefradil potency is affected by the concentration, and choice, of divalent cation, with less block in Ba^{2+} than in Ca^{2+} solutions (267). In 2 mM Ca^{2+} solutions, mibefradil blocked recombinant Ca,3,2 channels with an IC_{50} of 0.14 μM, whereas slightly higher concentrations were required for block of Ca,3,1 and Ca,3,3 (267). Mibefradil block of both HVA and LVA channels is state dependent (41, 278). Single-channel measurements indicate the presence of open channel block, and in addition, a longer lasting block that results in a reduction in active sweeps (280). Whole cell measurements indicate that inhibition is enhanced by holding the membrane at potentials where T-type channels inactivate, suggesting the drug has higher affinity for inactivated states. The apparent affinity for these states was estimated to be 83 nM using native T-type currents (278), and similar results were obtained with recombinant channels (267). This provides another mechanism for selectivity, since T-type channels inactivate near the resting membrane potential of most cells, while HVA channels require depolarization. A similar mechanism has been invoked to explain the selectivity of dihydropyridines for L-type channels in depolarized vascular beds over L-type channels in heart. These results suggest that mibefradil selectivity in vivo was probably greater than estimated in vitro since most studies used Ba^{2+} solutions, and a significant fraction of T-type channels is inactivated in vivo. Therapeutic plasma concentrations of mibefradil were found to be in the 0.5–1 μM range, with 99.5% bound to plasma proteins such as α1-acid glycoprotein (433). Therefore, a significant fraction of T-type channels in vascular smooth muscle myocytes would have been blocked during treatment. Subsequent studies have established that micromolar concentrations of mibefradil are capable of blocking a wide range of ion channels, including those for Na^{+} (106), K^{+} (138), and Cl− (304). It remains to be determined whether block of these other channels contributed to its antihypertensive activity. This ability to block other ion...
channels complicates the interpretation of mibefradil effects, and additional studies are required to establish the role of T-type channels in smooth muscle proliferation (355), cardiac remodeling (113, 422), and renal protection (28).

Akaike et al. (4) have extensively studied the sensitivity of neuronal T-type currents to calcium channel blockers. These studies demonstrated that many drugs blocked neuronal T-type currents at micromolar concentrations. The order of potency (IC\textsubscript{50} in parentheses, in \(\mu M\)) was flunarizine (0.7) > nifedipine (3.5) > nifedipine (5) > nimodipine (7) > methoxyverapamil (50) and diltiazem (70). A similar rank order was found in a study of septal neurons, except nicardipine was found to be more potent (IC\textsubscript{50} = 0.77 \(\mu M\;\text{Ref. 6}\)). Because early studies using 0.1–1 \(\mu M\) dihydropyridines had shown selective block of L-type channels with little block of T-type channels, these authors concluded that there was a subset of dihydropyridine-sensitive T-type channels. A similar conclusion was reached from a study of thalamic and mesenteric SMC T-type currents (246, 398). Felodipine is also a potent T-type blocker, displaying an apparent affinity for inactivated channels of atrial myocytes of 13 nM, but only 700 nM for those of pituitary GH\textsubscript{3} cells (83). Such high-affinity block led these authors to conclude that block of T-type channels might contribute to felodipine’s antihypertensive properties. Amiodipine (Norvasc, Pfizer), which is currently the most widely prescribed dihydropyridine, is ~12-fold selective for cardiac L-type channels (IC\textsubscript{50} = 0.5 \(\mu M\)), blocking T-type channels with an apparent IC\textsubscript{50} of 5.7 \(\mu M\) (323). Recombinant Ca\textsuperscript{2+} channels were slightly less sensitive (IC\textsubscript{50} = 31 \(\mu M\)). Because amiodipine serum concentrations are ~14 nM (432), at least 3% of the T-type channels will be blocked during therapy, and this fraction might be higher in depolarized cells.

The dihydropyridine structure-activity relationships of T- and L-type channels are different. This is most clearly demonstrated by the stereoisomers of BAY K 8644; T-type channels are weakly blocked by micromolar concentrations of both (237), while for L-type channels the (−)-isomer is an agonist (198). Stimulation of LVA currents by BAY K 8644 is difficult to interpret because this compound shifts gating of L-type channels to more negative potentials. In contrast, the stereoselectivity and potency of nifedipine were similar for both L- and T-type channels; the racemic mixture blocked T-type currents in guinea pig atrial myocytes with an IC\textsubscript{50} of 0.18 \(\mu M\) (341). Although the binding site for dihydropyridines is different between L- and T-type channels, the mechanism of block appears similar. Dihydropyridines appear to stabilize inactivated states of both channels, causing a negative shift in the steady-state availability curve (31, 83, 221, 336). In conclusion, dihydropyridines are selective for L-type channels, and some analogs are capable of blocking T-type channels in the 1–10 \(\mu M\) range.

### B. Antiepileptics

Generalized, or petit mal, absence epilepsies are characterized by periods of synchronized neuronal activity, generating a 3- to 4-Hz spike-wave pattern on the electroencephalogram. Considerable evidence suggests that spike-wave discharges are produced by synchronized oscillations of cortical, reticular thalamic, and corticothalamic neurons (92, 136, 378). Spike-wave discharges of corticothalamic neurons are in part mediated by T-type channels that produce LTS (93, 277, 377, 387). Ethosuximide is a first-line drug in the treatment of absence epilepsy (193). In vitro studies found that ethosuximide can block T-type channels with little effect of HVA channels in thalamic neurons (86). Because block occurred at therapeutically relevant concentrations (0.3 mM), it was concluded that T-type channel block might explain its mechanism of action. Support for this hypothesis came from the following studies with related analogs: 1) T-type current block was also observed at therapeutically relevant concentrations of methyl-phenylsucinimide (MPS, the active metabolite of the antiepileptic drug methsuximide, and 2) no block was observed using either the inactive analog succinimide or the convulsant analog tetra-methylsuccinimide (87). With one exception (215), most studies have failed to confirm ethosuximide block of T-type currents at therapeutically relevant concentrations (403), leading to the suggestion that block of other ionic channels may be more relevant (232).

Ethosuximide and MPS are capable of blocking all three recombinant T-type Ca\textsuperscript{2+} channels (137). As observed with mibefradil, block is enhanced up to 10-fold by holding the membrane at potentials that partially inactivate T-type channels. In addition, ethosuximide has greater affinity for open than rested channels, suggesting that it is both a gating modifier and an open channel blocker. The effect of ethosuximide on other recombinant Ca\textsuperscript{2+} channels has not been rigorously tested, although block of Ca\textsubscript{2.3} (IC\textsubscript{50} = 20 mM) has been reported (295). In particular, its affinity for partially inactivated HVA channels has not been studied, so its selectivity is unknown. Studies with MPS suggest that it is only about twofold selective for LVA over HVA channels in thalamic neurons (87). The affinity (K\textsubscript{i}) of ethosuximide for inactivated states was estimated to be 2 mM. Because therapeutic plasma concentrations are close to 0.5 mM (57), only a fraction of channels is expected to be inhibited during therapy. This partial block may be relevant due to “pharmacological amplification” (297). This is due to the role of thalamic T-type channels in gating Na\textsuperscript{+} channels, such that nominal block of the T-type channel is sufficient to block all-or-none action potentials (178).

T-type channels are also blocked by antiepileptics that are thought to work by blocking Na\textsuperscript{+} channels. Perhaps the best studied is phenytoin, which clearly blocks...
both channels at therapeutic concentrations (413). Phenytoin appears to be selective for LVA over HVA currents (413). The mechanisms of block are also similar, with higher affinity for inactivated states as evidenced by its ability to shift the $h_{\infty}$ curve (413). From this shift it can be calculated that phenytoin blocks inactivated channels with a $K_i$ of 7 $\mu$M. Studies using recombinant T-type channels indicated that Ca$_{\text{v3.2}}$ was the most sensitive, although block was variable even in a clonal cell line, suggesting that additional factors may be important for block (404). Zonisamide has also been reported to partially block T-type channels (60% max, IC$_{50}$ 404). From this study was that T-type channel mRNA was increased result of this study was that T-type channel mRNA was increased in treatment with phenytoin (409). Consistent with the enhanced currents, enhanced expression of Ca$_{\text{v3.2}}$ was detected in the reticular thalamic nuclei, although the changes in current density (50%) were larger than the changes in channel mRNA (20%; Ref. 394). In addition, a small increase in Ca$_{\text{v3.1}}$ expression in the ventrobasal nucleus of thalamus was detected at the message level, but not with currents (151). Another interesting result of this study was that T-type channel mRNA was very similar, and in fact rose slightly, between juvenile (15 days old) and adult (>40 days old) rats. Enhanced expression of Ca$_{\text{v3.2}}$ mRNA was noted at both time points. Because the absence epilepsy phenotype of these animals is not manifested until after 30 days, these data suggest that maturation of the thalamocortical circuit plays a more important role in determining the onset than the intrinsic properties of these neurons (419, 431). Increased expression of T-type currents has also been found in hippocampal CA1 pyramidal neurons in three different models: a kindling model of focal epilepsy (112), a pilocarpine model of temporal lobe epilepsy (380), and an in vitro model (301). These currents, as well as intrinsic bursting of these neurons, could be blocked by 100 $\mu$M Ni$^{2+}$ (380), suggesting the involvement of Ca$_{\text{v3.2}}$ channels (230).

C. Anesthetics

Although the precise mechanism of action of general anesthetics is unknown, many studies have demonstrated that they are capable of modulating the activity of ion channels, including ligand-gated GABA and glycine channels. A newly described mechanism for inhalation anesthetics is that they hyperpolarize neurons by potentiating the activity of leak K$^+$ currents carried by the two-pore domain channels such as TASK-1 (371). Isoflurane, halothane, and nitrous oxide block DRG T-type currents at therapeutically relevant concentrations of anesthetics (401, 403). Isoflurane block of either DRG or recombinant Ca$_{\text{v3.1}}$ occurs at concentrations (271–303 $\mu$M) that are lower than those achieved during anesthesia (404). Block is not totally selective, as block of HVA currents by halothane occurs at similar millimolar concentrations (160, 390). In contrast, nitrous oxide selectively blocked DRG LVA currents with no effect on HVA currents (401). In addition, nitrous oxide was found to be a selective blocker of Ca$_{\text{v3.2}}$ currents, with little effect on Ca$_{\text{v3.1}}$ currents.

Octanol (20 $\mu$M), an aliphatic alcohol, was reported to block LVA currents totally from inferior olivary neurons (245). Subsequent studies required much higher concentrations for block (IC$_{50}$ ~200 $\mu$M; reviewed in Ref. 158). Recombinant T-type channels can be totally blocked by octanol, with 50% inhibition occurring at 160 $\mu$M for Ca$_{\text{v3.1}}$ and 219 $\mu$M for Ca$_{\text{v3.2}}$ (404). Studies on hippocampal CA1 pyramidal neurons indicated that octanol is non-selective, blocking T-, N-, and L-type channels to a similar extent. In conclusion, T-type currents are blocked at therapeutically relevant concentrations of some anesthetics, and this block may contribute to their anesthetic and analgesic properties.

D. Antipsychotics

Antipsychotics are used in the treatment of psychotic disorders such as schizophrenia, the main phase of manic-depressive illness, and other acute idiopathic psychotic illnesses (20). Most antipsychotics (neuroleptics) act by blocking D$_2$ class dopaminergic receptors, although drugs of diphenylbutylpiperidinede class can also block T-type channels.

Among diphenylbutylpiperidines, penfluridol was the most effective at blocking T-type currents of human medullary thyroid cancer (TT) cells (108). Penfluridol blocked with an IC$_{50}$ of 224 nM, and similar results were obtained with adrenal zona fasciculata cells (109). It was also ~10-fold more selective for LVA currents over HVA currents as 500 nM penfluridol inhibited 82% of the LVA current but only 20% of the HVA. Block of other channels has not been studied in detail, although it should be noted that these drugs are also potent blockers of K$^+$ channels (139) and bind with subnanomolar affinity to L-type channels (208). Despite strikingly different structures, thioridazine, clozapine, and haloperidol have comparable activity in TT cells, but are still much less active than fluspirilene or penfluridol (108). Similar results have been obtained using recombinant rat Ca$_{\text{v3.3}}$ channels, although the apparent affinity was higher than observed with native channels (349). Ca$_{\text{v3.1}}$ channels were blocked with the following order of potency (IC$_{50}$ in parentheses, in nM):
pimozide (43) > penfluridol (110) > flunarizine (530) > haloperidol (1,163). Similar results were obtained with Ca,3,2 and Ca,3,3, although flunarizine block of Ca,3,2 required sevenfold higher concentrations. These compounds all shifted the steady-state inactivation curve to more negative potentials, indicating that they bind preferentially to the inactivated state. Use-dependent block has been noted previously in studies of native channels, suggesting open channels may also have a higher affinity than rested channels (15, 108). Pimozide binding to D2 receptors occurs over the same concentration range (Kd = 29 nM) as block of the recombinant Ca,3 channels, suggesting that T-type channels may be blocked during therapy (349).

VI. CONCLUSIONS

A. Physiological Roles

The expression of T-type channels in diverse cell types suggests that they play a role in various physiological functions. The voltage dependence of activation and inactivation provides clues to these roles and places constraints on when these channels will be active. In neurons where the resting membrane potential is in the −90 to −70 mV range, T-type channels can play a secondary pacemaker role; an excitatory postsynaptic potential (EPSP) opens T-type channels and generates a LTS, which in turn activates Na+-dependent action potentials and HVA Ca2+ channels. Therefore, T-type channels play an important role in the genesis of burst firing. In neurons where the resting membrane potential is relatively depolarized (greater than −70 mV), T-type channels are inactivated and an EPSP directly activates Na+ channels. Due to their fast recovery from inactivation, T-type channels can produce a rebound burst in depolarized neurons following an IPSP. Electrophysiological recordings indicate that these channels are preferentially localized to dendrites, and hence play a role in signal amplification (97, 256, 265, 328). Large T-type currents have been found in thalamic neurons, where they play an important role in oscillatory behavior. The thalamus acts as a gateway to the cerebral cortex, and inappropriate oscillations of these circuits, or thalamocortical dysrhythmias, have been implicated in a wide range of neurological disorders (242). T-type currents also appear to play a role in olfaction, vision, and pain reception (201, 317, 402).

Calcium influx not only depolarizes the plasma membrane but also acts as a second messenger, leading to the activation of a plethora of enzyme and channel activities. T-type channels can cause robust increases in intracellular Ca2+, especially in proximal dendrites (294, 460). Calcium and voltage synergistically open Ca2+-activated K+ channels, which contribute to spike repolarization and afterhyperpolarizations (40, 244, 416). In addition to transient increases in intracellular Ca2+, T-type window currents may play an important role in slower increases in basal Ca2+. This property appears to be important for hormone secretion from adrenal cortex and pituitary, myoblast fusion (45), and possibly smooth muscle contraction.

B. Future Directions

Cloning of the T-type channel α1-subunits has provided tools with which to study the distribution of these channels. As expected, mRNA for these channels was found to be distributed throughout the central nervous system; however, it was very surprising to find expression in organs such as liver and kidney. Future studies are required to discern their role in these tissues. Such studies would be aided by the development of high-affinity antibodies. Antibodies would also be useful in studying the distribution of these channels within neurons and in their purification. HVA channels are multisubunit complexes; therefore, it seems likely that T-type channels will also have auxiliary subunits. These hypothetical subunits may regulate surface expression or might be involved in hormonal regulation of channel activity.

Because mutations in many ion channel genes have been linked to inherited diseases, it seems likely that mutations in T-type channel genes would lead to a phenotype. Mutations that lead to enhanced expression of channels, or that reduce inactivation, might tip the balance toward overexcitability. Enhanced expression of T-type channels have been observed in animal models of epilepsy (409), neuronal injury (79), cardiac hypertrophy (308), and heart failure (362). The opposite approach is also being tested by creating transgenic mice that lack expression of Ca,3 genes. Studies with the Ca,3.1 knock-out mouse have established the central role this isoform plays in generating burst firing and thalamic spike-wave discharges (206). What will be the phenotype of other Ca,3 knock-out mice?

Somewhat surprisingly the transcriptional regulation of Ca2+ channel subunits has received scant attention. Preliminary studies indicate that the Ca,3.1 promoter is methylated in some tumors, which presumably leads to decreased expression (408). It has been noted that the Ca,3.2 gene contains a region with high homology to the consensus sequence of neural restrictive silencer elements, providing another avenue for future research (342). Transcriptional regulation also appears to be important for hormonal upregulation of T channels (22, 447). Studies on gene regulation may also provide insights into the control of T-channel expression during the cell cycle (220).

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