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Low-Voltage-Activated ("T-Type") Calcium Channels in Review

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The past 5 years has witnessed an advance in our understanding of alpha1G ($Ca_v3.1$), alpha1H ($Ca_v3.2$), and alpha1l ($Ca_v3.3$), the pore-forming subunits of T-type or low-voltage-activated calcium channels (LVAs). LVAs differ in their localization and molecular, biophysical, and biochemical properties, but all conduct a transient calcium current in a variety of cells. T-type currents mediate a number of physiological functions in developing and mature cells, and are implicated in neural and cardiovascular diseases. Hampered by a lack of selective antagonists, characterization of T-type channels has come from recombinant channel studies and use of pharmacological and electrophysiological methods to isolate endogenous T-type currents. The surprising heterogeneity in T-type currents likely results from differences in LVA molecular composition, temporal and spatial localization, and association with modulatory molecules. A fundamental knowledge of LVA biochemical properties, including the molecular composition of endogenous LVAs and spatial and temporal characterization of protein expression, is necessary to elucidate mechanisms for regulation of expression and function in normal and diseased cells.

KEY WORDS: t-type; review; alpha1G ($Ca_v3.1$); alpha1H ($Ca_v3.2$); alpha1I ($Ca_v3.3$); development; epilepsy; cardiac; vascular.

INTRODUCTION

History of T-Type Currents

The study of low-voltage-activated (LVAs) voltage dependent calcium channels has a long and fascinating history that impacts on many fields in neuroscience and pharmacology. In 1975, Hagiwara *et al*. recorded two inward calcium currents from fertilized starfish eggs. The biophysical properties of one channel type ("channel I") had a number of novel properties, including activation and inactivation at more negative membrane potentials than other known channels. In contrast, the second channel ("channel II") was similar to previously characterized calcium channels in adult tissues (Hagiwara *et al.*, 1975). Six years later, Llinás *et al.* published the first in a series of detailed in vitro electrophysiological studies of inferior olive (Jahnsen and Llinás, 1982; Llinás and Yarom, 1981a,b) and thalamic nuclei (Jahnsen and Llinás, 1982, 1984a,b; Llinás and Jahnsen, 1982) that correctly anticipated the function of an unusual mammalian inward calcium current similar to Hagiwara's novel current. Recordings from inferior olivary nuclei in brainstem slices revealed a complex action potential waveform composed of a early fast sodium spike and a late high-threshold calcium spike, followed by an after-hyperpolarizing potential (Llinás and Yarom, 1981a). Unexpectedly, they discovered that the after-hyperpolarization activated a novel "low-threshold" calcium current. This current, which had similar biophysical and pharmacological properties to the low-threshold calcium current recorded in starfish eggs (Hagiwara *et al.*, 1975), was postulated to help generate rebound sodium spikes following after-hyperpolarizing potentials (Llinás

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and Yarom, 1981a). The presence of two distinct types of calcium-dependent spikes led Llinás and Yarom to propose that two types of calcium channels with different kinetics and cellular localization mediated the low- and high-threshold calcium spikes (Llinás and Yarom, 1981b). Finally, they hypothesized that sequential activation of specific ionic conductances resulted in sustained oscillatory membrane potentials (Llinás and Yarom, 1981a,b).

The elegant electrophysiological studies by Llinás *et al.* also demonstrated that low-threshold calcium currents were responsible for the generation of paradoxical calcium-dependent spikes ("low-threshold spikes" (LTS)) recorded when thalamic neurons were hyperpolarized to levels more negative than rest. Prior to these landmark studies, intracellular recordings had revealed that thalamic neurons could generate both repetitive and burst-firing modes of action potentials through undefined ionic mechanisms (Andersen *et al.*, 1964). Llinás *et al.* demonstrated that depolarizations of thalamic neurons positive to −60 mV resulted in repetitive firing via sodium-dependent action potentials ("tonic firing"). In contrast, depolarization of thalamic cells from voltages more negative than −65 mV produced a short burst of sodium action potentials ("rebound burst firing") resulting from activation of a rapidly inactivating calcium-dependent spike (Llinás and Jahnsen, 1982). Furthermore, the novel calcium currents recorded by Llinás *et al.* were proposed to serve as the ionic basis of "postanodal exaltation" that had been described in a variety of neurons, including spinal ganglia, motoneurons, and hippocampal neurons (Andersen *et al.*, 1964; Coombs *et al.*, 1959; Ito, 1957; Kandel and Spencer, 1961).

Cultures of dorsal root ganglion cells and cardiac myocytes were useful model systems to characterize the biophysical properties of the novel calcium currents that were inactivated by strong membrane depolarizations. These currents were activated and inactivated (desensitized) by unusually negative voltages positive to −70 mV ("LVAs") (Carbon and Lux, 1984a,b; reviewed in Huguenard, 1996). The currents exhibited transient kinetics with rapid inactivation and relatively slow deactivation ("T-type") (Nowycky *et al.*, 1985) that gave rise to characteristic tail currents after depolarizing test pulses (Matteson and Armstrong, 1986). Furthermore, the responsible channels had small unitary conductances (∼8 pS in isotonic barium chloride) and were insensitive to known organic calcium channel antagonists (Carbone and Lux, 1984a; Fedulova *et al.*, 1985; Nowycky *et al.*, 1985; Swandulla and Armstrong, 1988). These currents failed to exhibit metabolic rundown displayed by other calcium channels (Carbone and Lux, 1984a; Hagiwara *et al.*, 1975). Finally, T-type currents exhibited a distinctive voltage dependence of both activation and inactivation, resulting in a characteristic crossing over pattern of currents in response to progressively larger depolarization steps (Randall and Tsien, 1997; reviewed in Perez-Reyes, 1998).

Also named "I_{fast}" (Bean, 1985), "type I" (Hagiwara *et al.*, 1975; Narahashi *et al.*, 1987), "low-threshold inactivating (LTI)" (Kostyuk *et al.*, 1988), and "slowly deactivating" currents (Armstrong and Matteson, 1985), T-type currents appear to underlie different physiological processes than currents carried by other known voltage dependent calcium channels (VDCCs). Unlike neuronal P-/Q-type and N-type high-voltage-activated voltage dependent calcium channels (HVAs), most neuronal T-type currents do not appear directly coupled to neurotransmitter release (Kato *et al.*, 1992; Terrian *et al.*, 1989; Stephens *et al.*, 2001; Pfrieger *et al.*, 1992; but see Pan *et al.*, 2001, which indicates that T-type channels mediate neurotransmitter release from retinal bipolar cells). T-type currents are recorded from cardiac myocytes from some species (Bean, 1985; Hagiwara *et al.*, 1988; Nilius *et al.*, 1985), and gastrointestinal (Vivaudou *et al.*, 1988; Smirnov *et al.*, 1992a; Xiong *et al.*, 1993), bronchial (Janssen, 1997), vascular (Akaike *et al.*, 1989a; Ganitkevich and Isenberg, 1991; Kuga *et al.*, 1990), and uterine smooth muscle cells (Young *et al.*, 1993), although excitation–contraction coupling is mainly mediated by L-type high-voltage dependent calcium currents.

Physiological Functions of T-Type Currents

Neuronal Functions

A number of functions have been attributed to T-type channels in neurons, as robust T-type currents are recorded from central and peripheral neurons. One of the best known functions of T-type channels is mediation of neuronal rebound burst firing. While characterizing thalamic neurons, Andersen *et al.* (1964) noted that relay neurons displayed postanodal exaltation after administration of endogenous or exogenous inhibitory postsynaptic potentials (IPSPs). The underlying low-threshold calcium-mediated depolarization and accompanying sodium action potentials were suggested to be responsible for alpha rhythms (Andersen *et al.*, 1964; Llinás and Jahnsen, 1982) and spindle waves (reviewed in McCormic and Bal, 1997; Steriade and Deschênes, 1984). Spindle waves are important in a number of processes including sleep (Crunelli *et al.*, 1989; Hernandez-Crus and Pape, 1989; Steriade *et al.*, 1994; Suzuki and Rogawski, 1989) and epilepsy (see below). During transition from the awake state to drowsiness, thalamocortical

relay cells are hyperpolarized because of decreased excitatory inputs. This hyperpolarization removes T-type channels from the inactivated state ("deinactivation"), resulting in membrane depolarization and calcium spikes (McCormick and Bal, 1997; Steriade *et al.*, 1993a,b). If the membrane depolarizations are sufficiently large, opening of voltage-gated sodium channels results in bursts of sodium action potentials (Bean and McDonough, 1998). LTS have been recorded throughout the brain in such diverse regions as cortical pyramidal neurons (Giffin *et al.*, 1991; Hamill *et al.*, 1991), hippocampal interneurons (Fraser and MacVicar, 1991), thalamic relay and reticular neurons (Jahnsen and Llinás, 1984a,b), and inferior olive cells (Jahnsen and Llinás, 1982; Llinás and Yarom, 1981b, 1982), suggesting low-threshold spikes are important in normal neuronal processes.

T-type channels are hypothesized to underline neuronal pacemaker activity and oscillations in neuronal membrane potential, partial, partially due to the biophysical properties of T-type channels that promote regenerative low-threshold calcium spikes (reviewed in Huguenard, 1996). Thalamic relay cells generate rhythmic bursts of action potentials, resulting in 7–14 Hz spindle activity during non-REM sleep detected by electroencephalograms (EEGs) ("sleep spindles") (Steriade and Llinás, 1988), and 0.5–4 Hz bursts of action potentials (delta waves) (McCormick and Pape, 1990; Soltesz *et al.*, 2001). The cellular basis for this rhythm is due to both the intrinsic biophysical properties of T-type channels, and the physiological interaction of T-type currents with a "hyperpolarization-activated" mixed sodium and potassium slow inward current ("I_h") (McCormick and Huguenard, 1992; McCormick and Pape, 1990). This later current normally contributes to resting membrane potential, but is inactivated by membrane depolarization. Following depolarization, inactivation of T-type channels repolarizes the membrane, and a hyperpolarizing overshoot occurs. This hyperpolarization deinactivates T-type channels and activates I_h (reviewed in Lüthi and McCormick, 1998; McCormick and Bal, 1997). The I_h current slowly brings the resting membrane potential towards the I_h equilibrium potential of approximately -30 mV, resulting in reactivation of T-type channels and a low-threshold calcium spike (reviewed in McCormick and Bal, 1997), repeating the cycle. Therefore, the rate of repolarization of the membrane potential by I_h current is thought to control the rate of rhythmic oscillations (reviewed in Lüthi and McCormick, 1998). T-type channels are likely to control the rate of action potentials in brain regions other than thalamus. For example, blockade of T-type channels by mibefradil, a nonspecific T-type channel antagonist, slows the rate of action potential generation in Purkinje neurons by $\sim$30% (Raman and Bean, 1999), suggesting modulation of T-type channels may be a common mechanism for mediating neuronal excitability.

A final example of the importance of T-type channels in controlling membrane excitability occurs in thalamic reticular cells. Opening of T-type channels results in generation of a marked after-hyperpolarizing current conducted by apamin-sensitive calcium-activated potassium channels (Avanzini *et al.*, 1993; Bal and McCormick, 1993). This potassium current can be sufficient to deinactivate T-type channels, resulting in another low-threshold calcium spike. Therefore, physiological interactions with other gated ion channels coexpressed in the same cell with T-type channels can result in rhythmic membrane oscillations (reviewed in McCormick and Bal, 1997). Moreover, this functional coupling may occur spontaneously in cell culture (Gu and Spitzer, 2001; Gutnick and Yarom, 1989). Taken together, these examples illustrate the importance of T-type channels in gating or controlling the activity of other voltage- and calcium-dependent ion channels (Llinás and Yarom, 1981b; White *et al.*, 1989; reviewed in Huguenard, 1996).

Because of the biophysical properties of activation and deactivation, T-type channels can also open in response to *depolarizing* membrane potentials depending on the resting membrane potential with respect to the inactivation curve of T-type channels. For example, depolarization of dorsal root ganglion neurons can generate an after-depolarizing potential that triggers a short burst of action potentials, potentially contributing significantly to intracellular calcium levels (White *et al.*, 1989). Voltage-clamp experiments of dorsal root ganglion neurons reveal that T-type current is the major source for calcium entry during the repolarization phase of brief action potentials (McCobb and Beam, 1991). In experiments carried out several years later, data resulting from genetic manipulation of T-type channel expression supported this conclusion. Antisense oligonucleotides against a conserved region found in alpha1G, alpha1H, and alpha1I transcripts attenuated T-type current in nodose ganglion neurons, and action potentials recorded from these neurons had a faster repolarization phase than cells transfected with a scrambled oligonucleotide (Lambert *et al.*, 1998). In contrast, overexpression of human alpha1G in HEK-293 cells results in sustained calcium influx during repolarization in response to action potential waveforms (Monteil *et al.*, 200a). These results imply that T-type currents control the action potential shape and promote calcium entry during membrane repolarization (Lambert *et al.*, 1998; McCobb and Beam, 1991; Monteil *et al.*, 2000a), a mechanism that allows for substantial calcium entry during short action potentials.

T-type currents also may strengthen local synaptic potentials and modulate synaptic efficacy. Both T-type currents and alpha1 LVA mRNA (discussed below) are localized to dendrites in many brain regions, and depolarizing dendritic synaptic potentials open T-type channels (Magee and Johnston, 1995; Sutor and Zieglgansberger, 1987). Opening of these channels may permit a localized increase in intracellular dendritic calcium, thus permitting integration of subthreshold synaptic inputs within a localized region (reviewed in Ghosh and Greenberg, 1995). Finally, T-type currents may underlie after-deplorizing potentials that occur after single action potentials in some neurons (Komatsu and Iwakiri, 1992; White *et al.*, 1989; Zhang *et al.*, 1993b), and mediate input signal amplification in some neurons (Williams *et al.*, 1997).

Nonneuronal Functions

T-type channels have a number of functions in nonneuronal tissues. The low voltage of activation of cardiac T-type channels permits substantial inward current during the late phase of diastole, potentially contributing to action potential initiation in pacemaker cells. Small concentrations of the nonspecific T-type channel antagonist nickel slow the beating rate of sinoatrial nodal and atrial pacemaker cells without attenuating L-type currents (Hagiwara *et al.*, 1988; Zhou and Lipsius, 1994), supporting the hypothesis that T-type channels underlie pacemaker activity. T-type currents may be involved in smooth muscle contraction, as vascular, bronchial, gastrointestinal, and uterine smooth muscle cells express T-type currents (Akaike *et al.*, 1989a; Friedman and Gutnick, 1987; Janssen, 1997; Xiong *et al.*, 1993; Young *et al.*, 1993). Furthermore, secretions from pituitary (Matteson and Armstrong, 1986), pancreatic beta (Zhuang *et al.*, 2000), and adrenal zona fasciculata, chromaffin, and glomerulosa cells (Barrett *et al.*, 1991a; Cohen *et al.*, 1988; Enyeart *et al.*, 1992; Mlinar *et al.*, 1993; Roussier *et al.*, 1993; Schrier *et al.*, 2001) requires T-type channel currents. However, this calcium-dependent secretion may depend not on a bolus of calcium resulting from membrane depolarization (as in synaptic vesicle release), but on a steady influx of calcium near resting membrane potential (Cohen *et al.*, 1988).

In addition to excitable and neuroendocrine cells, T-type channels may mediate steady calcium influx near resting membrane potentials in nonexcitable cells such as fibroblasts (Chen *et al.*, 1988; Cohen *et al.*, 1988) through the existence of a window current that occurs when channels are not fully inactivated (Perez-Reyes *et al.*, 1999). Although there is a low probability of T-type channel opening at these negative voltages, a strong driving force for calcium may result in substantial calcium entry. Finally, adhesion of sperm to the zona pellucida of oocytes opens VDCCs, and the resulting increased intracellular calcium is sufficient to trigger the sperm acrosome reaction. Currents recorded from spermatogenic cells display T-type channel activation and inactivation properties (Arnoult *et al.*, 1996, 1997; Hagiwara and Kawa, 1984; Liévano *et al.*, 1996), suggesting T-type channels are important mediators of fertilization.

Heterogeneity in Endogenous T-Type Currents

One of the first indications of VDCC diversity came from electrophysiological recordings of endogenous voltage dependent calcium currents, as currents had unique voltages of activation, inactivation, and deactivation and kinetic profiles. Comparisons of biophysical properties of calcium currents recorded either in the same neuron or under similar recording conditions lead to the conclusion that neurons conduct at least five major types of voltage dependent calcium currents (Nowycky *et al.*, 1985; reviewed in Catterall, 2000; Randall, 1998). Similarly, comparisons of T-type currents recorded from different neurons revealed an underlying heterogeneity in T-type currents (Matteson and Armstrong, 1986). Although some of the heterogeneity was attributed to differences in recording conditions, comparisons of channels recorded under the same conditions revealed biophysical differences. For example, the membrane potential at which T-type current is half-activated ("$V_{1/2}$") varies among cell types, although activation properties are recorded with the same conditions (Huguenard *et al.*, 1993; Huguenard and Prince, 1992). Neurons also differ markedly in their activation and inactivation kinetics and the voltage sensitivity of inactivation of T-type currents (Huguenard *et al.*, 1993; Huguenard and Prince, 1992; reviewed in Huguenard, 1996). These observations lead to one of the first suggestions for subtypes of T-type current: "$I(T_s)$," a slowly inactivating T-type current, such as that found in thalamic reticular inhibitory neurons, and "$I(T)$," a faster activating and inactivating T-type current, such as that recorded from thalamocortical relay cells (Huguenard and Prince, 1992). The variations in the voltage dependent biophysical properties of activation, deactivation, and inactivation of T-type currents have a profound impact in determining the rate and length of calcium entry and the total amount of calcium, thus shaping each neuron's physiological response to a given stimulus. Analysis of biophysical properties (such as inactivation kinetics) of other neurons including a dorsal root ganglion cell line (ND7–23) (Kobrinsky *et al.*, 1994), hippocampal CA3 pyramidal neurons (Avery and Johnston, 1996), and laterodorsal thalamic nuclei (Tarasenko *et al.*, 1997) also suggested that different types of voltage dependent calcium channels underlie T-type currents.

The use of pharmacological antagonists also provided early evidence for multiple types of LVAs. T-type currents recorded from different tissues such as aorta, skeletal muscle, and brain had different rank-order sensitivity to antagonism by cations and organic channel antagonists (Akaike *et al.*, 1989a,b; Satoh *et al.*, 1991; Takahashi and Akaike, 1991; reviewed in Yunker, 2003), suggesting that different channels could conduct current with similar biophysical characteristics. Taken together, these data provided compelling evidence for the presence of marked heterogeneity within a single type of voltage dependent calcium current.

Identification of Ca_v3 Alpha1 Subunits

In 1998, Perez-Reyes *et al.* published the first in a series of studies that ultimately described the cloning of rat alpha1G (Perez-Reyes *et al.*, 1998), alpha1H (Cribbs *et al.*, 1998), and alpha1I (Lee *et al.*, 1999), thus providing the foundation for detailed functional and biochemical characterization of the family of molecules that conduct T-type currents. Prior to cloning of alpha1G, all known HVA VDCCs were minimally composed of a pore-forming alpha1 subunit composed of four repeated domains (I–IV) predicted to each contain six transmembrane segments (S1–S6) that were evolutionarily related to both voltage-gated sodium and potassium channels. However, more traditional attempts to clone T-type channels by homology-based low-stringency hybridization and polymerase chain reaction screening techniques were unrequited. The Perez-Reyes group used a text-based search of partially sequenced cDNA fragments cloned from normalized cDNA libraries (expressed sequence tags, ESTs) to identify sequences with homology to known alpha1 voltage dependent calcium channel subunits. The initial identification and sequencing of an EST (clone H06096; GenBank no. AF029228) with low homology to a known alpha1 subunit proved to be critical, as this sequence had conserved structural motifs including a putative voltage sensor and a pore-forming (P) loop, but low homology to other cloned voltage dependent calcium channels (Perez-Reyes *et al.*, 1998). When a fragment of this clone was used to screen a rat brain library, a full-length cDNA for alpha1G (GenBank no. AF027984) ($Ca_vT.1$, Perez-Reyes *et al.*, 1998); $Ca_v3.1$, Ertel *et al.*, 2000)) (Table I) was obtained and found to be expressed

Table I. Summary of Low-Voltage-Activated Voltage Dependent Calcium Channel Complete mRNA Sequences to Date

Splice variant, species, tissue of origin	GenBank No.	Predicted length of open reading frame (in amino acids); chromosome location		Ref.
Alpha1G ($Ca_v3.1$) (*cacna1g*)				
3.1a,[a] rat brain	AF027984	2254		Perez-Reyes *et al.* (1998)
	AF290212	2254		McRory *et al.* (2001)
3.1bce, rat pancreas beta cells	AF125161	2288		Zhuang *et al.* (2000)
3.1ace, mouse cerebellum	AJ012569	2295	11	Klugbauer *et al.* (1999)
3.1a, human	AF126966[b]	2250		Monteil *et al.* (2000a)
	AF134985	2171		Mittman *et al.* (1999a)
	AF190860	2250	17q22	Cribbs *et al.* (2000)
3.1b, human	AF126965[c]	2243		Monteil *et al.* (2000a)
	AF134986	2374		Mittman *et al.* (1999a)
Alpha1H ($Ca_v3.2$) (*cacna1h*)				
3.2, rat brain	AF290213	2359		McRory *et al.* (2001)
3.2, mouse	NM021415	2365	17	Mittman *et al.* (unpublished)
3.2, human brain	AF051946	2353	16p13.3	Cribbs *et al.* (1998)
	AF073931	2353		Williams *et al.* (1999)
alpha1I ($Ca_v3.3$) (*cacna1i*)				
3.3, rat brain	AF086827	1835		Lee *et al.* (1999)
	AF290214	1834		McRory *et al.* (2001)
3.3, mouse	N/A[d]			
3.3, human brain	AF129133	2016	22q12.3–q13.2	Mittman *et al.* (1999b)
	AF211189	1981		Monteil *et al.* (2000b)
				Gomora *et al.* (2001)

[a] Refer to text for discussion of isoform nomenclature.

[b] Sequences AF227744-AF227746 represent alpha1G splice variants of the human "a" isoform (AF126966) that contain insert e (AF227744), insert f (AF227746), or both e and f (AF227745) (Monteil *et al.*, 2000a).

[c] Sequences AF227747-AF227751 encode alpha1G splice variants of the human "b" isoform resulting from an alternative 5'-splice donor site in exon 25 and acceptor site in exon 27 (AF126965) (Monteil *et al.*, 2000a). Sequence AF134986 harbors, c, d, e, and f inserts (Mittman *et al.*, 1999a).

[d] Not available at this time.

in organisms as diverse as *C. elgans* and human. Heterologous expression of the alpha1G cDNA in *Xenopus* oocytes resulted in currents with similar biophysical properties to those of previously reported endogenous T-type currents (Perez-Reyes *et al*., 1998), providing compelling evidence that the first subunit of the elusive LVA family had been identified.

The original EST clone was also crucial to clone alpha1H ($Ca_v3.2$) (Table I), the second member of the T-type family of VDCCs. When used to screen a human heart cDNA library, expression of the resulting alpha1H cDNA (GenBank no. AF051946, Cribbs *et al.*, 1998) gave rise to barium currents with similar kinetics to both endogenous T-type currents and those currents recorded from oocytes expressing recombinant alpha1G (Perez-Reyes *et al.*, 1998). However, the predicted protein sequence was only 57% identical to alpha1G and had a number of molecular differences when compared to alpha1G, suggesting a novel second LVA family member. Subsequent screening of a rat brain library with a probe derived from a human EST clone homologous to alpha1G (clone 50902; GenBank no. H19230) resulted in alpha1I ($Ca_v3.3$) cDNA (GenBank no. AF086827) (Lee *et al.*, 1999). Heterologous expression of alpha1I yielded barium currents with many characteristics of T-type channels, including activation and inactivation at negative voltages, and slow deactivation upon hyperpolarization. However, the rate of inactivation was approximately 10-fold slower than currents conducted by either alpha1G or alpha1H depending on the expression system (Lee *et al.*, 1999; McRory *et al.*, 2001). As the alpha1I sequence is homologous to both alpha1G and alpha1H (Lee *et al.*, 1999), these electrophysiological results suggested at least some observed heterogeneity observed in endogenous T-type currents (Coulter *et al.*, 1989a; Huguenard and McCormick, 1992; Huguennard and Prince, 1992) results from differences in the molecular identity of the channel pore.

MOLECULAR STRUCTURE OF LVA ALPHA1 (CA_V3) SUBUNITS

Molecular Analysis of Recombinant LVA Alpha1 (Ca_v3) Subunit Sequences

Analysis of the predicted protein sequences and structure of alpha1G, alpha1H, and alpha1I reveals similarities to alpha1 subunits of HVAs. Each LVA alpha1 subunit is composed of four repeated domains (I–IV) that each contains six transmembrane segments (S1–S6). The greatest sequence identity both among LVA alpha1 subunits and between HVA and LVA alpha1 subunits occurs in the predicted membrane-spanning segments (S1–S6) in each domain, including S4, the predicted voltage sensor of activation. Common to both LVA and HVA alpha1 subunits, the S4 region contains postively charged amino acid residues every three or four residues that are thought to rotate in response to changes in the electric field, thus initiating conformational changes to open the calcium pore (reviewed in Jones, 1998). The pore-lining hydrophobic sequence ("P loop") connecting S5 to S6 in each of the four domains of an alpha1 subunit is postulated to come together to form the pore and selectivity filter of the VDCC (Lipkind and Fozzard, 2001; reviewed in Catterall, 2000; Hering *et al.*, 2000). The selectivity of HVA channels for calcium depends on the presence of a ring of four negatively charged glutamate residues ("EEEE locus") to provide high-affinity calcium binding (reviewed in Talavera *et al.*, 2001). Generation of a systematic set of mutations to the EEEE locus eliminates high-affinity calcium binding (reviewed in Cibulsky and Sather, 2000). This results suggests that each glutamate interacts differently with the permeating ions, possibly indicating an asymmetrical arrangement of the four pore-forming regions of the channel (reviewed in Cibulsky and Sather, 2000). In contrast to HVA alpha1 subunits, aspartate replaces glutamate in the P loop of domains III and IV in alpha1G, alpha1H, and alpha1I (Cribbs *et al.*, 1998; Lee *et al.*, 1999; Perez-Reyes *et al.*, 1988). Interestingly glutamate in domain III has the strongest influence on calcium selectivity in HVAs (Yang *et al.*, 1993), and mutation of aspartate to glutamate in domain III of LVAs produces characteristic features usually observed in HVAs (Talavera *et al.*, 2001). Therefore, the presence of aspartate residues substituted for glutamate in the P loop of domains III and IV of LVAs maintains the ring of negative charges, but appears to alter properties of the current, including ion selectivity and permeability of LVAs. This may help to explain the observation of an equimolar ratio of barium to calcium peak current for most LVA channels, but a ratio of $\sim$2 for most HVA currents (Randall, 1998). Finally, replacement of two glutamates in P loops of domain II and IV with glutamine significantly alters steady-state inactivation (Hockerman *et al.*, 1997). In contrast, replacement of aspartate residues with glutamate in alpha1G modifies activation (Talavera *et al.*, 2001), indicating multiple roles for this calcium channel locus.

Although there is high sequence homology between LVA and HVA alpha1 subunits in the transmembrane regions, LVA alpha1 subunits are less than 60% identical to each other overall and less than 20% identical to HVA alpha1 subunits (Lee *et al.*, 1999). The regions of greatest diversity occur in the sequences that link transmembrane segments within a domain and especially in intracellular

loops that connect the domains (Cribbs *et al.*, 1998; Lee *et al.*, 1999; Perez-Reyes *et al.*, 1998). These regions are of special interest, because a number of intracellular molecules can interact with VDCCs to modulate their activity (reviewed in Catterall, 2000). For example, LVA alpha1 subunits lack a full alpha interacting domain (AID) consensus sequence for binding intracellular beta VDCC auxiliary subunits (QQ-E-D/EL-GY—WI—E; Walker and DeWaard, 1998) in the I–II intracellular loop (Cribbs *et al.*, 1998; Lee *et al.*, 1999; Pragnell *et al.*, 1994; Perez-Reyes *et al.*, 1998; see below). Thus far, sequence analysis of LVA alpha1 subunits has failed to reveal binding sites for synaptic vesicles ("synprint site," short for *syn*aptic *pro*tein *int*eraction site (Rettig *et al.*, 1996)) in the II–III loop of LVA alpha1 subunits, although such sites have been identified in alpha1 subunits of both Ca_v1 and Ca_v2 subclasses of HVA (reviewed in Catterall, 2000). Finally, cloned alpha1H, but not alpha1G or alpha1I, contains a consensus site for G protein binding (QXXER) in the I–II intracellular loop (De Waard *et al.*, 1997; Herlitze *et al.*, 1997; Page *et al.*, 1997; Williams *et al.*, 1999; Zamponi *et al.*, 1997). Beta-gamma G protein subunits bind to the C- and N-termini of some HVA (Canti *et al.*, 1999; Furukawa *et al.*, 1998; Page *et al.*, 1997; Qin *et al.*, 1997; Simen and Miller, 1998, 2000) to modulate channel activity. Functional studies are needed to determine if G proteins can bind to LVA alpha1 subunits to affect the activity of T-type channels (see Yunker, 2003).

Marked sequence differences are also observed in the N- and C-termini of alpha1G, alpha1H, and alpha1I when compared to each other or to the HVA subfamily. Although the functional significance is not yet known, differences in splice variants of LVA alpha1 subunit carboxyl termini may be important in membrane targeting, channel stability, and/or channel modulation. For example, some HVA alpha1 subunits contain a second beta interaction site in the carboxyl terminus (Birnbaumer *et al.*, 1998; Tareilus *et al.*, 1997; Walker *et al.*, 1998). Therefore, sequence differences in LVA alpha1 subunits may result in unique interactions of LVA alpha1 subunits with either known or currently unknown auxiliary subunits (see below). Finally, the carboxyl terminus is also important in calcium-dependent regulation of biophysical properties of some VDCCs (De Leon *et al.*, 1995). In contrast to HVA VDCCs, the carboxyl terminus of LVA alpha1 subunits is thought to lack both an EF hand region (Babitch, 1990; Perez-Reyes, 1998) and a calmodulin binding site (Lee *et al.*, 1999; Peterson *et al.*, 1999), consistent with the lack of reported calcium modulation of recombinant T-type channels (but see Klöckner *et al.*, 1999; reviewed in Yunker, 2003). In summary, the differences between LVA and HVA alpha1 subunit primary sequences suggest an early evolutionary divergence between the two families of voltage dependent calcium channels that may be responsible for coupling of different physiological processes to unique subfamilies of VDCCs. However, LVA alpha1 subunits appear conserved throughout evolution, consistent with the varied and crucial physiological functions ascribed to the T-type currents they mediate.

Functional Evidence for Auxiliary Subunits Associated With LVA Alpha1 (Ca_v3) Subunits

Biochemical purification of HVA channels reveals that calcium channels are composed of at least three subunits in a postulated equal stoichiometry of one pore-forming alpha1 to one auxiliary beta and one alpha2-delta subunit (Curtis and Catterall, 1984; Hosey *et al.*, 1987; Liu *et al.*, 1996; Martin-Moutot *et al.*, 1995, 1996; McEnery *et al.*, 1991; Witcher *et al.*, 1993). Recently our lab (Sharp *et al.*, 2001) and others (Kang *et al.*, 2001) have demonstrated that a transmembrane gamma auxiliary subunit is physically associated with at least some HVAs. Whereas alpha1 subunits confer the channel pore and electrophysiological and pharmacological properties, auxiliary subunits can modulate intracellular trafficking, assembly, and transmembrane incorporation of functional channels (Brice *et al.*, 1997; Chien *et al.*, 1995; reviewed in McEnery *et al.*, 1998a). Electrophysiological properties such as activation and inactivation can also be affected by association of alpha1 subunits with auxiliary subunits (reviewed in Jones, 1998; Qin *et al.*, 1998).

Beta Subunits

Although endogenous LVAs have yet to be purified, cloned alpha1G, alpha1H, and alpha1I lack full AID consensus sites in the I–II intracellular loops, as described above, suggesting they do not bind intracellular beta subunits. Four genes encoding beta subunit isoforms have been identified (reviewed in Birnbaumer *et al.*, 1998). Beta subunit expression strongly modulates recombinant HVA channel activity, resulting in marked alterations in current amplitude and kinetics (Neely *et al.*, 1993; Stea *et al.*, 1995). Beta subunits play crucial roles in anatomical and functional expression of HVAs in vivo, as targeted gene disruption and spontaneous mutations in genes encoding beta subunits result in altered calcium currents through HVA channels (reviewed in Freise *et al.*, 1999). Functional evidence for a lack of beta subunit function on T-type currents comes from targeted loss of either a single beta subunit (Gregg *et al.*, 2001; Strube *et al.*, 1996)

or antisense inhibition of beta expression in nodose ganglion neurons (Lambert *et al.*, 1997), and differentiated NG 108-15 neuroblastoma glioma cells (Leuranguer *et al.*, 1998). Whereas both N- and L-type HVA currents are attenuated following antisense inhibition of beta expression, T-type currents are not affected, implying that beta subunits are not necessary for either expression or function of T-type channels (Leuranguer *et al.*, 1998). As anticipated, overexpression of beta1B does not influence the electrophysiological properties of T-type currents in undifferentiated NG108-15 cells (Wyatt *et al.*, 1998). Finally, unlike heterologous expression of HVA alpha1 subunits that require cotransfection of auxiliary subunits for functional expression, transfection of only alpha1G, alpha1H, or alpha1I cDNA is necessary to obtain T-type currents in *Xenopus oocytes* (Cribbs *et al.*, 1998; Lee *et al.*, 1999; Perez-Reyes *et al.*, 1998). However, it should be noted that many cell types, including *Xenopus oocytes* express endogenous auxiliary subunits (Dolphin *et al.*, 1999) that may be sufficient to support heterologous alpha1 expression. This hypothesis is supported by functional studies of recombinant alpha1I, as the channel displayed strikingly different biophysical properties when expressed in oocytes compared to HEK-293 cells (Lee *et al.*, 1999).

A few intriguing functional studies have provided contradictory evidence that suggests beta subunits may modulate current conducted by alpha1 LVA subunits. Transfection of either beta2 or beta4 cDNA slows endogenous T-type current in *Xenopus oocytes* (Lacerda *et al.*, 1994), and exogenous beta2 expression induces a sustained T-type current in NG 108-15 cells (Wyatt *et al.*, 1998). Furthermore, coexpression of alpha1G with either beta1b or beta3 significantly potentiates alpha1G current density in COS-7 cells (Dolphin *et al.*, 1999). When expressed with either beta3 or beta4, both the membrane potential at which T-type current is half-activated and the steady-state inactivation of alpha1G is shifted to more depolarizing potentials (Dolphin *et al.*, 1999). These results suggest there may be functional interactions between alpha1G and auxiliary beta subunits in some cells. Alternatively, introduction of beta subunits may act to compete for another protein which itself modulates T-type currents. Although it is unclear if these biophysical changes result from a physical interaction of alpha1G with auxiliary beta proteins, it is intriguing that beta subunits tend to shift the voltage dependence of activation and steady-state inactivation to more hyperpolarized values for HVA channels, contrary to the reported biophysical alterations of the channel formed by alpha1G (Dolphin *et al.*, 1999). If beta subunits physically interact with LVA alpha1 subunits, it will be interesting to determine if LVA alpha1 subunits preferentially interact with certain beta subunits, similar to HVA alpha1 subunits that display differential association with beta subunit isoforms (Vance *et al.*, 1998; reviewed in Birnbaumer *et al.*, 1998).

Alpha-2/Delta Subunits

Functional analysis of heterologously coexpressed LVA alpha1 and alpha2/delta isoforms raises the possibility that alpha1G and other LVA alpha1 subunits may physically interact with alpha2/delta. Alpha2/delta is a highly glycosylated disulfide-linked protein that results from posttranslational cleavage of a single gene product (De Jongh *et al.*, 1990; Jay *et al.*, 1991). Overexpression of alpha2/delta modulates both activation and inactivation of T-type channels in undifferentiated NG108-15 cells (Wyatt *et al.*, 1998). Coexpression of alpha2/delta2 and alpha1G in COS-7 cells potentiates alpha1G localization to plasma membranes, although alpha1G is incorporated into plasma membranes in the absence of this auxiliary subunit (Dolphin *et al.*, 1999). Coexpression also increases alpha1G-mediated current density in COS-7 cells (Dolphin *et al.*, 1999), *Xenopus oocytes* (Gao *et al.*, 2000), and HEK-293 cells (Klugbauer *et al.*, 2000). However, differences in the cellular constituents may differ among these cell types, as expression of alpha2/delta2 shifts the steady-state inactivation curves toward more positive potentials in HEK-293 cells, but not in other cell types (Dolphin *et al.*, 1999; Klugbauer *et al.*, 2000). Similar results were not obtained when alpha1G was coexpressed with delta, suggesting that the alpha2 portion of alpha2/delta is both sufficient and necessary for increased functional alpha1G in the plasma membrane. Finally, the physiological modulation of T-type current by alpha2-delta appears dependent on the molecular identity of the alpha2-delta isoform. Changes in the biophysical parameters of current conducted by alpha1G in HEK-293 cells are not observed when alpha1G is cotransfected with either alpha2/delta1 or alpha2/delta3 (Lacinová *et al.*, 1999). The site of physical interaction between alpha1G and alpha2/delta has not been identified at this time, but it should be noted that early structural studies suggest that alpha2 can interact with transmembrane domain III of alpha1S HVA (Gurnett *et al.*, 1996).

Gamma Subunits

There is a current paucity of information regarding either the physical or physiological interaction of transmembrane gamma subunits with LVA alpha1 subunits. The gamma auxiliary subunit was first identified from purification of skeletal muscle L-type channels (Curtis and Catterall, 1984) and the first neuronal gamma isoform was

revealed by analysis of *stargazer* epileptic mice (Letts *et al.*, 1998). At least eight gamma isoforms have been cloned (Black and Lennon, 1999; Burgess *et al.*, 2001; Klugbauer *et al.*, 2000), and gamma isoforms have a differential distribution in the brain (Burgess *et al.*, 2001; Klugbauer *et al.*, 2000; Sharp *et al.*, 2001). Transfection of HEK-293 cells with a combination of alpha1G and various gamma isoforms reveals that the presence of some gamma isoforms can alter the biophysical properties of recombinant alpha1G channels depending on the stimulation parameters (Klugbauer *et al.*, 2000). For example, the membrane potential at which recombinant alpha1G-mediated barium current is half-inactivated is slightly depolarized in the presence of exogenous gamma-4, but not in the presence of gamma-2 or gamma-5 (Klugbauer *et al.*, 2000). In contrast, slowing of the speed of recovery from voltage dependent inactivation occurs with coexpression of either gamma-2 or gamma-4 (Klugbauer *et al.*, 2000). Gamma-5 alone does not affect any measured biophysical properties on alpha1G-mediated current but can modulate T-type currents when coexpressed with alpha2 (Klugbauer *et al.*, 2000). However, gamma-5 significantly accelerates both the time course of activation and inactivation when individual currents are analyzed over a wide range of voltages (Klugbauer *et al.*, 2000), thus providing a tantalizing suggestion that the recently discovered gamma family may be important modulators of at least some LVAs.

The functional relationship between gamma isoforms and alpha1I has also been examined. Neither the voltage dependence of activation or inactivation is altered when human gamma-1, gamma-2, gamma-3, or gamma-4 isoforms are expressed with recombinant alpha1I channels in stably-transfected HEK-293 cells (Green *et al.*, 2001). However, gamma-2, but not gamma-3 or gamma-4, significantly slows deactivation of alpha1I over a wide range of voltages (Green *et al.*, 2001). As gamma-2 protein is widely expressed in the brain (Sharp *et al.*, 2001) and overlaps the distribution pattern of alpha1I mRNA (Talley *et al.*, 1999), it is intriguing to speculate that coexpression of gamma subunits in at least some neurons may modulate T-type currents.

LVA ALPHA1 (CA_V3) SUBUNIT HETEROGENEITY

Different Molecular Subunits Underlie T-Type Currents

Multiple levels of complexity contribute to the reported heterogeneity of native T-type channels, and differential expression of alpha1 subunit genes accounts for the most basic source of channel diversity. Electrophysiological analyses of recombinant channels and the discovery of highly potent and selective antagonists have conclusively demonstrated that unique pore-forming subunits underlie each of the major calcium currents (reviewed in Catterall, 2000; Jones, 1998; Randall, 1998). At least three lines of evidence indicate that T-type calcium channels are formed by alpha1G, alpha1H, and alpha1I. As discussed in the previous sections, expression of recombinant alpha1G, alpha1H, or alpha1I is sufficient to generate T-type currents in either *Xenopus oocytes* or HEK-293 cells (Cribbs *et al.*, 1998; Lee *et al.*, 1999; Perez-Reyes *et al.*, 1998). Attenuation of alpha1G, alpha1H, or alpha1I mRNA expression by the use of antisense oligonucleotides selectively inhibits T-type current in isolated nodose ganglion neurons, indicating that the cloned Ca_v3 family of alpha1G, alpha1H, and alpha1I can underlie endogenous T-type currents (Lambert *et al.*, 1998). Finally, disruption of *cacna1g* by homologous recombination decreases the density of T-type calcium currents (Kim *et al.*, 2001), further supporting the conclusion that T-type currents are conducted by LVA alpha1 subunits in vivo.

Surprisingly, additional molecules may form T-type channels. Alpha1B ($Ca_v2.2$), alpha1C ($Ca_v1.2$), and alpha1E ($Ca_v2.3$) normally form HVA subunits that conduct N-type, L-type, and R-type currents, respectively (reviewed in Burgess *et al.*, 1997; Catterall, 2000; Randall, 1998). In the absence of detectable beta, alpha2-delta, and gamma auxiliary subunits, these alpha1 subunits may form small-conductance channels that conduct T-type-like currents in COS7 cells (Meir and Dolphin, 1998; reviewed in Bean and McDonough, 1998). It is currently unknown if this mechanism is used to form functional channels in vivo, although it is tempting to speculate such channels may be expressed during dynamic periods of development or dysfunction.

Finally, it should be noted that alpha1E shares properties with both HVA and LVA subfamilies. Both alpha1E channels and T-type channels inactivate at negative potentials and are blocked by low concentrations of nickel (12–30 μM IC50 for recombinant alpha1E) (Soong *et al.*, 1993; Williams *et al.*, 1994). In contrast, recombinant alpha1E channels have fast deactivation and much larger single-channel conductances in comparison to T-type channels which deactivate slowly and have small single channel conductances in barium. These data suggest that channels formed by alpha1E are more similar to R-type channels found in cerebellar granule cells (Randall, 1998; Williams *et al.*, 1994; Zhang *et al.*, 1993a). Comparison of either endogenous T-type currents (Randall and Tsien, 1997) or recombinant alpha1E channels to recombinant alpha1G (Perez-Reyes *et al.*, 1998), alpha1H, or alpha1I (Lee *et al.*,

1999) channels reveal marked differences, indicating that alpha1E does not normally conduct T-type currents.

The Molecular Pore Identity Contributes to Biophysical Differences in T-Type Current

Functional studies on recombinant T-type channels reveal that differences in the molecular identity of the pore confer unique biophysical and pharmacological properties to the resulting current (see Yunker, 2003). Comparison of alpha1G, alpha1H, and alpha1I recombinant LVA channels stably expressed in HEK-293 cells without exogenous auxiliary subunits indicates that each recombinant T-type channel has unique kinetics and voltage dependence of activation (alpha1G $\geq$ alpha1H $\gg$ alpha1I), inactivation (alpha1G $\geq$ alpha1H $\gg$ alpha1I), and deactivation (alpha1I $>$ alpha1H $\geq$ alpha1G) depending on the identity of the pore-forming subunit (Klöckner *et al.*, 1999; Kozlov *et al.*, 1999). Furthermore, LVA alpha1 subunits differ in their modulation by calcium (Klöckner *et al.*, 1999), and in their ability to display facilitation (Klöckner *et al.*, 1999; Kozlov *et al.*, 1999). However, it should be noted that although there are many similarities among the currents conducted by Cav3 alpha1 subunits, T-type channels are not probably interchangeable with each other, as small shifts in either the amplitude or voltage dependence of T-type channels can have a large effect on physiological processes, such as generation of rhythmic LTS (Destexhe *et al.*, 1993; McCormick and Huguenard, 1992). In summary, although recombinant LVA alpha1 subunits differ in their biophysical characteristics, the current lack of selective antagonists prevents positive identification of the molecules responsible for endogenous T-type current generation. Therefore, differences between neuronal types may result from differences in the molecular identity of the underlying pore. Alternatively, biophysical differences in endogenous currents may also arise because of alternative splicing of a single transcript, posttranslational modifications, or modulation by intracellular molecules such as auxiliary subunits (see above) and second messengers (see Yunker, 2003).

Alternative Splicing of LVA Alpha1 Subunits May Contribute to Heterogeneity

The initial findings that the skeletal muscle alpha1 subunit existed as multiple isoforms (De Jongh *et al.*, 1989, 1991) served as an accurate predictor for isoform diversity of neuronal alpha1 subunits (reviewed in Catterall, 2000). Although the presence of isoforms has been suggested to occur as a result of posttranslational proteolysis (Hell *et al.*, 1996), accumulating evidence reveals that much diversity in calcium channel expression results from alternative splicing of transcripts arising from a single alpha1 subunit gene (Perez-Reyes *et al.*, 1990; Snutch *et al.*, 1991; reviewed in Snutch and Reiner, 1992). Analysis of LVA alpha1 subunit expression also suggests the possibility of unique splice variants for each alpha1 subunit gene. As splice variants can differ in their spatial and temporal expression patterns and electrophysiological properties, the presence of splice variants could yield further T-type channel heterogeneity than originally predicted.

Alpha1G

The possibility for alternative splicing of alpha1G was first suggested upon comparison of mouse alpha1G cDNA sequence to rat brain alpha1G (Klugbauer *et al.*, 1999), and detailed PCR studies of human DNA have supported this conclusion. The human gene encoding alpha1G, *CACNA1G* ($CA_v3.1$), is composed of at least 38 exons, and analysis of PCR products reveals at least six sites for alternative splicing that could generate 24 putative unique alpha1G isoforms (Mittman *et al.*, 1999a). Both the publication of full-length alpha1G cDNA sequences (Table 1) and use of PCR techniques reveal that some isoforms are very common whereas others may be rare. For example, inclusion of exon 14 in the II–III intracellular loop of alpha1G results in an additional 69 nucleotides found in some rodent cDNA sequences and PCR products from human tissues (Mittman *et al.*, 1999a; Monteil *et al.*, 2000a) (Table II). When included, the 23 amino acid sequence (named insertion "e;" Monteil *et al.*, 2000a) provides an additional phosphorylation site for protein kinase C, thus potentially acting as an important regulatory site. Furthermore, alpha1G recombinant channels with this insert display faster inactivation rates than do channels without the "e" insert (Chemin *et al.*, 2001a), suggesting that additional variability in endogenous T-type currents could result from differences in alternative splicing of a single LVA alpha1 gene product.

A more complicated splicing event can occur in the III–IV intracellular loop of alpha1G, as an alternative 5′ splice donor site of exon 25 can combined with an acceptor site on exon 27, excluding a portion of exon 25 (Mittman *et al.*, 1999b; Monteil *et al.*, 2000a). Comparison of full-length cloned alpha1G cDNA sequences reveal that the short form of exon 25, called either "exon 25b" (Mittman *et al.*, 1999b) or "form b" (Monteil *et al.*, 2000a), appears to exist either with exon 26, as in alpha1G isolated from a rat-insulin-producing cell line (AF125161; Zhuang *et al.*, 2000) or without exon 26, as in alpha1G cloned from a human brain library (AF126965; Monteil

Table II. Comparison of Common Splice Variants of Full-Length alpha1G CDNA Cloned From Rat, Mouse, and Human Tissues

Species GenBank # (Ref.)	Putative splice variant sites; name of insert					Estimated size difference compared to smallest hypothetical protein without inserts (kDa)
	Exon 14 II–III e	Exon 25 III/IV full sequence a	Exon 26 III/IV c	Exon 34 C-term. f	Exon 35 C-term. d	
Rat AF125161 (Zhuang *et al.*, 2000)	+	−	+	−	−	∼4.5
Rat "a" AF027984 (Perez-Reyes *et al.*, 1998)	−	+	−	−	−	∼0.8
Mouse AJ012569 (Klugbauer *et al.*, 1999)	+	+	+	−	−[a]	∼5.2
Human "a" AF126966 (Monteil *et al.*, 2000)	−[b]	+	−	−[b]	−[b]	∼0.8
Human "b" AF126965 (Monteil *et al.*, 2000)	−[c]	−	−[c]	−	−[c]	0
Total size	69 nt 23 aa ∼2.5 Da	21 nt 7 aa ∼0.8 Da	54 nt 18 aa ∼2 Da	144 nt 48 aa ∼5.3 Da	135 nt 45 aa ∼5 Da	∼16

Note. The exon/intron structure and exon numbering is based on Mittman *et al.* (1999a) and Monteil *et al.* (2000a). Splice variants isoforms "a" and "b" are mutually exclusive, as isoform "b" results from use of an alternative 5' splice donor of exon 25 combined with an acceptor in exon 27.

[a] denotes a partial mouse sequence with both exons 34 ("f insert") and 35 ("d insert") (Mittman *et al.*, unpublished, AF227530).

[b] denotes the presence of splice variants of the "a" form of alpha1G. Specifically, forms ae (AF227744), aef (AF227745), and af (AF227746) have been recovered by PCR and DNA sequencing (Monteil *et al.*, 2000a).

[c] denotes the presence of splice variants of the "b" form of alpha1G. Specifically, forms bc (AF227747), bcd (AF227748), bce (AF227749), bcef (AF227750), be (AF227751), and bcedf (AF134986) have been recovered by PCR and DNA sequencing (Mittman *et al.*, 1999a; Monteil *et al.*, 2000a).

et al., 2000a). Similarly, the long form of exon 25, called "exon 25a" (Mittman *et al.*, 1999b) or "form a" (Monteil *et al.*, 2000a), can combine with either an acceptor site in exon 26 as in mouse (AJ012569, (Klugbauer *et al.*, 1999)) or an acceptor site in constitutive exon 27, as in the original rat sequence (AF027984; Perez-Reyes *et al.*, 1998) (Table II). Inclusion of exon 26, irrespective of the identity of the 5′ end, has been named "form c" (Monteil *et al.*, 2000a). However, Monteil *et al.* (2000a) never observed a PCR product to be composed of both long exon 25 (exon25a; Mittman *et al.*, 1999b) and exon 26; conversely Mittman *et al.*, (1999b) never observed a PCR product to be composed of both short exon 25 (exon 25b) and exon 26. Although the full significance of these splice variants remains unclear, the presence of full-length exon 25 confers activation at more negative membrane potentials and faster rates of activation compared to other alpha1G splice variants (Chemin *et al.*, 2001a).

There is a surprising potential for marked heterogeneity in the carboxyl terminus of alpha1G. Comparison of human alpha1G RT-PCR products with genomic *CACNA1G* suggests at least three alternative splice sites involving exons 34, 35, and 38 (Mittman *et al.*, 1999b). Both RT-PCR data and sequence alignments reveal that many alpha1G sequences lack exons 34 and 35 (Table II), or express exon 34 without exon 35 (Klugbauer *et al.*, 1999). Coexpression of exons 34 and 35 has been observed in a human alpha1G sequence (Mittman *et al.*, 1999b). The final possibility of exclusive expression of exon 34 has not been reported in a published paper, but comparison of human alpha1G sequence from the Human Genome Project with other human alpha1G sequences (Mittman *et al.*, 1999a; Monteil *et al.*, 2000a) suggest that some alpha1G sequences may exclude exon 35 (NCBI annotation project, XM_037981). At this time, most of the variability exists in comparison of human sequences, potentially suggesting that this region may not be a target of dynamic expression in rodents. Alternatively, all species may exhibit differential expression of this region of the C terminus, and splice variants may confer unique biophysical or modulatory properties to the resulting alpha1G channel. For example, inclusion of exon 35 introduces two phosphorylation consensus sites for casein kinase II (Mittman *et al.*, 1999b), leading to intriguing possibilities for intracellular regulation by intracellular messengers (see Yunker, 2003).

The final sites of potential alpha1G heterogeneity occur in the distal region of the C terminus. For example, most, but not all, alpha1G isoforms contain an 80 amino acid insert resulting from use of exon 38 (Mittman *et al.*, 1999b). Furthermore, alignment of cloned alpha1G sequences indicate that small sequence variations are observed in regions corresponding to exons 36 and 37 (Mittman *et al.*, 1999a; Monteil *et al.*, 2000a). Additional heterogeneity in the structure of alpha1G may yet be revealed, as the shortest predicted alpha1G protein would have 2171 amino acids, and the longest alpha1G would have 2377 amino acids (Mittman *et al.*, 1999b), with a difference of 206 amino acids. Therefore, it is likely that alternative splicing of alpha1G is a common mechanism used to generate a heterogeneous family of alpha1G channels that may mediate diverse cellular functions due

to differences in the biophysical properties of the pore-forming alpha1 subunit.

Alpha1H

Alignment of alphaH cDNA isolated from human (Cribbs *et al.*, 1998; Williams *et al.*, 1999), rat (McRory *et al.*, 2001), and mouse (NM_021415, Mittman *et al.*, unpublished) reveals a high degree of identity among these sequences throughout both membrane spanning and intracellular regions of alpha1H. At this time, few studies have examined if alternative splicing events generate intra- and/or interspecies diversity of alpha1H transcripts. Recently, analysis of alpha1H mRNA in human testicular cells recovered two alpha1H products, found to result from omission of exon 26 in the III–IV linker of alpha1H (Jagannathan *et al.*, 2002b). This 18-b deletion has also been reported in rat brain (McRory *et al.*, 2001), suggesting it is not restricted to either human tissues or male germ cells.

Alignment of alpha1H cDNA sequences also suggests potential regions of heterogeneity in the N and C-termini and II–III intracellular loops. For example, a 20 amino acid insert in the N terminus of human alpha1H is found in some (Daniels *et al.*, 2001) but not all (Cribbs *et al.*, 1998; Williams *et al.*, 1999) human sequences. Although single amino acid substitutions are found in both the N terminus and I–II intracellular loop, the II–III intracellular loop contains two regions of significant divergence in the alignment of predicted alpha1H sequences (Fig. 1), possibly occurring as a result of unidentified alternative splicing in these regions. In contrast, four

```
IIS6
VAILVEGFQA  EGDANRSDTD  EDKTSVHFEE  DFHKLRELQT  TELKMCSLAV  TPNGHLEGRG  SLSPPLIMCT
VAILVEGFQA  EGDANRSDTD  EDKTSVHFEE  DFHKLRELQT  TELKMCSLAV  TPNGHLEGRG  SLSPPLIMCT
VAILVEGFQA  EGDATRSDTD  EDKTSTHLEE  DFDKLRDVRA  TEMKMYSLAV  TPNGHLEGRG  SLPPPLITHT
VAILVEGFQA  EGDATRSDTD  EDKTSTQLEG  DFDKLRDLRA  TEMKMYSLAV  TPNGHLEGRG  SLPPPLITHT


AATPMPTPKS  SPFLDAAPSL  PDSRRGSSSS  GDPPLGDQKP  PASLRSSPCA  PWGPSGAWSS  RRSSWSSLGR
AATPMPTPKS  SPFLDAAPSL  PDSRRGSSSS  GDPPLGDQKP  PASLRSSPCA  PWGPSGAWSS  RRSSWSSLGR
AATPMPTPKS  SPHLDMAHTL  LDSRRSSSGS  VDPQLGDQKS  LASLRSSPCA  PWGPNSAGSS  RRSSWNSLGR
AATPMPTPKS  SPNLDVAHAL  LDSRRSSSGS  VDPQLGDQKS  LASLRSSPCT  PWGPNSAGSS  RRSSWNSLGR

        **  ***
APSLKRRGQC  GERESLLSGE  GKGSTDDEAE  ---------D  GRAAPGRATP  -LRRAESLDP  RPL-------
APSLKRRGQC  GERESLLSGE  GKGSTDDEAE  ---------D  GRAAPGPRAT  PLRRAESLDP  RPL-------
APSLKRRSQC  GERESLLSGE  GKGSTDDEAE  DSRPNSGTHP  G-ASPGPRAT  PLRRAESL--  ---GHRSTMD
APSLKRRNQC  GERESLLSGE  GKGSTDDEAE  DSRPSTGTHP  G-ASPGPRAT  PLRRAESL--  ---DHRSTLD


----RPAALP  PTKCRDRDGQ  VVALPSDFFL  RIDSHREDAA  ELDDDSEDSC  CLRLHKVLEP  YKPQWCRSRE
----RPAALP  PTKCRDRDGQ  VVALPSDFFL  RIDSHREDAA  ELDDDSEDSC  CLRLHKVLEP  YKPQWCRSRE
LCPPRPATLL  PTKFRDCNGQ  MVALPSEFFL  RIDSHKEDAA  EFDDDIEDSC  CFRLHKVLEP  YAPQWCSSRE
LCPPRPAALL  PTKFHDCNGQ  MVALPSEFFL  RIDSHKEDAA  EFDDDIEDSC  CFRLHKVLEP  YAPQWCRSRE

                           IIISI
AWALYLFSPQ  NRFRVSCQKV  ITHKMFDHVV  LVFIFLNCVTI
AWALYLFSPQ  NRFRVSCQKV  ITHKMFDHVV  LVFIFLNCVTI
SWALYLFPPQ  NRLRVSCQKV  IAHKMFDHVV  LVFIFLNCITI
SWALYLFPPQ  NRLRVSCQKV  IAHKMFDHVV  LVFIFLNCITI


Human NM_021098 (Cribbs et al., 1998; Williams et al., 1999)
Human AF223563 (Mittman et al., unpublished)
Mouse NM_021415 (Mittman et al., unpublished)
Rat AF290213 (McRory et al., 2001)
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Fig. 1. Comparison of the predicted amino acid sequences in the II–III loop of alpha1H reveals variations between human (first two lines) and rodent alpha1H (last two lines). Note the presence of a G protein binding consensus sequence (QXXER) indicated by ******** (Williams *et al.*, 1999). Predicted transmembrane regions indicated by underscore.

```
IVS6
VTFVLVAQFV  LVNVVVAVLM  KHLEESNKEA  REDAELDAEI  ELEMAQGPGS  ARRVDADRPP  LPQESPGAR-
VTFVLVAQFV  LVNVVVAVLM  KHLEESNKEA  REDAELDAEI  ELEMAQGPGS  ARRVDADRPP  LPQESPGAR-
VTFVLVAQFV  LVNVVVAVLM  KHLEESNKEA  REDAEMDAEI  ELEIAQGSTA  ------QPPS  TAQESQGTDP
VTFMLVAQFV  LVNVVVAVLM  KHLEESNKEA  REDAEMDAEI  ELEMAQGSTA  ------QPPP  TAQESQGTQP

DAPNL-VARK  VSVSRMLSLP  NDSYMFRPVV  PASAPHPRPL  QEVEMETYGA  GTPLGSVASV  HSPPAESCA
DAPNL-VARK  VSVSRMLSLP  NDSYMFRPVV  PASAPHPRPL  QEVEMETYGA  GTPLGSVASV  HSPPAESCA
DTPNLLVVRK  VSVSRMLSLP  NDSYMFRPVA  PAAAPHSHPL  QEVEMETY--  ---TGPVTSA  HSPSLEPRT
DTPNLLVVRK  VSVSRMLSLP  NDSYMFRPVA  PAAAPHSHPL  QEVEMETY--  ---TGPVTSA  HSPPLEPRA

SLQIPLAVSS  PARSGEPLHA  LSPRGTARSP  SLSRLLCRQE  AVHTDSLEGK  IDSPRDTLDP  ----------
SLQIPLAVSS  PARSGEPLHA  LSPRGTARSP  SLSRLLCRQE  AVHTDSLEGK  IDSPRDTLDP  ----------
SFQVPSAASS  PARASDPLCA  LSPRDTPRSL  SLSRILYRQE  AMHAESLEGQ  ID--------  DAGEDGIPDY
SFQVPSAASS  PARVSDPLCA  LSPRGTPRSL  SLSRILCRQE  AMHSESLEGK  VD--------  DVGGDSIPDY

----------  --------AE  PGEKTPVRPV  TQGGSLQSPP  RSPRPASVRT  RKHTFGQHCV  SSRPAAPGGE
----------  --------AE  PGEKTPVRPV  TQGGSLQSPP  RSPRPASVRT  RKHTFGQHCV  SSRPAAPGGE
TEPAENISMS  QAPLGTLR--  ----------  -------SPP  CSPRPASVRT  RKHTFGQHCI  SSRPPTLGGD
TEPAENMSTS  QASTGAPR--  ----------  -------SPP  CSPRPASVRT  RKHTFGQRCI  SSRPPTLGGD

EAEASDPADE  EVSHITSSAC  PWQPTAEPHG  PEASPVAGGE  RDLRRLYSVD  AQGFLDKPGR  ADEQWRPSAE
EAEASDPADE  EVSHITSSAC  PWQPTAEPHG  PEASPVAGGE  RDLRRLYSVD  AQGFLDKPGR  ADEQWRPSAE
DAEAADPADE  EVSHITSSAH  PW-PATEPHS  PEASP-----  ----------  ----------  ----------
EAEAADPADE  EVSHITSSAH  PW-PATEPHS  PEASP-----  ----------  ----------  ----------

LGSGEPGEAK  ----------  ----------  ----------  ----------  ----------  ----------
LGSGEPGEAK  ----------  ----------  ----------  ----------  ----------  ----------
----------  TASPAKGTVG  SGRDPHRFCS  VDAQSFLDKP  GRPDAQRWSS  VELDNGDGHL  ESGEVRARAS
----------  TASPVKGTMG  SGRDPRRFCS  VDAQSFLDKP  GRPDAQRWSS  VELDNGESHL  ESGEVRGRAS

AWGPEAEPAL  GARRKKKMSP  PCISVEPPAE  DEGSARPSAA  EGGSTTLRRR  TP
AWGPEAEPAL  GARRKKKMSP  PCISVEPPAE  DEGSARPSAA  EGGSTTLRRR  TP
----ELEPAL  GARRKKKMSP  PCISIDPPTE  DEGSSRPPAA  EGGNTTLRRR  TP
----ELEPAL  GSRRKKKMSP  PCISIEPPTK  DEGSSRPPAA  EGGNTTLRRR  TP

Human NM_021098 (Cribbs et al., 1998; Williams et al., 1999)
Human AF223563 (Mittman et al., unpublished)
Mouse NM_021415 (Mittman et al., unpublished)
Rat AF290213 (McRory et al., 2001)
```

Fig. 2. Comparison of the predicted amino acid sequences in the carboxyl terminus of alpha1H reveals variations between human (first two lines) and rodent (last two lines). Predicted transmembrane regions indicated by underscore.

regions of sequence differences exist between human and rodent alpha1H carboxyl terminus sequences, although mouse and rat sequences are almost identical throughout this region (Fig. 2). At this time, it is unknown if alternative splicing of alpha1H can generate channels with different biophysical properties.

Alpha1I

Comparison of human alpha1I cDNA to *CACNA1I* suggests the gene encompasses at least 36 exons (Mittman *et al.*, 1999b), and alignment of human and rat alpha1I cDNA reveals three main regions of divergence in the I–II intracellular loop and carboxyl terminus. The presence of exon 9 in human alpha1I contributes 35 amino acids to the I–II intracellular domain in some (NM_021096, Mittman *et al.*, 1999b; AF142567, Mittman *et al.*, unpublished), but not all (AF211189; Monteil *et al.*, 2000b), human alpha1I sequences (Mittman *et al.*, 1999b, unpublished; Monteil *et al.*, 2000b). The electrophysiological significance of this insert is not known at this time. However, this region includes a consensus site for phosphorylation by protein kinase C (Mittman *et al.*, 1999b) suggesting alpha1I channels with this insert may be differentially regulated by intracellular messengers. This sequence has not been

observed in rat alpha1I sequences to date (Lee *et al.*, 1999; McRory *et al.*, 2001).

The carboxyl terminal is the largest source for potential variations in alpha1I. For example, analysis of the predicted rat alpha1I amino acid sequence reveals an unusual cysteine and proline rich region encoded by exon 32 that is not observed in human alpha1I sequences (Lee *et al.*, 1999; Mittman *et al.*, 1999b). Furthermore, alternative splicing to an acceptor site in exon 33 can lead to deletion of 13 amino acids (ENLWLDSVSLIIK) in the C terminus of humans (Chemin *et al.*, 2001b; Mittman *et al.*, 1999b), and this variant is associated with an additional alanine residue coded by exons 36 (Chemin *et al.*, 2001b). Although this deletion has not been recovered in full-length alpha1I cDNAs reported to date, comparison of the electrophysiological properties of the alpha1I variant to that of an intact alpha1I sequence with the 13 amino acids suggests that this sequence can alter current kinetics but not steady-state activation or inactivation properties (Chemin *et al.*, 2001b).

The most striking potential differences in alpha1I occur distal to these exons. Alignment of alpha1I predicted protein sequences suggests three potential length C-termini (Fig. 3 and Table III). Independent cloning of rat alpha1I by two different groups recovered a short C terminus compared to all human sequences (Lee *et al.*, 1999; McRory *et al.*, 2001). However, longer rat alpha1I sequences have been cloned (AF203697; AF346817, Mittman, unpublished), suggesting the short alpha1I isoform may be the consequence of a frameshift introduced by alternative splicing in this region (Monteil *et al.*, 2000b). Cloning of human alpha1I by two independent research groups reveals that human alpha1I contains an 169 amino acid substitution encoded by exons 34–36, resulting in replacement of the last 11 amino acid residues in rat (Mittman *et al.*, 1999b; Monteil *et al.*, 2000b). Finally, a sequence cloned by Mittman (AF142567, unpublished) suggests a third "long" variation of alpha1I (Fig. 3 and Table III) that may result from use of an alternative splice site, thus permitting translation through exon 36. The recent discovery of an additional 37th exon in both mouse (AY026384; Mittman, unpublished) and human alpha1I extends the earlier studies, as this exon is composed of 3969 nucleotides and encodes an additional 214 amino acids in the C terminus (Gomora *et al.*, 2002) (Fig. 3). Interestingly, recombinant channels formed by this clone have greater current density and prepulse facilitation than channels formed by truncated alpha1I (Gomora *et al.*, 2002). As there are a number of differences in the biophysical properties of currents conducted by rat and human recombinant alpha1I proteins (Chemin *et al.*, 2001b; Monteil *et al.*, 2000b), sequence differences between species may yield significant functional differences in current. Alternatively, differences in the molecular sequence of the pore-forming subunit resulting from alternative splicing may render the channel susceptible to posttranslational modifications or modification by intracellular second messengers.

Additional controversy regarding the structure of alpha1I results from independent cloning of rat alpha1I, as both initial cloning and expression studies suggest widespread distribution of alpha1I throughout the adult rat brain (Lee *et al.*, 1999; Talley *et al.*, 1999). In contrast, cloning by a second group yielded an almost identical alpha1I sequence, but their Northern blot analysis suggested that alpha1I is exclusively restricted to straitum in adult rats (McRory *et al.*, 2001). Furthermore, this alternative rat alpha1I clone had unique voltage dependent properties compared to the original rat alpha1I isoform (Lee *et al.*, 1999), although the rat sequences were almost identical.

SPATIAL AND TEMPORAL PATTERNS OF ENDOGENOUS NEURAL LVA EXPRESSION

T-Type Channels Are Expressed in the Adult Central Nervous System

Functional studies demonstrate a widespread distribution of T-type currents in the brain (reviewed in Akaike, 1991). For example, neurons throughout the cortex (Friedman and Gutnick, 1987; Giffin *et al.*, 1991; Hamill *et al.*, 1991; Komatsu and Iwakiri, 1992; Magistretti and de Curtis, 1998; Sayer *et al.*, 1993), olfactory bulb (Charpak *et al.*, 2001; Wang *et al.*, 1996b), hippocampus (Beck *et al.*, 1997, 1998; Takahashi *et al.*, 1989a,b, 1991), neostriatum (Hoehn *et al.*, 1993), thalamus (Coulter *et al.*, 1989a; Huguenard and Prince, 1992; Jahnsen and Llinás, 1984a; Steriade and Llinás, 1988), lateral habenula (Wilcox *et al.*, 1988), hypothalamus (Akaike *et al.*, 1989b; Fan *et al.*, 2000), lateral geniculate nucleus (Crunelli *et al.*, 1989), amygdala (Kaneda and Akaike, 1989), pontine reticular formation (Greene *et al.*, 1986), dorsal raphe nucleus (Burlhis and Aghajanian, 1987), inferior olive (Llinás and Yarom, 1981a), vestibular nucleus (Smith *et al.*, 2002), and cerebellum (Aizenman and Linden, 1999; Mouginot *et al.*, 1997; Raman and Bean, 1999) conduct T-type currents, and many of these regions exhibit LTS.

Although current antagonists are not able to identify the molecular nature of the pore in any of these channels, alpha1G, alpha1H, and alpha1I transcripts are widely and differentially distributed throughout the brain by both Northern blot (Lee *et al.*, 1999; McRory *et al.*, 2001; Monteil *et al.*, 2000a,b; Perez-Reyes *et al.*, 1998) and in

```
(Fourth Domain)
(1) TFIICLNVVTMSLEHYNQPTSLETALKYCNYMFTTVFVLEAVLKLVAFGLRRFFKDRWNQLDLAIVLL
(2) TFIICLNVVTMSLEHYNQPTSLETALKYCNYMFTTVFVLEAVLKLVAFGLRRFFKDRWNQLDLAIVLL
(3) TFIICLNVVTMSLEHYNQPTSLETALKYCNYMFTTVFVLEAVLKLVAFGLRRFFKDRWNQLDLAIVLL
(4) TFIICLNVVTMSLEHYNQPTSLETALKYCNYMFTTVFVLEAVLKLVAFGLRRFFKDRWNQLDLAIVLL
(5) TFIICLNVVTMSLEHYNQPTSLETALKYCNYMFTTVFVLEAVLKLVAFGLRRFFKDRWNQLDLAIVLL
(6) TFIICLNVVTMSLEHYNQPTSLETALKYCNYMFTTVFVLEAVLKLVAFGLRRFFKDRWNQLDLAIVLL

(1) SVMGITLEEIEINAALPINPTIIRIMRVLRIARVLKLLKMATGMRALLDTVVQALPQVGNLGLLFMLL
(2) SVMGITLEEIEINAALPINPTIIRIMRVLRIARVLKLLKMATGMRALLDTVVQALPQVGNLGLLFMLL
(3) SVMGITLEEIEINAALPINPTIIRIMRVLRIARVLKLLKMATGMRALLDTVVQALPQVGNLGLLFMLL
(4) SVMGITLEEIEINAALPINPTIIRIMRVLRIARVLKLLKMATGMRALLDTVVQALPQVGNLGLLFMLL
(5) SVMGITLEEIEINAALPINPTIIRIMRVLRIARVLKLLKMATGMRALLDTVVQALPQVGNLGLLFMLL
(6) SVMGITLEEIEINAALPINPTIIRIMRVLRIARVLKLLKMATGMRALLDTVVQALPQVGNLGLLFMLL

(1) FFIYAALGVELFGKLVCNDENPCEGMSRHATFENFGMAFLTLFQVSTGDNWNGIMKDTLRDCTHDERS
(2) FFIYAALGVELFGKLVCNDENPCEGMSRHATFENSARAFLTLFQVSTGDNWNGIMKDTLRDCTHDERT
(3) FFIYAALGVELFGKLVCNDENPCEGMSRHATFENFGMAFLTLFQVSTGDNWNGIMKDTLRDCTHDERS
(4) FFIYAALGVELFGKLVCNDENPCEGMSRHATFENFGMAFLTLFQVSTGDNWNGIMKDTLRDCTHDERS
(5) FFIYAALGVELFGKLVCNDENPCEGMSRHATFENFGMAFLTLFQVSTGDNWNGIMKDTLRDCTHDERS
(6) FFIYAALGVELFGKLVCNDENPCEGMSRHATFENFGMAFLTLFQVSTGDNWNGIMKDTLRDCTHDERS

(1) CLSSLQFVSPLYFVSFVLTAQFVLINVVVAVLMKHLDDSNKEAQEDAEMDAEIELEMAHGLGPCPGPC
(2) CLSSLQFVSPLYFVSFVLTAQFVLINVVVAVLMKHLDDSNKEAQEDAEMDAEIELEMAHGLGPCPGPC
(3) CLSSLQFVSPLYFVSFVLTAQFVLINVVVAVLMKHLDDSNKEAQEDAEMDAELELEMAHGLGP
(4) CLSSLQFVSPLYFVSFVLTAQFVLINVVVAVLMKHLDDSNKEAQEDAEMDAELELEMAHGLGP
(5) CLSSLQFVSPLYFVSFVLTAQFVLINVVVAVLMKHLDDSNKEAQEDAEMDAELELEMAHGLGP
(6) CLSSLQFVSPLYFVSFVLTAQFVLINVVVAVLMKHLDDSNKEAQEDAEMDAELELEMAHGLGP
(7)         SPLYFVSFVLTAQFVLINVVVAVLMKHLDDSNKEAQEDAEMDAEIELEMAHGLGPCPGPC
(8)         SPLYFVSFVLTAQFVLINVVVAVLMKHLDDSNKEAQEDAEMDAEIELEMAHGLCPCPGPC

(1) PGPCPCPCPCPCPGPRLPTSSPGAPGRGSGGAGAGGDTESHLCRHCYSPAQETLWLDSVSLIIKDSLE
(2) PGPCPCPCPCPCAGPRLPTSSPGAPGRGSGGAGAGGDTESHLCRHCYSPAQETLWLDSVSLIIKDSLE
(3)               GPRLPTGSPGAPGRGPGGAGGGGDTEGGLCRRCYSPAQENLWLDSVSLIIKDSLE
(4)               GPRLPTGSPGAPGRGPGGAGGGGDTEGGLCRRCYSPAQENLWLDSVSLIIKDSLE
(5)               GPRLPTGSPGAPGRGPGGAGGGGDTEGGLCRRCYSPAQENLWLDSVSLIIKDSLE
(6)               GPRLPTGSPGAPGRGPGGAGGGGDTEGGLCRRCYSPAQENLWLDSVSLIIKDSLE
(7) PGPCPCPCPCPCPGPRLPTSSPGAPGRGSGGAGAGGDTESHLCRHCYSPAQETLWLDSVSLIIKDSLE
(8) PGPCPCPCPCPCPGPRLPTSSPGAGPRGSGGAGAGGDTESHLCRHCYSPAQETLWLDSVSLIIKDSLE

(1) GELTIIDNLSGSVFHHYASPDGCGKCHHDKQETGLHPSCWGMT------------------------
(2) GELTIIDNLSGSVFHHYASPDGCGKCHHDKQETGLHPSCWGMT------------------------
(3) GELTIIDNLSGSIFHHYSSPAGCKKCHHDKQEVQLAETEAFSLNSDRSSSILLGDDLSLEDPTACPPG
(4) GELTIIDNLSGSIFHHYSSPAGCKKCHHDKQEVQLAETEAFSLNSDRSSSILLGDDLSLEDPTACPPG
(5) GELTIIDNLSGSIFHHYSSPAGCKKCHHDKQEVQLAETEAFSLNSDRSSSILLGDDLSLEDPTACPPG
(6) GELTIIDNLSGSIFHHYSSPAGCKKCHHDKQEVQLAETEAFSLNSDRSSSILLGDDLSLEDPTACPPG
(7) GELTIIDNLSGSVFHHYASPDGCGKCHHDKQEVQLAETEAFSLNSDRSSSILLGDDLSLEDPTACPQG
(8) GELTIIDNLSGSVFHHYASPDGCGKCHHDKQEVQLAETEAFSLNSDRSSSILLGDDLSLEDPTACPQG

(1) ------------------------------------------------------------------
(2) ------------------------------------------------------------------
(3) RKDSKGELDPPEPMRVGDLGECFFPLSSTAVSPDPENFLCEMEEIPFNPVRSWLKHDSSQAPPSPFSP
(4) RKDSKGELDPPEPMRVGDLGECFFPLSSTAVSPDPENFLCEMEEIPFNPVRSWLKHDSSQAPPSPFSP
(5) RKDSKGELDPPEPMRVGDLGECFFPLSSTAVSPDPENFLCEMEEIPFNPVRSWLKHDSSQAPPSPFSP
(6) RKDSKGELDPPEPMRVGDLGECFFPLSSTAVSPDPENFLCEMEEIPFNPVRSWLKHDSSQAPPSPFSP
(7) PKESKGELEPPEPMQAGDLDECFFPPFASEPVSTGPESLLCEMGAIPFNPVQSWLKHESSQAPQSPFSP
(8) PKESKGELEPPEPMQAGDLDECFFPPFASEPVSTGPESLLCEMGAIPFNPVQSWLKHESSQAPQSPFSP
```

Fig. 3. Alignment of the predicted amino acid sequences in the forth domain and carboxyl terminus of alpha1I cloned from different species reveals sequence heterogeneity suggestive of alternative splicing. The sixth membrane spanning region of the forth domain is underlined.

```
(1) ------------------------------------------------------------------
(2) ------------------------------------------------------------------
(3) DASSPLLPMPAEFFHPAVSASQKGPEKGTGTGTLPKIALQGSWASLRSPRVNCTLLRQVPTPPRP---
(4) DASSPLLPMPAEFFHPAVSASQKGPEKGTGTGTLPKIALQGSWASLRSPRVNCTLLRQVPTPPRP---
(5) DASSPLLPMPAEFFHPAVSASQKGPEKGTGTGTLPKIALQGSWASLRSPRVNCTLLRQATGSDTSLDA
(6) DASSPLLPMPAEFFHPAVSASQKGPEKGTGTGTLPKIALQGSWASLRSPRVNCTLLRQATGSDTSLDA
(7) DGSSPLLQMPAEFFHPAVSASQKGQEPGMSAGTLPKIALQGSWASLRSPSVNCTLLRQVQAPPGF---
(8) DGSSPLLQMPAEFFHPAVSASQKGQEPGMSASTLPKIALQGSWASLRSPSVNCTLLRQATVSDTSLDA
(9)                                                 SPSVNCTLLRQATVSDTSLDA

(1) ------------------------------------------------------------------
(2) ------------------------------------------------------------------
(3) ------------------------------------------------------------------
(4) ------------------------------------------------------------------
(5) SPSSSAGSLQTTLEDSLTLSDSPRRALGPPVQVPGPRAGLSPAARRRLSLRGRGLFSLRGLRAHQRSH
(6) SPSSSAGSLQTTLEDSLTLSDSPRRALGPPAPAPGPRAGLSPAARRRLSLRGRGLFSLRGLRAHQRSH
(7) ------------------------------------------------------------------
(8) SPSSSAGSLQTTLEDSLTLSDSPRRALGPPVQVPGPRASLSPATRRRLSLRGRGLFSLRGLRAHQRSH
(9) SPSSSAGSLQTTLEDSLTLSDSPRRALGPPVQVPGPRASLSPATRRRLSLRGRGLFSLRGLRAHQRSH
(10)                           PVQVPGPRASLSPATRRRLSLRGRGLFSLRGLRAHQRSH

(1) ------------------------------------------------------------------
(2) ------------------------------------------------------------------
(3) ------------------------------------------------------------------
(4) ------------------------------------------------------------------
(5) SSGGSTSPGCTHHDSMDPSDEEGRGGAGGGGAGSEHSETLSSLSLTSLFCPPPPPPAPGLTPARKFSS
(6) SSGGSTSPGCTHHDSMDPSDEEGRGGAGGGGAGSEHSETLSSLSLTSLFCPPPPPPAPGLTPARKFSS
(7) ------------------------------------------------------------------
(8) SSGGSTSPGCTHHDSMDPSDEEGRGGAGGGGAGSEHSETLSSLSLTSLFCLPPTLPPPGLTPARKFNS
(9) SSGGSTSPGCTHHDSMDPSDEEGRGGAGGGGAGSEHSETLSSLSLTSLFCLPPTLPPPGLTPARKFNS
(10)SSGGSTSPGCTYHDSMDPSDEEGRGGAGGGGAGSEHSETLSSLSLTSLFCLPPTLPPPGLTPARKFSS

(1) ------------------------------------------------------------------
(2) ------------------------------------------------------------------
(3) ------------------------------------------------------------------
(4) ------------------------------------------------------------------
(5) TSSLAA PGRPHAAALAHGLARSPSWAADRSKDPPGRAPLPMGLGPLAPPPQPLPGELEPGDAASKRKR
(6) TSSLAA PGRPHAAALAHGLARSPSWAADRSKDPPGRAPLPMGLGPLAPPPQPLPGELEPGDAASKRKR
(7) ------------------------------------------------------------------
(8) TSSLAAGPGRPGSTVSARGLVRSPSWAADRSKDPPGQAQLVSGLGSSAPGPQPPPGEST  DAASKRKR
(9) TSSLAAGPGRPGSTVSARGLVRSPSWAADRSKDPPGQAQLVSGLGSSAPGPQPPPGEST  DAASKRKR
(10)TSSLAAGPGRPGATVSVRGLARSPSWAADRSKDPPGQAQLASGFGSSAPEPQPPPGEST  DAASKRKR

(1)  AF086827 Rat alpha1I (Lee et al., 1999)
(2)  AF290214 Rat alpha1I (McRory et al., 2001)
(3)  AF129133 Human alpha1I (Mittman et al., 1999b)
(4)  AF211189 Human alpha1I (Monteil et al., 2000b)
(5)  AF142567 Human alpha1I, delta36b variant (Mittman et al., unpublished)
(6)  AF393329 Human alpha1I (Gomora et al., 2002)
(7)  AF203697 Rat alpha1I, delta37 variant (Mittman, unpublished)
(8)  AF203697 Rat alpha1I, delta 36b variant (Mittman, unpublished)
(9)  AF346817 Rat alpha1I, delta36b variant (Mittman, unpublished)
(10) AY026384 Mouse alpha1I, exon37 (Mittman, unpublished)
```

Fig. 3. (*Continued*).

Table III. Summary of C Terminus Variants in Alpha 1l Sequences

Species	GenBank No.	Number of predicted amino acids	Number of additional amino acids in carboxyl terminus compared to original rat alpha1I sequence (AF086827)	Refs.
rat	AF086827	1835	—	Lee *et al.*, (1999);
	AF290214	1834	—	McRory *et al.*, (2001)
"short"				
human	AF129133	2016	158	Mittman *et al.*, (1999b)
	AF211189	1981	158	Monteil *et al.*, (2000b)
rat (Δ37)	AF203697[a]	[a]	158	Mittman, (unpublished)
"intermediate"				
human (Δ36b)	AF142567	2198	365	Mittman *et al.*, (unpublished)
	AF393329	2188	365	Gomora *et al.*, (2002)
rat (Δ36b)	AF203697[a]	[a]	364	Mittman, (unpublished)
"long"				

[a] Indicates predicted protein based on a partial sequence.

situ hybridization (Craig *et al.*, 1999; Kase *et al.*, 1999; Klugbauer *et al.*, 1999; Talley *et al.*, 1999; Williams *et al.*, 1999). For example, detailed in situ hybridization studies by Talley *et al.* (1999) suggested that alpha1G and alpha1H mRNA are expressed in a reciprocal manner with either the presence or absence of alpha1I mRNA (Talley *et al.*, 1999). Furthermore, these studies indicate that the brain has a graded distribution of both alpha1G and alpha1H, with alpha1G transcripts most abundant in the hindbrain and alpha1H transcripts more commonly expressed in forebrain structures (Talley *et al.*, 1999). Finally, with the notable exception of a few regions such as olfactory mitral cells and cerebellar granule cells, the presence of LVA alpha1 mRNA transcripts corresponded with the detection of T-type currents (Talley *et al.*, 1999). Taken together, studies of the distribution of LVA alpha1 transcripts suggests that many cellular functions mediated by T-type channels occur through selective expression of LVA alpha1 isoforms. Although the biophysical properties of recombinant alpha1G and alpha1H are more similar to each other than to alpha1I (discussed above), modeling studies suggest that each LVA alpha1 subunit has unique effects on the resulting electrical activity. Furthermore, LVA alpha1 subunits are not interchangeable with each other (Chemin *et al.*, 2002a). Therefore, spatial differences in expression of alpha1G, alpha1H, and alpha1I may confer unique biophysical properties in a regionally dependent manner.

T-Type Channels Are Expressed in the Retina

T-type currents are recorded from many retinal cell types by electrophysiological methods. For example, T-type currents are recorded from bovine ciliary body epithelial cells (Jacob, 1991); goldfish, bass, frog, and rat horizontal cells (Akopian *et al.*, 1997; Pan and Lipton, 1995; Pfeiffer-Linn and Lasater, 1993; Sullivan and Lasater, 1992); salamander, mouse, rat, and cat bipolar cell terminals (de la Villa *et al.*, 1998; Kaneko *et al.*, 1989; Liu and Lasater, 1994; Maguire *et al.*, 1992; Pan *et al.*, 2001); turtle, mouse, and rat retinal ganglion cells (Karschin and Lipton, 1989; Rothe *et al.*, 1999); and retinal glial (Muller cells) (Bringmann *et al.*, 2000). Although voltage dependent calcium currents are recorded from both retinal pigment epithelial (Struass and Wienrich, 1994; Ueda and Steinberg, 1993) and amacrine cells (Cohen, 2001; Koizumi *et al.*, 2001), at this time it is unclear if these cell types express T-type channels.

As many retinal cell types express T-type channels, detailed studies are needed to determine the molecular identity of the pore-forming subunits responsible for T-type currents in the retina. RT-PCR of whole mouse retina reveals the presence of all three LVA alpha1 subunits (Pan *et al.*, 2001). Similarly, single cell RT-PCR demonstrates that bipolar cells can express all three LVA alpha1 transcripts, although many bipolar cells express combinations of fewer than three alpha1 transcripts (Pan *et al.*, 2001), suggesting heterogeneity in expression of T-type channels.

It should be noted that T-type channels in the retina may be unusual in that they appear to be important for neurotransmitter release. Unlike T-type currents in many other cells, T-type currents in bipolar cells activate in a range favorable for neurotransmitter release (Kaneko *et al.*, 1989). This hypothesis has been recently supported by two pieces of evidence. Capacitance measurements demonstrate that T-type currents result in exocytosis in rat bipolar cells (Pan *et al.*, 2001). Secondly, GABA-mediated responses resulting from reciprocal synaptic transmission between bipolar cells and amacrine cells are recorded from bipolar cells (Pan *et al.*, 2001). As T-type currents appear to be responsible for the presence of burst firing and mediate transient oscillations in some, but not in all cells (Akopian

et al., 1997), T-type channels may play multiple roles in transduction of visual information in the retina.

Subcellular Neuronal Distribution of T-Type Channels

The neuronal subcellular localization of LVAs has been elucidated by functional studies of T-type currents, as optical imaging and electrophysiological studies suggest that T-type channels are preferentially located on dendrites of many neurons. For example, surgical separation of distal dendrites from CA1 hippocampal neuronal soma eliminates T-type, but not other voltage dependent ionic currents recorded in the soma (Karst *et al.*, 1993). T-type channels mediate a larger fraction of calcium influx into dendrites than into hippocampal (Kavalali *et al.*, 1997) and deep cerebellar somata (Gauck *et al.*, 2001), and stimulation of NMDA receptors on neocortical pyramidal cells results in a brief increase in dendritic intracellular calcium conducted by T-type channels (Markram and Sakmann, 1994). Analysis of single channel activity reveals that 70% of recordings from Purkinje cell dendrites contain T-type channels, compared to only 25% of somatic recordings (Mouginot *et al.*, 1997). Dendritic currents are larger than somatic T-type currents (Mouginot *et al.*, 1997), and the presence of T-type currents increase as the distance from the Purkinje cell somata increases (Pouille *et al.*, 2000). However, it should be noted that the distribution of T-type channels may be determined by neuronal identity. Intracellular recordings from inferior olive neurons (Llinás and Yarom, 1981b) and single channel recordings from thalamic relay neurons (Suzuki and Rogawski, 1989) and CA3 pyramidal neurons (Fisher *et al.*, 1990) indicate the presence of T-type channels in somatic, not dendritic, membranes. Alternatively, the molecular identity of the LVA alpha1 subunit and association with unidentified auxiliary molecules may differentially target the resulting calcium channel complex within the neuron.

At this time, the physiological significance of preferential targeting of T-type channels is under investigation. The presence of T-type channels on dendrites can contribute to voltage- and space-clamp errors in recordings, thus altering the observed voltage- and time-dependent properties of T-type currents, contributing to the appearance of T-type current heterogeneity (Destexhe *et al.*, 1996, 1998). Modeling studies reveal that T-type calcium currents in distal dendrites of hippocampal dentate gyrus neurons contribute to the development of afterdepolarizing potentials (Aradi and Holmes, 1999), potentially leading to oscillatory behavior. Dendritic T-type calcium channels have also been implicated in long-term potentiation in CA1 hippocampal neurons (Song *et al.*, 2001). Perhaps the most intriguing role of T-type channels results from recordings of dendrites in cerebellar Purkinje cells, as dendritic T-type currents underlie low-threshold calcium-dependent action potentials recorded from cerebellar Purkinje cell dendrites and not somata (Pouille *et al.*, 2000). These LTS can occasionally trigger bursts of action potentials in the soma, providing the tantalizing possibility for active electrical dendtictic communication with somata (Cavelier *et al.*, 2002; Pouille *et al.*, 2000).

The recent development of antibodies against LVA alpha1 subunits has begun to permit anatomical localization of T-type channels, as well as aid in identification of the pore-forming subunit in the absence of selective pharmacological agents. Alpha1G immunoreactivity is found throughout neuronal somata and processes in rat brain sections (Craig *et al.*, 1999) and cerebellar slices (Pouille *et al.*, 2000). More recently, we have raised, affinity-purified, and characterized antibodies against alpha1G and alpha1I (Yunker *et al.*, 2003), and our results suggest alpha1G is more restricted to dendrites than previously reported (Craig *et al.*, 1999). It is likely that the use of these reagents and the development of selective pharmacological agents will be useful in the identification of differentially localized T-type channels.

Glial Expression of T-Type Channels

Although glial cells have been viewed as support cells of the nervous system, accumulating evidence indicates that these cells are not as passive as originally assumed. Cultured astrocytes display calcium spikes (MacVicar, 1984), and voltage dependent calcium currents are recorded from glial cells in both the central and peripheral nervous systems (reviewed in Barres *et al.*, 1990a). Fluorescent imaging and electrophysiological recordings suggest that both gray and white matter glial cells may express T-type channels. T-type calcium currents are recorded from highly purified cultured cortical astrocytes treated with some batches of fetal calf serum (Barres *et al.*, 1989) and cortical astrocytes cocultured with neurons (Corvalan *et al.*, 1990). In contrast, T-type calcium currents are not observed in control astrocytes in culture, raising the possibility that these cells may not normally express functional T-type channels (Barres *et al.*, 1989; Corvalan *et al.*, 1990). T-type calcium currents are recorded from presumptive astrocytes in immature hippocampal slices (Akopian *et al.*, 1996) and glial precursors in developing corpus callosum (Berger *et al.*, 1992), suggesting regional and developmental heterogeneity may affect glial expression of T-type channels. At this time, the molecular identity of T-type channels expressed by glial cells is not known.

Other types of glial cells may also express functional T-type channels. Cortical glial precursor cells and mature oligdendrocytes display calcium transients consistent with expression of functional T-type channels (Kirischuk *et al.*, 1995), and T-type currents can be recorded from some cells in cultures of astrocytes from pure white matter tracts (Barres *et al.*, 1990b). In contrast to the sporadic T-type channels observed in many glial cells, Muller glial cells in the retina express robust T-type currents (Bringmann *et al.*, 2000). Finally, T-type currents are recorded from embryonic Schwann cells in culture (Beaudu-Lange *et al.*, 1998), and the detection of these currents is correlated with the presence of neurites or treatment with cAMP analogs (Beaudu-Lange *et al.*, 1998). Taken together, these data suggest that T-type channels may have a functional role in normal glial physiology in a cell-dependent manner.

Although the functional significance of T-type channel expression by glial cells is unclear, expression of these channels may be important in activating calcium-dependent enzymes, channels, and second messenger systems (Sontheimer, 1994). Alternatively, T-type channels may be involved in the genesis and/or maintenance of complex intracellular calcium oscillations and intercellular calcium waves observed in many glial cultures (reviewed in Deitmer *et al.*, 1998; Verkhratsky *et al.*, 1998). Interestingly, similar to the heterogeneous expression of T-type channels in neurons, T-type channels may also be unevenly distributed within individual glial cells, as calcium transients in glial processes are larger than those observed in cell bodies (Kirischuk *et al.*, 1995). This asymmetric distribution of T-type channels has been postulated to be important for glial functions such as secretion (Barres *et al.*, 1989) and myelin formation (Kirischuk *et al.*, 1995). Finally, an intriguing role for T-type channels has been indicated for calcium-mediated cell death during ischemia. Anoxia–ischemia produces severe neuronal injury, and ischemia induces T- and L-type calcium influx in astrocytes from the optic nerve (Fern, 2002). The calcium influx through T-type channels occurs prior to cell death, and treatment with nickel, a nonspecific T-type channel blocker, attenuates ischemic-induced damage (Fern, 2002).

T-Type Channels Are Expressed in the Adult Peripheral Nervous System

Dorsal Root Ganglia

A strong relationship has existed between sensory neurons and T-type channels since the early and detailed electrophysiological studies of T-type currents (Bossu and Feltz, 1986; Carbone and Lux, 1984a,b, 1987; Nowycky *et al.*, 1985; Swandulla and Armstrong, 1988). For example, sensory neurons of the dorsal root ganglia heterogeneously express T-type channels, as some neurons do not contain detectable current, while the magnitude of detectable T-type currents varies from neuron to neuron (Cardanas *et al.*, 1995; Schroeder *et al.*, 1990). Low-voltage-activated alpha1 mRNA transcripts are also heterogeneously distributed throughout dorsal root neurons, as robust levels of alpha1H transcripts and moderate levels of alpha1I transcripts are found scattered in scattered medium-sized neurons, but not in large neurons (Talley *et al.*, 1999). In contrast, little alpha1G mRNA is found in adult rat dorsal root ganglia (Talley *et al.*, 1999).

The presence of T-type currents in dorsal root ganglia is especially intriguing. Small capsaicin-sensitive dorsal root ganlia neurons that respond to chemical and thermal stimuli commonly express functional T-type channels (Todorovic *et al.*, 2001), suggesting that T-type channels may be important in transduction of painful sensations. At least two pieces of experimental evidence support this hypothesis. Redox agents increase T-type currents and exacerbate painful stimuli in rodent models (Todorovic *et al.*, 2001). Conversely, mibefradil, ethosuximide, and redox agents that attenuate T-type currents reduce perception of painful stimuli (Bilici *et al.*, 2001; Matthews and Dickenson, 2001; Todorovic *et al.*, 2001). Finally, coadministration of miberfradil enhances the antiociceptive effects of morphine and attenuates development of tolerance (Dogrul *et al.*, 2001, 2002), suggesting further studies into the role of T-type channels and pain perception may yield promising new treatments.

Olfaction

Accumulating data reveal that T-type channels are involved in transduction of olfactory stimuli to the central nervous system. T-type currents are recorded from receptor cells in the olfactory epithelium in a variety of species (Kawai *et al.*, 1997, 1999; Kawai and Miyachi, 2001b; but see Trombley and Westbrook, 1991), and odorant-evoked action potentials are blocked by T-type channel blockers (Kawai and Miyachi, 2001a). Electrophysiological studies have sugested that odorants affect T-type channels in two distinct manners. Some studies of newt olfactory receptor cells indicate that odorants suppress T-type currents by shifting their inactivation curves to more negative values (Kawai, 1999; Kawai *et al.*, 1997). Paradoxically, other studies by some of the same investigators report that T-type channels lower the action potential threshold (Kawai *et al.*, 1996; Kawai and Miyachi, 2001a) and

contribute significantly to inward current during action potentials (Kawai and Miyachi, 2001a) in newt olfactory receptor cells. As T-type channels are also recorded from output neurons in the olfactory bulb (Charpak *et al.*, 2001; Wang *et al.*, 1996b), these data suggest that T-type currents are important in olfactory signal transduction.

Autonomic Ganglia

T-type calcium currents are recorded from some autonomic neurons such as those in submandibular (Endoh and Suzuki, 1998; Hayashi *et al.*, 2002) and pelvic (Akasu *et al.*, 1990; Park *et al.*, 2001; Zhu *et al.*, 1995) ganglia. Similar to the distribution of T-type channels in dorsal root ganglia, it is unlikely that all neurons within a ganglion express T-type channels, and the presence of T-type channels may be correlated with phenotypic markers of subpopulations of neurons (Zhu *et al.*, 1995). Although the molecular identity of most T-type channels has not been identified, low levels of alpha1G, but not alpha1H or alpha1I, mRNA are present in adult rat superior cervical ganglion (Talley *et al.*, 1999). In contrast, alpha1H mRNA is found in pelvic ganglia (Lee *et al.*, 2002). The functional significance of T-type channel expression is not known at this time. However, hyperpolarization of major pelvic ganglion neurons produces LTS, similar to central neurons (Lee *et al.*, 2002). Furthermore, transfection of nodose ganlia primary sensory neurons with antisense oligonucleotides against LVA alpha1 subunits increases the rate of repolarization during action potentials and blocks calcium influx during spikes (Lambert *et al.*, 1998). These data suggest that T-type channels may have a similar role in some neurons of the sympathetic and parasympathetic nervous systems.

Expression of T-Type Channels Varies During Neural Development

Changes in intracellular calcium can influence a plethora of events necessary for neuronal maturation including neuronal survival, neurite outgrowth, gene expression, and synapse formation and plasticity (reviewed in Ghosh and Greenberg, 1995). Expression and assembly of HVAs are under tight developmental and metabolic regulation (reviewed in McEnery *et al.*, 1998b), and both functional and anatomical data indicate that expression of T-type channels is also controlled during neural development.

T-type currents are commonly observed in the early development of cells (reviewed in Gu *et al.*, 1999), including neurons. T-type currents are recorded from embyronic dorsal root ganglia (Carbone and Lux, 1984a; Desmadryl *et al.*, 1998; Gottmann *et al.*, 1988; Lux, 1988), hippocampal neurons (Yaari *et al.*, 1987), neural plate neurons (Gu and Spitzer, 2001), and spinal motoneurons (Barish, 1991b; McCobb *et al.*, 1989; Martin-Caraballo and Greer, 2001). T-type currents are also observed in fetal neurons from a variety of regions including dorsal root ganglia (Fedulova *et al.*, 1991), visual cortical neurons (Tarasenko *et al.*, 1998), hippocampal neurons (Takahashi *et al.*, 1989a), and laterodorsal thalamic nuclei (Tarasenko *et al.*, 1997).

Similar to T-type currents observed in other tissues, the density, amplitude, and properties of neuronal T-type channels change over time. In some neurons, T-type currents increase during development. Both the amplitude and density of T-type currents increase in embryonic dorsal root ganglia (Desmadryl *et al.*, 1998; Gottmann *et al.*, 1988) and autonomic ciliary ganglia (Gottmann *et al.*, 1988). Potentiated T-type channel currents are observed after birth in cat thalamocortical cells (Pirchio *et al.*, 1990), rat dorsal root ganglia (Fedulova *et al.*, 1991), and retinal Muller cells (Bringmann *et al.*, 2000). A more complex potentiation of T-type currents is observed in some thalamic nuclei. Homogeneous T-type currents in laterodorsal thalamic nuclei are recorded at P12, but heterogeneous LVA channels are observed on the basis of pharmacological sensitivities and kinetic profiles by P14 (Tarasenko *et al.*, 1997), suggesting the appearance of a second functional T-type channel. Finally, studies of hippocampal neurons support the hypothesis that the identity of the T-type channel may change with development, as perinatal neurons have different kinetics than older T-type currents in culture (Thompson and Wong, 1991). Alternatively, perinatal T-type channels may differentially associate with modulatory molecules, thus accounting for heterogeneous channel kinetics.

Some neuronal populations exhibit decreased expression of T-type currents during development. The percentage of *Xenopus* and rat neural plate neurons with T-type currents decreases with the age of the culture (Gu and Spitzer, 2001) or animal (Frischkrecht and Randall, 1998). In rat visual cortical neurons, the density of T-type currents declines in early postnatal development, while HVA current density progressively increases until they are the exclusive calcium current observed (Tarasenko *et al.*, 1998). Similar patterns of calcium current expression are also observed in hypoglossal (Berger *et al.*, 1996), phrenic (Martin-Caraballo and Greer, 2001), and spinal (McCobb *et al.*, 1989) motoneurons in situ, and cultured hippocampal neurons (Chameau *et al.*, 1999; Thompson and Wong, 1991) and neuroblastoma (NB-l) cells (Kito

et al., 1999). For example, approximately 60% of NB-l cells express T-type currents after 2 days in culture, but only ~20% have T-type currents after 2 weeks in culture (Kito *et al.*, 1999). Concomitant with the loss of LVA currents, the percentage of cells with HVA currents increases over time (Kito *et al.*, 1999). Finally, a gradual decline in T-type current densities or in the number of neurons with T-type currents is observed a few weeks after birth in rat dorsal root ganglia (Fedulova *et al.*, 1991, 1994; but see Lovinger and White, 1989) and Muller cells (Bringmann *et al.*, 2000). However, T-type currents are still present in adult cells (Bringmann *et al.*, 2000; Fedulova *et al.*, 1991). Interestingly, a recent study suggests that T-type currents regulate expression of HVA currents (Chemin *et al.*, 2002b). Treatment either nickel, a nonspecific T-type channel antagonist, or antisense alpha1H cDNA reduces HVA current independent of neurite elongation (Chemin *et al.*, 2002b), providing a tantalizing clue into mechanisms responsible for coordinated LVA and HVA expression.

Although T-type currents are robustly expressed during formation of the nervous system, the molecular identity of the pore-forming subunits is largely unknown at this time. Alpha1H mRNA is observed in the developing central nervous system at embryonic day 9 by in situ hybridization (Son *et al.*, 2002), and alpha1G (Monteil *et al.*, 2000a) and alpha1I (Monteil *et al.*, 2000b) transcripts are observed in dot blots of fetal human tissues. Comparison of the relative intensities of these signals suggests differential regulation of individual LVA alpha1 transcripts in development. Whereas whole brain extracts contain similar levels of alpha1G mRNA compared to fetal tissues (Monteil *et al.*, 2000a), fetal brains contain more alpha1I mRNA than fetal brain extracts (Monteil *et al.*, 2000b).

More recently, we have used our affinity-purified antipeptide antibodies against alpha1G or alpha1I to examine the spatial and temporal patterns of LVA alpha1 expression in postnatal mouse development (Yunker *et al.*, 2003). At postnatal (P1), little alpha1G immunoreactivity is observed in either forebrain or hindbrain homogenates (Fig. 4(A)). In contrast, robust alpha1I immunoreactivity is found in both forebrain and hindbrain homogenates (Fig. 4(B)). Over the next three postnatal weeks, alpha1G immunoreactivity increases in forebrain and hindbrain homogenates, and differences are observed between these two regions. First, hindbrain homogenates contain more alpha1G immunoreactivity than forebrain homogenates by P10, and heterogeneity in the distribution of alpha1G is maintained into adulthood (Fig. 4(A)). Second, hindbrain alpha1G isoforms have different average molecular sizes (~260 kDa, intense band; ~227 kDa, faint band) compared to alpha1G in forebrain homogenates (~240 kDa).

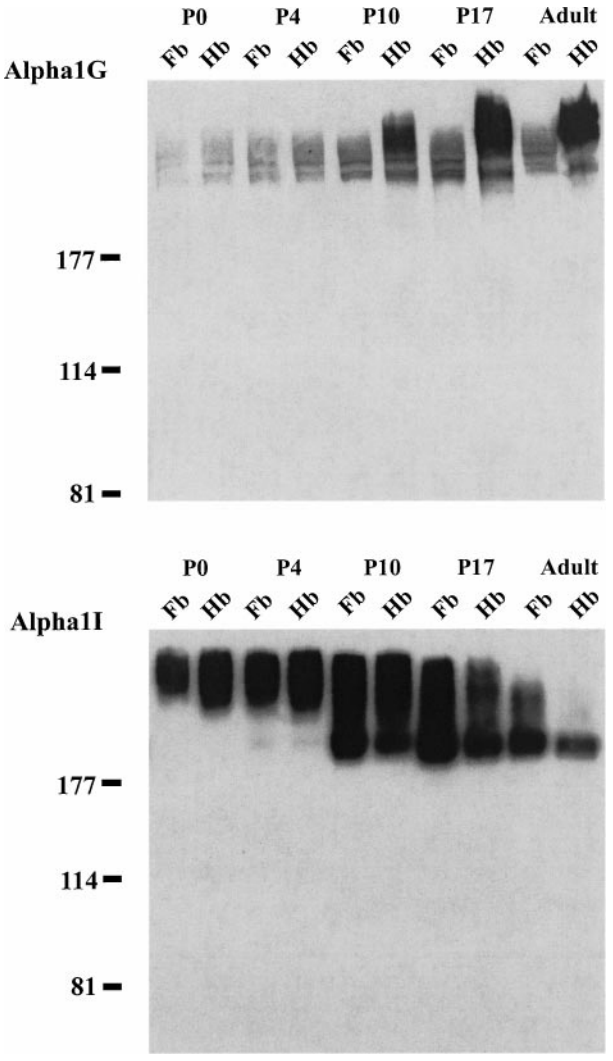

Fig. 4. Low-voltage-activated calcium channel alpha1G and alpha1I subunit proteins are differentially expressed during development of murine forebrain (Fb) and hindbrain (Hb). Tissues were harvested from postnatal (P) mice, where P0 is defined as the day of birth. Homogenates were prepared and analyzed as described previously (McEnery *et al.*, 1998b) using affinity-purified antipeptide antibodies raised against alpha1G or alpha1I (Yunker *et al.*, 2003). The apparent molecular size markers (kDa) are indicated on the left.

More surprising was the distribution of alpha1I immunoreactivity during postnatal development (Fig. 4(B)). Over time, the presence of the robust large (~260 kDa) alpha1I immunoreactive band decreases in both forebrain and hindbrain homogenates (Fig. 4(B)). Concomitant with this decrease is the gradual appearance of a smaller alpha1I immunoreactive isoform (~190 kDa), whose expression is positively correlated with the age of the animal into adulthood (Fig. 4(B)). Therefore, although antibodies against

alpha1G appear to react with the same proteins in perinatal developement and adulthood, alpha1I expression in both forebrain and hindbrain is markedly affected by developmental age. Although the underlying cause of the heterogeneously sized alpha1G and alpha1I bands is unclear at this time, both alpha1G and alpha1I transcripts undergo alternative splicing (see above). Differences in the length of the mRNA transcripts can result in profound differences in the length of the protein (see Tables II and III), suggesting that unique alpha1G and alpha1I protein isoforms are subjected to regional and temporal regulation of expression.

T-Type Currents Are Affected by Aging

A few studies suggest that T-type channels are altered by aging. Old rat neurons from various septal nuclei display increased T-type current density, but not different biophysical properties, compared to young neurons (Murchison and Griffith, 1995). In contrast, whereas T-type and HVA currents are recorded in cultures of dorsal root ganglia from neonatal and adult rats, cultures of dorsal root ganglia from old rats only exhibit HVA currents (Kostyuk *et al.*, 1993). These results imply that temporal expression of T-type channels is under regional control and that unique expression profiles of T-type channels in old age may contribute to age-dependent alterations in calcium homeostasis.

Putative Functions of T-Type Channels in Neural Development

At this time, there is much speculation on the role of T-type channels in development of the nervous system. Studies of cultured nonneuronal cells suggest that T-type currents are an important source of intracellular calcium during proliferation. Robust T-type currents are recorded from blastocyst-derived embroyonic stem cells (Day *et al.*, 1998), and proliferating fibroblasts (Chen *et al.*, 1988; Estacion, 1991), vascular smooth muscle cells (Schmitt *et al.*, 1995, 1996), and cardiac myocytes (Guo *et al.*, 1998). T-type channel antagonists block both smooth muscle cell proliferation (Schmitt *et al.*, 1995; but see Chemin *et al.*, 2002b) and cell cycle progression of endothelial cells (Bertolesi *et al.*, 2002; Nilius *et al.*, 1997), suggesting calcium influx through T-type channels is important for cell cycle regulation. Furthermore, functional T-type channels are detected in G1 and S phases, but not in G2 and M in cultured smooth muscle and cardiac myocytes (Day *et al.*, 1998; Guo *et al.*, 1998; Kuga *et al.*, 1996; Richard *et al.*, 1992), indicating both the presence and level of T-type channel expression correlates to cell cycle.

Although overexpression of either alpha1G or alpha1H does not affect proliferation of HEK-293 cells (Chemin *et al.*, 2000), at least three lines of preliminary evidence suggest that T-type channels are also important for neuronal proliferation. Many mitogens, including angiotensin II (McCarthy *et al.*, 1993), endothelin (Furukawa *et al.*, 1992; Yokokawa *et al.*, 1990), and platelet-derived growth factor (Wang *et al.*, 1993), potentiate T-type currents and induce proliferation (reviewed in Ertel *et al.*, 1997). Expression of both T-type channels and LVA alpha1 mRNA decreases after differentiation of some cultured cells (Asem *et al.*, 2002; Hirroka *et al.*, 2002), implying that T-type currents are no longer needed by the cell. Finally, antibodies against ganglioside GM1 stimulate neural proliferation and activate a T-type channel in Neuro2a cells (Ravichandra and Joshi, 1999). As treatment with amiloride, a nonspecific T-type channel blocker, prevents induction of proliferation, T-type channels may be important in coupling calcium to intracellular events necessary for cell division (Ravichandra and Joshi, 1999).

The early expression of functional T-type channels may also be involved in morphological differentiation, as T-type channels are present in growth cones of differentiating neuronal precursor cells (Gottmann *et al.*, 1991; Gottmann and Lux, 1990) and regenerating neurities in culture (Gottmann and Lux, 1995). Morphological differentiation of dorsal root ganglion neurons with fetal calf serum or dibutyryl cAMP enhances T-type currents (Pirchio *et al.*, 1990), and differentiation of pheochromocytoma (PC12) cells with nerve growth factor and dexamethasone induces the appearance of T-type channels (Garber *et al.*, 1989). In contrast, untreated PC12 cells predominantly conduct HVA currents (Garber *et al.*, 1989).

A different pattern of developmental expression can be observed in other cell types, as undifferentiated SN56 cells (a fusion of septal neurons with neuroblastoma cells) express robust T-type currents (Kushmerick *et al.*, 2001). Differentiation of SN56 cells with dibutyryl-cAMP induces HVA currents, and alters the pharmacological and kinetic profiles of T-type currents (Kushmerick *et al.*, 2001), again suggesting a relationship between calcium channel expression and cell morphology. Finally, increased T-type currents are recorded from all N1E-115 neuroblastoma cells treated with dimethylsulphoxide to induce morphological differentiation (Silver and Bolsover, 1991), although some undifferentiated N1E-115 cells do not express detectable T-type currents (Silver and Bolsover, 1991). However, these results may vary depending on the methods and cells used, as other

investigators report that differentiation of NIE-115 cells induces HVA channel expression (Chemin *et al.*, 2002b; Leuranguer *et al.*, 1998), resulting in coexpression of LVA and HVA calcium currents.

Although there is a clear correlation between T-type channel expression and morphological differentiation, at this time a few studies directly support the hypothesis that T-type channels are necessary for neuronal differentiation. Optical imaging studies suggest that calcium influx through T-type channels present in somata, neurites, and growth cones of cultured embryonic *Xenopus* spinal neurons contributes to rapid depolarization-induced calcium transients in neurites and growth cones (Barish, 1991a). However, nickel does not affect neurite initiation or extension in N1E-115 cells (Audesirk *et al.*, 1990), although nickel sensitive T-type currents are recorded from these cells (Audesirk *et al.*, 1990). In contrast, nickel, miberfradil, and antisense alpha1H cDNA reduce neuritogenesis, but not neurite elongation, in NG108-15 cells (Chemin *et al.*, 2002b). Additional support for a role of T-type channels in differentiation comes from studies of neuroendocrine differentiation of prostate cancer epithelial cell line. Long-term treatment of these cells with cyclic AMP analogs increases neuroendocrine differentiation and induces the appearance of T-type currents resulting from increased number of alpha1H transcripts (Mariot *et al.*, 2002). Long-term treatment with nickel attenuated neurite extension (Mariot *et al.*, 2002), suggesting that calcium entry near resting membrane potentials may promote (but not initiate) morphological differentiation (Mariot *et al.*, 2002). Finally, the neuroprotective glycoprotein erythropoietin enhances neurite outgrowth of retinal ganglion cells, and this effect is totally inhibited by ethosuximide, a postulated T-type channel antagonist (Bocker-Meffert *et al.*, 2002). Interestingly, erythropoietin enhances T-type currents in a neuroblastoma cell line (Assandri *et al.*, 1999), implying that T-type channels may be important for neurite initiation and extension in a cell-dependent manner.

The embryonic and fetal expression of T-type channels may be necessary for the generation of many types of electrical signals needed in developing neurons. Weak excitatory inputs can open T-type channels, as T-type channels in embryonic neurons have a low threshold of activation. The presence of T-type channels lowers the action potential threshold by 15 mV in mathematical models (Gu and Spitzer, 2001), and experiments using digital waveforms modeled after natural action potentials demonstrate that T-type calcium channels can contribute ~50% of total calcium entry during brief action potentials in dorsal root ganglion neurons (McCobb and Beam, 1991). T-type channels can also contribute to resting calcium levels, as overlap of activation and inactivation curves of T-type channels can result in "window currents" that result in continual calcium influx and substantial changes in intracellular calcium levels (reviewed in Bernheim and Bader, 2002). T-type channels mediate burst firing in postnatal hypoglossal motoneurons (Berger *et al.*, 1995) and spontaneous transient calcium spikes in *Xenopus* neural plate neurons in vivo and in vitro (Gu *et al.*, 1994; Gu and Spitzer, 2001). For both cell types, the frequency of these spikes is correlated with the presence of T-type channels (Berger *et al.*, 1995; Gu *et al.*, 1994) and decreases with developmental age. Furthermore, these spikes can be triggered by depolarization, resulting in depletion of intracellular calcium stores in *Xenopus* neural plate neurons (Gu *et al.*, 1994), indicating that T-type channels couple extracellular calcium influx to intracellular calcium stores in immature neurons. T-type channels are also responsible for generation of spontaneous calcium-dependent slow waves postulated to be important for phenotypic expression and neurite growth (Gu *et al.*, 1994). Finally, T-type channels modulate after-depolarizing potentials in postnatal dorsal root ganglion neurons (Lovinger and White, 1989), suggesting that the presence of functional T-type calcium channels can have profound effects on the electrical properties of nascent neurons.

T-Type Channels Are Implicated in Neuronal Diseases

T-type channels have been implicated in a number of neuronal and nonneuronal diseases, and virtually nothing is known regarding the cellular or molecular mechanisms that control expression of T-type channels. Although the best evidence for T-type channels in disease comes from the study of absence seizures (discussed below), T-type channels have been postulated to be involved in other neurological diseases, including Parkinson's disease. Parkinson's disease is accompanied by a 3–6 Hz resting tremor correlated with rhythmic firing of ventral lateral thalamocortical neurons that project to premotor cortex. The rhythmic firing may result from rebound burst firing of T-type channels in response to GABA-mediated IPSPs (Pare *et al.*, 1990; Steriade and Llinás, 1988; reviewed in Llinás, 1988; Perez-Reyes, 1998), and ethosuximide treatment reduces MPTP-induced resting tremor (Gomez-Mancilla *et al.*, 1992). In contrast, diltiazem and verpamil did not reduce resting tremors. As ethosuximide has been postulated to be a T-type channel antagonist (reviewed in Yunker, 2003), these data suggest that T-type channels may be involved in resting tremors (Gomez-Mancilla *et al.*, 1992).

T-type channels may also be involved in other neurological diseases. For example, the gene encoding alpha1G is located near the *teetering* locus (Perez-Reyes, 1998 Perez-Reyes *et al.*, 1998, 1999), and mutations in *teetering* mice are associated with cerebellar atrophy and ataxia (Meier, 1967). In contrast, *alpha1H* maps near the tuberous sclerosis locus TSC2 (16p13.3) (Perez-Reyes *et al.*, 1999; T.E.P.K.D. Consortium, 1994), and lies in a region linked to many diseases including asthma, epilepsy, autism, and susceptibility to bipolar disorder (Daniels *et al.*, 2001). The gene encoding alpha1I maps to 22q12.3–q13.2, the site of several neuronal disorders including the susceptibility locus for familial schizophrenia (Pulver *et al.*, 1994) and spinocerebellar ataxia 10 (Matsuura *et al.*, 2000; Zu *et al.*, 1999). Interestingly, spinocerebellar ataxia 10 is associated with a pentanucleotide repeat expansion, similar to trinucleotide repeat expansions observed in other forms of spinocerebellar ataxias (Matsuura *et al.*, 2000). Although the cellular consequences of such repeats are largely unknown, transfection of a neuroblastoma-motor neuron hybrid cell with a cDNA harboring a trinucleotide repeat results in altered steady-state activation of T-type currents and increased cell death that is prevented by treatment with nickel (Schulptoreanu *et al.*, 2000). Taken together, we suggest that T-type channels may contribute to cellular toxicity in neuropathies resulting from polynucleotide expansion.

Seizures

In addition to their role underlying normal physiological processes, alterations in VDCC expression and/or regulation are likely to play a role in some seizure disorders. Intracellular calcium concentrations increase and extracellular calcium concentrations decrease during seizures (Heinemann *et al.*, 1977), implying altered calcium homeostasis and/or signaling contribute to the underlying etiology of these diseases. This hypothesis has been strengthened in recent years by the discovery that spontaneous mutations in HVA alpha1 or auxiliary subunits result in absence seizures and ataxia (reviewed in Fletcher *et al.*, 1998), potentially as a consequence of altered calcium influx and homeostasis.

Absence epilepsy is a generalized, nonconvulsive seizure disorder characterized by epileptic attacks associated with bilaterally synchronous 3 Hz cortcial spike-wave discharges recorded by EEG, occurring simultaneously with behavioral immobility (reviewed in Avoli *et al.*, 2001; Burgess and Noebels, 1999). This spindle activity is similar to sleep wave spindles observed from EEG recordings of normal individuals (discussed above). The spindles are postulated to result from cyclic opening and closing of thalamocortical T-type channels (reviewed in Huguenard, 1996). A thalamocortical circuit composed of neocortical pyramidal cells, thalamic relay neurons, and thalamic reticular neurons is sufficient to sustain abnormal oscillatory rhythms similar to spike-wave discharges, and T-type channels are recorded from these neurons in all three locations (Coulter and Lee, 1993; Zhang and Coulter, 1996).

Pharmacological treatment of absence seizures supports the hypothesis for increased calcium entry, as some classes of anticonvulsant drugs inhibit T-type currents in thalamic and dorsal root ganglion neuronal cultures (Coulter, 1997; Coulter *et al.*, 1989b, 1990; Kostyuk *et al.*, 1992; reviewed in Yunker, 2003) and T-type currents conducted by recombinant LVA alpha1 subunits (Gomora *et al.*, 2001; Todorovic *et al.*, 2000). Furthermore, some of these drugs, such as ethosuximide, trimethadione, and methsuximide, attenuate seizure activity in humans, with an effective pharmacological blood concentration that is sufficient to reduce T-type currents, but not HVA currents, in thalamic relay and reticular neurons in vitro (Coulter, 1997; Coulter *et al.*, 1989b, 1990). These drugs appear to decrease total T-type current without altering gating kinetics of the affected channels (Coulter *et al.*, 1991), and increase threshold for burst generation in thalamic neurons (Huguenard and Prince, 1994). Taken together, these data suggest that increased T-type currents contribute to the underlying cause of absence seizures.

Additional support for the hypothesis that absence epilepsy results from increased T-type current comes from study of rodent models of absence epilepsy. Increased T-type currents were first observed in select thalamic nuclei from genetic absence epileptic rats from Strasbourg model (GAERS), an animal model of absence epilepsy (Tsakiridou *et al.*, 1995). In contrast, the amplitude of L-type currents was not affected (Tsakiridou *et al.*, 1995). Treatment of WAG jj rats, a second rodent model of general absence seizures, with ethosuximide reduces both the number and total duration of spike-wave discharges (van Luijtelaar *et al.*, 2000). Similarly, a single dose of ethosuximide suppresses absence seizure behavior and spike wave discharges in *lethargic* mice harboring a mutation in the auxiliary subunit beta4 (Burgess *et al.*, 1997; Ishige *et al.*, 2001). Anatomical studies suggest that thalamic reticular nuclei from juvenile GAERS animals display increased alpha1H mRNA, whereas adult GAERS animals harbor elevated levels of alpha1G mRNA in ventral posterior thalamic relay nuclei in addition to increased alpha1H mRNA (Talley *et al.*, 2000). T-type peak current densities and peak current amplitudes are increased in thalamic lateral dorsal nuclei in epileptic mice harboring mutations in beta4, gamma2, or alpha1A, although there is no difference in the

localization of LVA alpha1 transcripts by in situ hybridization (Zhang *et al.*, 2002). T-type currents in thalamic lateral dorsal nuclei from all of these mutant mice also display a depolarizing shift in stead-state inactivation (Zhang *et al.*, 2002), implying that a greater percentage of T-type channels are available for opening (Zhang *et al.*, 2002). Finally, although mice harboring spontaneous mutations in LVA alpha1 subunits have not been identified to date, the *teetering (tn)* mouse mutation maps near the *cacna1g* locus (Meier, 1967; Montgomery *et al.*, 1997). Interestingly, teetering mice have abnormal brain-stem and spinal cord development, ataxia, and progressive cerebellar neuronal atrophy (Meier, 1967), similar to some mice with mutations in HVA subunits (reviewed in Fletcher *et al.*, 2001).

The most compelling evidence for a role of LVA alpha1 subunits in generalized absence seizures to date comes from analysis of mice engineered to lack the gene encoding alpha1G (Kim *et al.*, 2001). When compared to wild-type littermates, alpha1G-deficient mice are more resistant to drug-induced seizures (Kim *et al.*, 2001). Although the molecular mechanisms by which increased T-type currents may contribute to disease is unknown at this time, T-type channels may act at thalamocortical circuits to promote electrical activity (Coulter, 1997; Coulter *et al.*, 1989a; Huguenard and McCormick, 1992; McCormick and Huguenard, 1992; Pellegrini *et al.*, 1989). However, increased T-type currents are observed in other brain regions such as hippocampal dentate gyrus neurons (Beck *et al.*, 1998) and CA1 pyramidal neurons (Su *et al.*, 2002) in some models of epilepsy. The upregulation of T-type channels in these neurons may result in the de novo appearance of burst firing in inappropriate brain regions (Su *et al.*, 2002), contributing to abnormal electrical activity and behavior.

In addition to altering the electrophysiological properties of individual cells and neuronal circuits, T-type channels may also be involved in regulation of gene expression in pathophysiological states such as epilepsy. Calcium is an important regulator of gene activity and multiple DNA regulatory elements are activated by binding calcium (reviewed in Bading, 1999). For example, cyclic AMP responsive element binding protein (CRE) and activator protein 1 (AP-1) bind specific sequences in DNA promoters and are important modulators in activity-dependent plasticity of the central nervous system (reviewed in Montminy *et al.*, 1990). The DNA binding activity of CRE- and AP-1 are elevated in thalamus and cortex from *lethargic* and gamma-butyrolactone-induced epileptic mice (Ishige *et al.*, 1996, 1998, 1999), suggesting alterations in transcription may contribute to the genesis and/or maintenance of seizure activity. Interestingly, T-type channel antagonists reduce elevated DNA binding activities of CRE and AP-1 following seizures (Ishige *et al.*, 1996, 1998, 2001), indicating that alterations in gene transcription may be regulated by calcium entry through T-type channels, potentially contributing to molecular disease mechanisms. However, as mutations in HVA subunits including alpha1A (*tottering, leaner, rolling mouse Nagoya, alphaA*$^{-}$), beta4 (*lethargic*), gamma2 (*stargazer*), and alpha2/delta2 (*ducky*) result in absence seizures (reviewed in Burgess and Noebels, 1999; Fletcher *et al.*, 1998; Frankel, 1999), it will be of great interest to determine if these separate mutations converge into a common disease mechanism, such as altered T-type channel expression. Alternatively, different genetic lesions may each result in unique disease mechanisms.

SPATIAL AND TEMPORAL PATTERNS OF ENDOGENOUS CARDIAC AND SMOOTH MUSCLE T-TYPE CHANNEL EXPRESSION

T-Type Channels Are Expressed in Adult Cardiac Tissues

Cardiac Myocytes

Whereas electrophysiological data clearly indicated the presence of multiple types of T-type calcium channels in neurons, it was assumed that there was only one HVA isoform in cardiac cells. However, recordings from canine atrial cells were some of the first data to demonstrate T-type currents in cardiac myocytes (Bean, 1985), and T-type calcium currents have been recorded from atrial myocytes in a variety of species (Bonvallet, 1987; Xu and Best, 1990; reviewed in Ertel *et al.*, 1997). In contrast, canine ventricular cells exhibit little T-type currents compared to atrial cells (Bean, 1985), suggesting heterogeneity in the localization and function of T-type channels within cardiac tissues. Comparison of calcium currents recorded from different cardiac cells from many species also reveals that the presence of functional T-type channels depends on the species examined. For example, robust T-type calcium currents are observed in ventricular cells from adult guinea pigs (Droogmans and Nilius, 1989; Mitra and Morad, 1986; Nilius *et al.*, 1985) and sharks (Maylie and Morad, 1995), but not in adult ventricular cells from many other species (Bean, 1985; Osaka and Joyner, 1991; Richard *et al.*, 1990; reviewed in Bootman *et al.*, 2001), indicating both bona fide anatomical and species differences in functional T-type channel expression.

It is unlikely that healthy adult human atrial or ventricular myocytes express T-type channels. Although

alpha1G (Monteil *et al.*, 2000a) and alpha1H (Cribbs *et al.*, 1998; Williams *et al.*, 1999) transcripts are detected in Northern blots of human heart, T-type calcium currents are not detected in adult human atrial (Ouadid *et al.*, 1991) or ventricular (Beuckelmann *et al.*, 1991) myocytes. Furthermore, LVA alpha1 mRNA transcripts are not detected in adult human myocytes by in situ hybridization (Jagannathan *et al.*, 2002b), supporting the conclusion that T-type currents are not present normal human adult myocytes.

At this time, the physiological function of T-type channel expression in cardiac myocytes is unclear. T-type channels were originally thought not to play a role in excitation–contraction coupling in myocytes (Bootman *et al.*, 2001). However, T-type channel blockers such as mibefradil can shorten the duration of guinea pig ventricular action potentials (Bénardeau *et al.*, 2000) and attenuate guinea pig ventricular myocyte contractions (Emanuel *et al.*, 1998; Hoischen *et al.*, 1998; Sipido *et al.*, 1998). T-type currents can induce calcium release from ventricular sarcoplasmic reticulum in some species (Brotto and Creazzo, 1996; Emanuel *et al.*, 1998). However, it should be noted that T-type currents are much less effective than L-type currents in evoking calcium release, implying that T-type currents play only a minor role in normal excitation–contraction coupling (reviewed in Lipsius *et al.*, 2001).

Cardiac Pacemaker Cells

In contrast to myocytes, robust T-type currents are observed in Purkinje cells (Hirano *et al.*, 1989; Tseng and Boyden, 2002), sinoatrial node cells (Hagiwara *et al.*, 1988), and latent pacemaker cells (Zhou and Lipsius, 1994) from a variety of species, suggesting that T-type channels are important in cardiac conduction and pacemaking (reviewed in Massie, 1998; Triggle, 1998). T-type currents are severalfold larger in atrial pacemaker cells than in nonpacemaker atrial myocytes (Zhou and Lipsius, 1994), and blockade of T-type calcium currents slows the firing rate and pacemaker cycle (Fareh *et al.*, 1999; Hagiwara *et al.*, 1988; Hüser *et al.*, 2000; Li *et al.*, 1997; Zhou and Lipsius, 1994). These data indicate that T-type channels aid in depolarization of pacemaker cells (Bean, 1985; Hagiwara *et al.*, 1988; Zhang *et al.*, 2000), action potential initiation, and sinus node automaticity (Hagiwara *et al.*, 1988; Hüser *et al.*, 2000; Nilius *et al.*, 1985; Officer and Ewert, 1998; Xiao *et al.*, 2000). Furthermore, T-type channels may conduct a significant calcium influx during the plateau phase of cardiac pacemaker action potentials (Bean, 1985), suggesting multiple roles for T-type channels in cardiac atrial cells.

Accumulating evidence reveals that similar to some ventricular myocytes, T-type channel currents induce intracellular calcium release in both cardiac Purkinje and feline atrial pacemaker cells. Electrophysiological studies using a perforated patch technique show that calcium influx through either T-or L-type calcium channels triggers calcium release from the sarcoplasmic reticulum and induces canine Purkinje cells shortening although T-type channels are less effective than L-type channels (Zhou and January, 1998). Similarly, T-type calcium influx in atrial pacemaker cells triggers a local release of subsarcolemmal intracellular calcium that in turn stimulates an inward sodium-calcium exchange current, resulting in further membrane depolarization and firing of action potentials (Hüser *et al.*, 2000; reviewed in Lipsius *et al.*, 2001). As predicted, some T-type channel blockers prevent intracellular calcium release from atrial nodal and latent atrial pacemaker cells, but not atrial myocytes (Hüser *et al.*, 2000; reviewed in Lipsius *et al.*, 2001), suggesting that the high density of T-type channels on specific cells in the cardiovascular system lead to profound physiological consequences.

Expression of T-Type Channels Varies During Cardiac Development

Additional insights into the complexity of T-type channel expression come from developmental studies that analyze the functional and/or anatomical expression of T-type channels over time. Cardiac contractility changes during development (Nair and Nair, 2001), and similar to the patterns observed in both neural (see above) and smooth muscle (see below) cells, cardiac T-type channel expression depends on the developmental age of the tissues analyzed. T-type currents are commonly recorded from embryonic tissues such as chick ventricle cells (Kawano and DeHaan, 1989, 1990, 1991), and neonatal tissues, including rodent atrial (Leuranguer *et al.*, 2000; Xu and Best, 1992) and ventricular (Ferron *et al.*, 2002; Guo *et al.*, 1998; Leuranguer *et al.*, 2000; Nuss and Marbàn, 1994; but see Davies *et al.*, 1996) myocytes. At least two different patterns of developmental changes are observed in cardiac T-type channels. Electrophysiological recordings from some tissues indicate that the pharmacological and/or biophysical characteristics of T-type channels change during development (Kawano and DeHaan, 1991), potentially as a result of endogenous modulators or developmental switching of the molecular identity of the pore-forming subunit. The density of T-type currents can

also change. The density of peak T-type currents is maximum near birth, and then decreases concomitant with maturation (Ferron *et al.*, 2002; Guo *et al.*, 1998; Leuranguer *et al.*, 2000; Xu and Best, 1992; but see Wetzel *et al.*, 1991), perhaps reflecting attenuated levels of functional T-type channels.

Embryonic and postnatal T-type currents are frequently larger than L-type currents (Kawano and DeHaan, 1989; Leuranguer *et al.*, 2000), suggesting that T-type currents are an important source of calcium influx for developing cells. Embryonic ventricular myocytes are capable of automatic activity, unlike adult cells (Cribbs *et al.*, 2001), implying the presence of functional T-type channels may promote spontaneous electrogenic activity. Alternatively, T-type channels may be important in control of cardiac cell cycle, similar to smooth muscle cells (see below), as T-type ventricular myocytes in S phase express a very high density of T-type channels compared to cells in other stages of mitosis (Guo *et al.*, 1998). T-type current density increase over twofold prior to onset of cardiac hypertrophy in adult atrial myocytes isolated from rats with growth hormone-secreting tumors (Xu and Best, 1990). In contrast, L-type currents in atrial and ventricular myocytes are not altered, supporting the hypothesis that T-type channel expression is involved in myocyte proliferation (see above and below). Furthermore, attenuation of T-type currents, but not L-type currents, is associated with both loss of proliferative ability (Guo *et al.*, 1998) and differentiation (Fares *et al.*, 1996), indicating a relationship between cell cycle progression, differentiation, and T-type channel expression. Finally, it should be noted that the T-type currents may also be involved in nonproliferative, nonelectrogenic processes. Blockade of neonatal atrial T-type channels reduces basal and depolarization-evoked atrial natriuretic factor release (Leuranguer *et al.*, 2000). As atrial natriuretic factor is important in controlling salt and water homeostasis, this finding reveals an indirect manner in which T-type channels may help modulate blood pressure (see below).

There is currently much interest in defining the molecular identity of the T-type channel pore(s) in developing and adult cardiac tissues. Northern blot analyses (Cribbs *et al.*, 1998; Leuranguer *et al.*, 2000; Monteil *et al.*, 2000a; Perez-Reyes *et al.*, 1998; Williams *et al.*, 1999), RT-PCR amplification (Ferron *et al.*, 2002; Leuranguer *et al.*, 2000; but see Cribbs *et al.*, 2001; Satin and Cribbs, 1999) and in situ hybridization (Bohn *et al.*, 2000) of cardiac tissues from a number of species reveal the presence of alpha1G and alpha1H transcripts in embryonic, neonatal, and/or adult heart. Furthermore, regional dissections of cardiac tissues indicate that atrial and ventricular tissues from neonatal and adult rats express both alpha1G and alpha1H transcripts (Leuranguer *et al.*, 2000; Ferron *et al.*, 2002; but see Cribbs *et al.*, 2001), and that the relative levels of alpha1G and alpha1H transcripts decreases during development (Ferron *et al.*, 2002; Huang *et al.*, 2000). However, it should be noted that T-type currents are not recorded from many adult ventricular myocytes (Leuranguer *et al.*, 2000), suggesting that paucity of T-type current in adult ventricular cells does not result from a lack of LVA alpha1 gene transcription.

Interestingly, the developmental data on T-type channel function and expression in rat may mimic the normal developmental pattern of T-type channel expression in humans. As described previously, alpha1G and alpha1H transcripts are present in adult human heart by Northern blot analysis (Cribbs *et al.*, 1998; Monteil *et al.*, 2000a; Williams *et al.*, 1999), but T-type currents are not observed (Beuckelmann *et al.*, 1991; Ouadid *et al.*, 1991). However, recent comparisons of LVA transcript levels during human development reveals that the quantity of both alpha1G (Monteil *et al.*, 2000a) and alpha1H transcripts decreases during human development (Qu and Boutjdir, 2002), indicating that T-type channels may transiently contribute to intracellular calcium levels during early human cardiac development.

As a complimentary method to determine the molecular identity of T-type channels, some investigators have compared the biophysical properties and pharmacological sensitivities of recombinant alpha1G and alpha1H channels to endogenous tissues in cardiac tissue (Cribbs *et al.*, 2001; Leuranguer *et al.*, 2000; Perchenet *et al.*, 2000) or AT-1 cells (Satin and Cribbs, 1999) derived from murine right atrial myocytes. These data have yielded conflicting opinions, as some data suggest that cardiac T-type channels are formed by alpha1G (Cribbs *et al.*, 2001; Satin and Cribbs, 1999; Leuranguer *et al.*, 2000), whereas other data imply that alpha1H forms cardiac T-type channels (Perchenet *et al.*, 2000). Preliminary analysis of alpha1H-deficient mice supports the later hypothesis, as alpha1H-deficient mice have abnormal electrocardiograms and small fibrotic regions (Chen *et al.*, 2002), suggesting abnormal cardiac function. Alternatively, different LVA alpha1 isoforms may be more prevalent during specific times, as both alpha1G and alpha1H may form T-type channels prior to birth, whereas postnatal T-type channels may be formed exclusively by alpha1G (Ferron *et al.*, 2002). Additional complexity in the molecular identification of cardiac T-type channels may arise if cardiac and neuronal T-type channels are formed by differential alternative splicing. Northern blot analysis of adult rodent heart indicates that cardiac alpha1G transcripts may be larger than neuronal alpha1G transcripts (Perez-Reyes *et al.*, 1998), and at least five alternative

splice sites have been identified in alpha1G, as described previously (Table II). Therefore, cardiac T-type channels formed by alpha1G (or alpha1H) may have different properties than the corresponding neuronal T-type channels. It is interesting to note that only the "d" isoform of alpha1G is amplified from both embryonic mouse heart and AT-1 cells (Cribbs *et al.*, 2001) although RT-PCR primers were designed against a consensus sequence found in all cloned LVA alpha1 subnits to date. Taken together, these data indicate that T-type channels are expressed in at least some cardiac cells. Development of both specific antagonists and antibodies against specific Ca_v3 isoforms may be necessary to conclusively identify the molecular pore of endogenous T-type channels.

T-Type Channels Are Implicated in Cardiac Disease

Calcium influx through VDCC determines cardiac muscle tone and cardiac pacemaker activity, and abnormalities in calcium channels and calcium homeostasis have been implicated in some cardiac diseases (reviewed in Richard *et al.*, 1998; Weisman, 1993). Analyses of T-type currents in at least four models of cardiac hypertrophy suggest either increased or de novo functional expression of T-type channels may contribute to the underlying disease (lto *et al.*, 1991, 1994; Nuss and Houser, 1993; Sen and Smith, 1994; Xu and Best, 1990). For example, T-type currents are recorded from adult feline and rat ventricular cells isolated from hearts with pressure–overload-induced hypertrophy, but not from control ventricular cells (Martinez *et al.*, 1999; Nuss and Houser, 1993; but see Ahmmed *et al.*, 2000). Similarly, increased T-type current densities are observed in ventricular cells from Syrian hamsters, a genetic model of cardiac calcium overload and congestive heart failure (Hoischen *et al.*, 1998; Sen and Smith, 1994). T-type currents from these hypertrophied cells display altered biophysical properties (Sen and Smith, 1994), potentially reflecting either de novo expression of a second type of T-type channel or differential modulation of T-type channels in hypertrophied heart. In contrast, L-type current densities are not affected (Sen and Smith, 1994), implying that altered T-type currents contribute to abnormal calcium homeostasis and/or cardiac function.

Treatment with mibefradil, a nonspecific T-type channel antagonist (reviewed in Yunker, 2003), may prevent disease-evoked anatomical alterations in myocardial tissues (Villame *et al.*, 2001). Although treatment with mibefradil does not appear to prevent the cellular appearance of necrosis and calcifications (Paquette *et al.*, 1999), mibefradil treatment appears beneficial in many animal models of heart failure (Clozel *et al.*, 1999). Mibefradil reduces ectopic ventricular activity (reviewed in Buhler, 1997) and promotes survival of rats with chronic heart failure (Mulder *et al.*, 1997), further suggesting that aberrant T-type channel expression contributes to cardiac malfunction. Interestingly, accumulating evidence suggests that cardiac hypertrophy shifts the expression of many genes towards a fetal program of gene expression (reviewed in Elvan, 2000), consistent with the recapitulation of T-type channel expression to a more immature state, such as increased expression of T-type channels (see above).

Although the physiological consequences of increased T-type currents in cardiac hypertrophy in unknown, increased T-type channel expression may increase the probability of spontaneous action potentials, thus increasing the likelihood for arrhythmias (Nuss and Houser, 1993). Alternatively, increased T-type currents may promote proliferation, as increased atrial T-type currents are observed prior to hypertrophy in rats harboring growth-hormone producing tumors (Xu and Best, 1990), and treatment with endothelin-1 induces both cardiac hypertrophy and increased T-type currents (Furukawa *et al.*, 1992; lto *et al.*, 1991, 1994). As expression of T-type channels is correlated with early development and cell cycle progression (see above), it is tempting to speculate that increased expression of functional T-type channels contributes to common cellular mechanisms important in both normal cardiac development and development of hypertrophy. If true, then the development of specific T-type channel blockers may be a useful therapeutic treatment for cardiac hypertrophy.

Ischemic Heart Damage

A handful of studies also suggest that T-type channels may be involved in ischemia-induced cardiac disease. Both T-type channel amplitude and density are acutely reduced in Purkinje myocytes isolated from ischemic hearts (Boyden and Pinto, 1994; Pinto *et al.*, 1999). In contrast, increased quantity of alpha 1G transcripts and T-type currents are recorded from ventricular myocytes after post-myocardial infarction (Huang *et al.*, 2000). Pharmacological studies suggest that blockade of T-type channels may be useful in preventing anatomical changes and improving cardiac performance after ischemia, as treatment with mibefradil improves a number of cardiac parameters after myocardial infarction (Sandmann *et al.*, 1998, 1999, 2000).

Atrial Fibrillation and Congestive Heart Failure

Whereas altered T-type currents are not observed in a canine model of atrial fibrillation (Yue *et al.*, 1997), mibefradil treatment prevents electrophysiological remodeling of the heart induced by atrial tachycardia, suggesting that T-type currents may provide sufficient calcium into cardiac cells to promote atrial arrhythmias (Fareh *et al.*, 1999). This finding is important because calcium overload and tachycardia-induced remodeling are thought to induce changes that further favor atrial arrhythmias (reviewed in Goette *et al.*, 1996; Nattel, 1999) and contribute to congestive heart failure (Nattel, 1999). However, T-type currents are not altered in atrial myocytes (Li *et al.*, 2000) or Purkinje cells (Han *et al.*, 2001) in experimental models of congestive heart failure (reviewed in Nattel and Li, 2000), implying that T-type channels have a complicated role in cardiac diseases.

Vascular and Nonvascular Smooth Muscle Express T-Type Channels

Electrophysiological recordings demonstrate that many smooth muscle cells isolated from different tissues and species display T-type currents. For example, T- and L-type currents are recorded from vacular smooth muscle cells isolated from cerebral, coronary, and peripheral blood vessels of varying diameters (Akaike *et al.*, 1989b; Bean *et al.*, 1986; Benham *et al.*, 1987; Brixius *et al.*, 1998; Friedman *et al.*, 1986; Ganitkevich and Isenberg, 1990, 1991; Hermsmeyer, 1991; Kuga *et al.*, 1990, 1996; Matsuda *et al.*, 1990; Neveu *et al.*, 1993, 1994; Ohya *et al.*, 1993; Schmitt *et al.*, 1995; Smirnov and Aaronson, 1992; but see Quignard *et al.*, 2001; Richard *et al.*, 1992). T-type currents are also observed in smooth muscle cells isolated from lymphatic ducts (Thornbury, 1999), adult bronchi (Janssen, 1997; Sui *et al.*, 2001), bladder (Nowycky *et al.*, 1985), stomach (Vivaudou *et al.*, 1988), proximal and distal intestine (Huizinga *et al.*, 1991; Smirnov *et al.*, 1992b; Xiong *et al.*, 1993, 1995), and uteri (Young *et al.*, 1993). Although the functional significance of T-type channels in nonvascular smooth muscle cells is under investigation, these channels may be important for calcium-dependent calcium release from intracellular stores and myogenic pacemaking activity (Thornbury, 1999).

At this time, the molecular identity of T-type channel pores is unknown in nonvascular smooth muscle. A few studies have suggested that T-type channels in vascular smooth muscle cells are composed of alpha1G or alpha1H. RT-PCR analyses of isolated mesenteric arterioles and arteries (Gustafsson *et al.*, 2001) and afferent and efferent glomerular vessels (Hansen *et al.*, 2001) reveal the presence of alpha1G and alpha1H transcripts. However, as vascular endothelial cells may also express functional T-type channels (see below), additional studies are necessary to elucidate both the molecular identity and anatomical localization of LVA alpha1 subunits in both vascular and nonvascular smooth muscle.

Putative Functions of T-Type Channels in Vascular Smooth Muscle

At least two physiological functions have been proposed for T-type channels in the vasculature. Vascular smooth muscle expression of T-type channels may be important for smooth muscle proliferation. The presence of T-type currents is associated with proliferation of aortic smooth muscle myocytes (Akaike *et al.*, 1989a; Richard *et al.*, 1992), and expression of T-type currents is correlated with cell cycle of smooth muscle cells, as functional T-type channel expression is highest during S phase during mitosis of rat aortic smooth muscle cells, (Kuga *et al.*, 1996). Injury of rat carotid arteries with an inflated embolectomy catheter induces smooth muscle cell proliferation that is antagonized by mibefradil but not L-type calcium channel blockers (Schmitt *et al.*, 1995, 1996; but see Quignard *et al.*, 2001). Mibefradil also antagonizes smooth muscle cell proliferation in vitro, as T-type channel blockers, but not L-type channel blockers, prevent smooth muscle cell proliferation induced by fetal calf serum (Schmitt *et al.*, 1995).

Accumulating pharmacological evidence suggests that T-type channels are also important mediators of vascular smooth muscle cell contractions. Mibefradil reduces peripheral blood pressure (Schmitt *et al.*, 1995), presumably by blocking T-type channels in smooth muscle cells preferentially over L-type channels (Mishra and Hermsmeyer, 1994). Furthermore, vasoconstriction of many resistance arterioles are antagonized by mibefradil and nickel (Angus *et al.*, 2000; Lam *et al.*, 1998; Sarsero *et al.*, 1998; but see van der Lee *et al.*, 1999). For example, local and conducted vasoconstrictions of rat mesenteric arteries are not affected by tetrodotoxin (a voltage dependent sodium channel blocker) or L-type channel antagonists, but are attenuated by nickel or mibefradil (Gustafsson *et al.*, 2001).

It is unlikely that T-type channels are involved in neurotransmitter release, but may be part of a signal

transduction pathway for some vasoconstrictors. For example, endothelin appears to constrict vascular smooth muscle through an action of T-type channels, as agonist-mediated vasoconstrictions are antagonized by nickel (Blackburn and Highsmith, 1990; Gustafsson *et al.*, 2001). Furthermore, whereas norepinephrine-mediated local vasoconstrictions are not affected by mibefradil, mibefradil prevented conduction of the vasoconstrictions away from the site of norepinephrine application (Gustafsson *et al.*, 2001), implicating a role from T-type channels in the spread of local changes in membrane potential of smooth muscle cells. Finally, some drugs that relax resistance vessels may act at least partially through T-type channels. Seventeen beta-estradiol inhibits smooth muscle cell contractions, potentially by modulating or blocking T-type channels, as application of the drug to smooth muscle cells attenuates peak L- and T-type currents (Zhang *et al.*, 1994).

T-Type Channels Are Implicated in Vascular Disease

Additional evidence that T-type channels are important in blood pressure control results from studies of hypertensive animals. Analysis of newborn and adult stroke-prone spontaneously hypertensive rats reveals elevated T- and L-type peak calcium current amplitude and altered biophysical channel properties, indicating that genetic aberrations in calcium channel expression can lead to hypertension and increased risk of strokes (Self *et al.*, 1994). Systemic treatment of stroke-prone spontaneously hypertensive rats with mibefradil reduces blood pressure, development of hypertension, anatomical alterations in resistance vessels, occurrence of strokes, and mortality rates of these rats (Vacher *et al.*, 1996), suggesting that aberrant T-type channel expression and/or function in smooth muscle cells and endothelium contribute to the developmental susceptibility to both hypertension and strokes. Finally, until its removal from the market due to inadvertent side effects, mibefradil was used clinically to treat mild-to-moderate essential hypertension (reviewed in Billups and Carter, 1998; Oparil, 1998), and appeared useful in the treatment of chronic stable angina pectoris, a condition resulting from impaired oxygen supply to the heart (reviewed in Massie, 1998). Clinically relevant doses of mibefradil (200 μM) preferentially attenuate T-type current over L-type current and reduced blood pressure by decreasing peripheral and coronary resistance in humans (reviewed in Massie, 1998; Oparil, 1998). Taken together, these data imply that T-type channels are important regulators of blood flow in both healthy and diseased resistance vessels.

T-Type Channels Are Expressed by Vascular Endothelial Cells

A handful of studies reveal that smooth muscle cells are not the only source of T-type currents in the vasculature, as endothelial cells also exhibit T-type currents. Endothelial cells isolated from a variety of tissues (Bossu *et al.*, 1989, 1992a, 1992b; Fisher *et al.*, 2002; Nilius *et al.*, 1997; Vinet and Vargas, 1999) have T-type calcium currents in addition to other ion currents (Bossu *et al.*, 1992a,b; Nilius *et al.*, 1997). Although the function of these channels in the endothelium is not known, mibefradil prevents endothelial cell proliferation in culture (Nilius *et al.*, 1997). Furthermore, T-type currents are attenuated in confluent cultured endothelial cells (Bossu *et al.*, 1992a), similar to studies of smooth muscle cells (Sui *et al.*, 2001), supporting the hypothesis that T-type currents may aid in cellular proliferation.

FUNCTIONAL AND BIOCHEMICAL EVIDENCE FOR T-TYPE CHANNEL EXPRESSION IN NONNEURAL, NONCARDIOVASCULAR CELLS

T-Type Channels Are Expressed Throughout the Body

Endocrine Tissues

Although most studies of T-type calcium channels and LVA alpha1 subunit expression focus on analysis of neural and cardiac tissues, accumulating evidence reveals that T-type channels are found throughout the body in a host of non-neural, noncardiovascular tissues. For example, T-type currents are recorded from many endocrine cells, including anterior pituitary lactotrophs (Lingle *et al.*, 1986), anterior pituitary GH3 clonal cells (Herrington and Lingle, 1992; Matteson and Armstrong, 1986), pituitary intermedia cells (Cota, 1986), thyroid C cells (Biagi and Enyeart, 1991; Williams *et al.*, 1999), pancreatic beta cells (see below), decidual cells of the uterus (Couderc *et al.*, 1995; Sartor *et al.*, 1992), and adrenal glomerulosa (Barrett *et al.*, 1991a,b; Cohen *et al.*, 1988; McCarthy *et al.*, 1993) and zona fasciculata (Barbara and Takeda, 1995; Enyeart *et al.*, 1992; Mlinar *et al.*, 1993) cells.

Although the molecular identity of the pore has not been determined for all of these tissues, alpha1H and alpha1I transcripts are found in thyroid cells (Monteil *et al.*, 2000b; Williams *et al.*, 1999), and alpha1G, alpha1H, and alpha1I mRNA have been identified in anterior pituitary and adrenal gland by either Northern blot (Monteil *et al.*, 2000b) or in situ hybridization (Schrier *et al.*, 2001;

Talley *et al.*, 1999). Endocrine T-type calcium currents have been implicated in both hormone synthesis (Enyeart *et al.*, 1992; Ritchie, 1993; Rossier *et al.*, 1998) and hormone release (Barrett *et al.*, 1991a; Biagi *et al.*, 1992; Cohen *et al.*, 1988; Mlinar *et al.*, 1993). As discussed previously, T-type channels have unusual electrophysiological properties, including an overlap of voltage dependent activation and inactivation curves. Therefore, within a restricted range of specific membrane potentials, significant calcium influx can occur, and this calcium is sufficient for hormone secretion (Barrett *et al.*, 1991b; Cohen *et al.*, 1988; Enyeart *et al.*, 1992; Mlinar *et al.*, 1993; Rossier *et al.*, 1993).

Fibroblasts

T-type currents are commonly recorded from many fibroblast cell lines, and studies of fibroblasts provided some of the first evidence that nonexcitable cell lines express functional T-type channels. Fibroblasts (such as 3T3 cells) express functional T- and L-type calcium currents (Chen *et al.*, 1988), whereas other fibroblast cell lines (such as C3H 10T1/2) lack HVA currents (Estacion and Mordan, 1993). The physiological role of these channels is not known, but fibroblast membrane potentials can oscillate between −70 and −10 mV, and the T-type channels may be important in gating the activity of other voltage dependent channels, such as in neurons. The ultimate consequence of such oscillations, however, remains unknown.

T-type calcium channels are important in the response to mitogens and entry into the S phase of cell cycle, as both nordihydroguariaretic acid, a nonspecific inhibitor of T-type channels (Wang *et al.*, 1993) and lanthanum (Estacion and Mordan, 1993) inhibits the platelet-derived growth factor- (PDGF) induced rise in intracellular calcium and progression to S phase in cell cycle. Surprisingly, both the presence of T-type currents and the magnitude of the response to PDGF are positively correlated with fibroblast cell density, with maximal responses recorded at confluence (Estacion and Mordan, 1993). Furthermore, oncogenic transformation of fibroblasts has provided some of the strongest evidence thus far for a role of T-type channels in carcinogenesis (see below), indicating the value of these cells in the study of T-type channels.

Bone

Osteoblasts respond to hormones (Fritsch and Chesnoy-Marchais, 1994), growth factors, and vitamins (Caffrey and Farach-Carson, 1989) by changing intracellualr calcium levels, and VDCC currents are recorded from both osteoblast-like cells (Amagai and Kasai, 1989) and osteoblast primary cultures (Chesnoy-Marchais and Fritsch, 1988; Gu *et al.*, 1999). Osteoblasts may express both HVA and LVAs, as two currents with different kinetics and voltage dependence are recorded from rat osteoblasts from trabecular femur load-bearing bones (Gu *et al.*, 1999). Although the LVA currents display more positive voltage dependence than do neuronal T-type channels, alpha1G mRNA is observed by RT-PCR, suggesting that T-type channels may be important in transduction of extracellular signals, including hormones and mechanical loading (Gu *et al.*, 1999).

Lung

As described previously, many types of smooth muscle cells express T-type channels, including smooth muscle cells isolated from bronchi (Janssen, 1997; Sui *et al.*, 2001). Preliminary evidence suggests that lung endothelial cells may also express functional T-type currents under pathophysiological conditions (Fisher *et al.*, 2002). Although the molecular identity of the pore-forming subunit is unknown for either cell type, alpha1G transcripts are detected in rat lung by Northern blot following long exposures (Perez-Reyes *et al.*, 1998), and dot blot analysis of alpha1G transcripts in human lungs suggest robust and transient expression after birth (Monteil *et al.*, 2000a). Northern blots of adult human lung reveal that lung tissues may also contain alpha1H transcripts (Williams *et al.*, 1999), but not alpha1I mRNA (Monteil *et al.*, 2000b). Although the functional significance of T-type channels is not known at this time, these channels may play a role in regulating airflow resistance (Janssen, 1997; Muramatsu *et al.*, 1997) and in ischemia-reperfusion injuries (Fisher *et al.*, 2002).

Spleen

Physiological studies suggest at least two splenic functions may be modulated by T-type channels. First, potassium-evoked depolarization of rat splenic muscle stimulates contractions through activation of a voltage dependent calcium channel that is not antagonized by toxins against N- or P-/Q-type calcium channels (Ko *et al.*, 1997). These splenic contractions are inhibited by inorganic channel blockers (Cd^{2+}, Ni^{2+}, Co^{2+}), pimozide, a mixed antagonist that affects both T- and L-type channels, and by dihydropyridine antagonists such as nifedipine,

verapamil, diltiazem, and R-(+)-BayK8644. Although these are L-type channel antagonists, the concentrations used to block splenic contractions are each over 50-fold higher than that needed to block aortic contractions mediated by L-type channels (Ko *et al.*, 1997).

Proliferation and activation of splenic lymphocytes is dependent on influx of extracellular calcium (reviewed in Lewis and Cahalan, 1995). Although the identity of this calcium channel on the plasma membrane is not known, at least two types of voltage dependent calcium currents have been recorded from lymphocyte-derived cells (reviewed in Lewis and Cahalan, 1995). Furthermore, 1,4-dihydropyridine antagonists can inhibit both proliferation and activation of splenic lymphocytes at concentrations greater than the concentration necessary for half-maximal inhibition of aortic L-type channels (Alivizatos *et al.*, 1993; Briede *et al.*, 1999; Kunert-Radek *et al.*, 1990). As studies of recombinant T-type channels have also found that 1,4-dihydropyridines and inorganic calcium channel blockers can antagonize T-type channels (reviewed in Yunker, 2003), it is tempting to speculate that T-type channels may be important in splenic lymphocyte proliferation and/or maturation, as well as splenic muscle contractions.

Liver

At least two different LVA alpha1 transcripts have been observed in liver, as robust alpha1H mRNA (Cribbs *et al.*, 1998; Williams *et al.*, 1999), and weak alpha1G mRNA (Klugbauer *et al.*, 1999) levels are observed by Northern blot analysis. Although the functional significance is unknown at this time, it is possible that these signals result from the presence of alpha1G and alpha1G and alpha1H transcripts in vascular smooth muscle cells and/or endothelium, as these tissues are predicted to have rebust levels of T-type channels (see above).

Gastrointestinal Tract

The gastrointestinal tract is a complex organ composed of epithelial and immune cells, interstitial cells of Cajal, nerves from the parasympathetic, sympathetic, and enteric nervous systems, and vascular and smooth muscle cells. Although the distribution of T-type channels has not been examined in detail, adult human small intestine and colon express low levels of alpha1G by Northern blot analysis (Monteil *et al.*, 2000a) and RT-PCR (Toyota *et al.*, 1999). In contrast, the presence of alpha11 mRNA is not detected (Monteil *et al.*, 2000b), and alpha1H mRNA levels have not been examined to date.

T-type calcium channels likely have multiple physiological functions in the gut, as T-type calcium currents have been recorded from smooth muscle of stomach (Vivaudou *et al.*, 1988) and small (Smirnov *et al.*, 1992b) and large (Huizinga *et al.*, 1991; Xiong *et al.*, 1993, 1995) intestine from a number of species. Furthermore, mathematical modeling of electrical activity of the intestine suggests that T-type channels are involved in peristalsis (Miftakhov *et al.*, 1996, 1999a,b; Miftakhov and Abdusheva, 1996). T-type channels may also be important in both normal and diseased state of the mucosa, as they are implicated in muscularis mucosae contractions (Uchida *et al.*, 1998) and gastrointestinal carcinomas (see below).

Pancreas

Calcium influx through voltage dependent calcium channels controls secretion from pancreatic alpha and beta cells (reviewed in Satin, 2000). T-type calcium currents have been recorded from human pancreatic islet cells (Barnett *et al.*, 1996; Misler *et al.*, 1992), rat pancreatic beta cells (Ashcroft *et al.*, 1990; Sala and Matteson, 1990), and pancreatic cell lines (Bhattacharjee *et al.*, 1997; Horvath *et al.*, 1998; Satin and Cook, 1988). Similar to their role in neuronal physiology, T-type channels play a role in spike-burst generation in beta cells (Kitasato *et al.*, 1996), as activation of T-type channels can decrease the latency of action potential onset and increase the frequency of action potentials, potentially contributing to increased secretion (Bhattacharjee *et al.*, 1997). Although Northern blot studies have not detected LVA alpha1 subunit mRNA in adult pancreas (Monteil *et al.*, 2000a,b; Perez-Reyes, 1998), an alpha1G splice variant (Tables I and II) has recently been cloned from INS-1, an insulin-secreting cell line (Zhuang *et al.*, 2000).

Kidney

At least two types of T-type channels may be expressed in kidney. Alpha1G transcripts are found in both adult (Andreasen *et al.*, 2000; Klugbauer *et al.*, 1999; Perez-Reyes *et al.*, 1998; Zhuang *et al.*, 2000) and fetal (Monteil *et al.*, 2000a) kidney. Similar to other tissues, alpha1G expression appears developmentally regulated in the kidney. Alpha1G mRNA levels are higher during development than during adulthood by dot blot analysis (Monteil *et al.*, 2000a). Furthermore, different types of alpha1G transcripts are found in fetal kidney by RT-PCR (forms “b”; “bc”; “bcd”) compared to brain (Monteil *et al.*,

2000a), suggesting tissues may differentially express alpha1G isoforms. Northern blot analysis reveals that kidney is one of the richest sources of alpha1H in human (Cribbs *et al.*, 1998; Williams *et al.*, 1999), implying that T-type channels in this organ are likely to be composed of alpha1H. In contrast, alpha1I mRNA has not been detected in kidney (Monteil *et al.*, 2000b; but see Lee *et al.*, 1999).

The physiological significance of the presence of LVA alpha1 transcripts is not clear at this time. Alpha1G mRNA is found in most regions of the rat kidney (Andreasen *et al.*, 2000; Hansen *et al.*, 2001), whereas alpha1G immunoreactivity using an antibody raised against the amino terminus indicates a more restricted distribution of protein to regions such as distal convoluted tubule, connecting tubule, and inner medullary collecting duct (Andreasen *et al.*, 2000). It has been suggested that T-type channels in these regions may respond to hormones to regulate calcium entry in vascular smooth muscle cells and tubular epithelial cells (Andreasen *et al.*, 2000). T-type currents are recorded from renal arteries (Gordienko *et al.*, 1994), and treatment with either mibefradil or nickel inhibits potassium-induced increases in calcium (Hansen *et al.*, 2001) and potassium- and angiotensin-II evoked vasoconstriction in afferent and efferent arteriles (Hansen *et al.*, 2001; Honda *et al.*, 2001; Ozawa *et al.*, 2001; Miftakhov *et al.*, 1996). Taken together, these data indicate that T-type channels are important contributors to resistance vessel tone in the kidney.

T-type channels in the kidney may also play a role in some kidney diseases, as the gene encoding alpha1H maps to the region for both the polycystic kidney disease 1 gene (T.E.P.K.D.Consortium, 1994) and the tuberous sclerosis gene (Daniels *et al.*, 2001; Perez-Reyes *et al.*, 1999). The former gene has significant homology to a repeat of a VDCC (Mochizuki *et al.*, 1996; T.E.P.K.D. Consortium, 1994), but does not appear to conduct calcium currents when expressed as a recombinant protein (reviewed in Perez-Reyes *et al.*, 1999). Finally, T-type channel blockers may be useful in the treatment of chronic renal disease (Balyis *et al.*, 2001; Griffin *et al.*, 2001; Nakamura *et al.*, 1999), suggesting that a better understanding of the biochemical, anatomical, and pharmacological properties of T-type channels in the kidney is important for treatment of pathophysiological states.

Ovary

Northern blot analysis of alpha1G (Monteil *et al.*, 2000a), but not alpha1I (Monteil *et al.*, 2000b), reveals that the ovary contains low levels of LVA alpha1 mRNA. (The presence of alpha1H mRNA has not been examined to date.) Ovarian granulosa cells are excitable and capable of generating calcium-dependent action potentials (Mealing *et al.*, 1994). Granulosa cells express a variety of functional voltage dependent ion channels, including L- and T-type VDCC (Asem *et al.*, 2002; Kusaka *et al.*, 1993; Schwartz *et al.*, 1988, 1989). Calcium influx into granulosa cells may be important for steroid biosynthesis, protein production, and/or secretion (Danisova *et al.*, 1995; Schwartz *et al.*, 1989). Specially, progesterone induces calcium influx through T-type calcium channels (Lieberherr *et al.*, 1999), whereas estradiol increases L-type currents (Lieberherr *et al.*, 1999). T-type calcium channels may also mediate purinergic and muscarinic acetylcholine-induced suprathreshold calcium oscillations that have an unknown physiological function (Morley *et al.*, 1992, 1994).

Testis

Spermatozoa interactions with the egg extracellular matrix stimulates calcium influx into human spermatoza through T-type channels (Arnoult *et al.*, 1996; Shoimi *et al.*, 1996). T-type calcium currents are recorded from mouse and rat spermatogenic cells (Arnoult *et al.*, 1996; Hagiwara and Kawa, 1984; Santi *et al.*, 1996), pachytene spermatocytes (Liévano *et al.*, 1996), round spermatids (Liévano *et al.*, 1996), and human germ cells (Jagannathan *et al.*, 2002b), indicating that both immature and mature sperm from a variety of species express functional T-type calcium channels.

The initial cloning of LVA alpha1 subunits reported the presence of alpha1G, but not alpha1I in mouse and human testis by Northern blot analysis (Klugbauer *et al.*, 1999; Monteil *et al.*, 2000a,b). These findings have been strengthened by independent investigations, some of which demonstrate the presence of alpha1G mRNA in mouse spermatogenic cells and human tests and germ cells (Espinosa *et al.*, 1999; Jagannathan *et al.*, 2002b; Son *et al.*, 2000,2002). Although alpha1I mRNA has not been observed to date (Son *et al.*, 2000, 2002), RT-PCR analysis and in situ hybridization detect the presence of alpha1H mRNA in human tests and germ cells (Jagannathan *et al.*, 2002b; Son *et al.*, 2000), and mouse pachytene spermatocytes (Espinosa *et al.*, 1999) and spermatids (Son *et al.*, 2002).

It is likely that T-type calcium channels expressed by mature sperm are involved in the acrosome reaction (reviewed in Jagannathan *et al.*, 2002a). Sperm undergo a calcium-dependent exocytosis in response to interaction with an egg's extracellular matrix, and T-type calcium channel blockers prevent exocytosis (Arnoult *et al.*,

1996; Bai and Shi, 2002; LiéAvano *et al.*, 1996), suggesting that T-type calcium channel antagonists may be useful antifertility compounds. However, the presence of T-type channels on immature sperm cells is also intriguing, and may indicate that LVA alpha1 subunits are important in the maturation of these cells.

Skeletal Muscle

T-type currents are recorded from immature skeletal muscle cells (Beam and Kundson, 1988; Fratacci *et al.*, 1996; Garcia and Beam, 1994; Imbert *et al.*, 2001; Kano *et al.*, 1992; Vandebrouck *et al.*, 2002; Satoh *et al.*, 1991) in vitro and in situ. During myocyte development, both the density of T-type channels decreases (Beam and Knudson, 1988; Berthier *et al.*, 2002; Gonoi and Hasegawa, 1988; Rohwedel *et al.*, 1994; Shimahara and Bournaud, 1991; Strube *et al.*, 2000), and the presence of alpha1G, alpha1H, and alpha1I mRNA transcripts decreases (Berthier *et al.*, 2002), concomitant with increased L-type currents (Beam and Knudson, 1988; Gonoi and Hasegawa, 1988; Rohwedel *et al.*, 1994; Shimahara and Bournaud, 1991; Strube *et al.*, 2000; Wingertzahn and Ochs, 2001). T-type currents can result in intracellular calcium release from the sarcoplasmic reticulum (Garcia and Beam 1994), indicating that T-type currents are an important source of calcium during maturation. Pharmacological data support this conclusion, as T-type channel blockers such as mibefradil (Liu *et al.*, 1999), amiloride, and nickel (Bijlenga *et al.*, 2000) attenuate myoblast fusion, a process necessary for both muscle growth and repair (reviewed in Bernheim and Bader, 2002). Furthermore, injection of antisense alpha1H cDNA, but not antisense alpha1G or alpha1I cDNA, attenuates ~80% T-type currents in newly formed myotubes (Bijlenga *et al.*, 2000), implying that calcium through T-type channels formed by alpha1H is important in muscle differentiation. However, it should be noted that myoblasts, myotubes, and embryonic and postnatal muscle fibers can also contain alpha1G and alpha1I transcripts detected by RT-PCR (Berthier *et al.*, 2002; Bijlenga *et al.*, 2000), suggesting that multiple T-type channels may be transiently expressed by smooth muscle cells.

T-Type Channels Are Implicated in Diseases of Nonexcitable Cells

Cancer

A weak association has almost always existed between T-type calcium channels and cancer. Neuroblastoma cell lines were used to characterize the electrophysiological properties of T-type channels (Carbone *et al.*, 1989, 1990; Tang *et al.*, 1988), and expression of T-type channels have been implicated repeatedly in proliferation and development. Robust T-type currents are recorded from a variety of tumor cell lines, and T-type currents are the sole calcium current recorded from a human medullary thyroid carcinoma cell line (Biagi *et al.*, 1992; deBustros *et al.*, 1986, 1990), and these cells were used as the source to clone alpha1H by one group (Williams *et al.*, 1999). However, some of the most compelling evidence came from oncogenic transformation of 3T3 fibroblast cells. Transformation of fibroblasts by a number of molecules including ras (c-H-*ras*, EJ-*ras*), v-*fms*, or polyoma middle T oncogene depresses T-type, but not L-type calcium currents, suggesting that decreased expression or function of T-type calcium channels may be involved in transformation (Chen *et al.*, 1988). In contrast, transformation by *src* does not affect T-type currents but induces a calcium-activated potassium current (Draheim *et al.*, 1995), implying T-type channels are preferentially modulated by some oncogenes.

Analysis of downstream pathways revealed that Ras mutants lacking the ability to activate the mitogen-activated protein kinase pathway fail to exhibit attenuated T-type calcium currents (Strobeck *et al.*, 1999). Conversely, attenuated T-type calcium currents are observed in 3T3 cells harboring a gain-of-function mutation in mitogen-activated protein kinase kinase (MAPK kinase; also known as extracellular signal-regulated kinase kinase), a direct activator of MAPK (Strobeck *et al.*, 1999). As predicted, treatment of *ras*-transformed cells with an MAPK kinase-specific inhibitor (PD98059) restores T-type calcium channel function, whereas inhibition of T-type currents with mibefradil is essential for the induction and/or maintenance of spindle-shaped transformation morphology of fibroblasts (Strobeck *et al.*, 1999). At this time, the mechanism by which *ras*-transformation attenuates T-type calcium currents is unknown, as MAPK can phosphorylate transcription factors, thus potentially altering expression of T-type calcium channels or unidentified accessory proteins. Alternatively, LVA alpha1 isoforms such as alpha1G harbors MAPK phosphorylation consensus sites, potentially marking it for direct regulation by MAPK (Strobeck *et al.*, 1999).

An independent line of investigation has also implicated decreased T-type calcium channel expression in cancer. Hypermethylation of CpG islands is a common mechanism in which tumor suppressor genes are inactivated (Ueki *et al.*, 2000), and PCR analysis of colorectal cancers, gastric cancer, myelogenous leukemia, pancreatic carcinomas, and hepatocellular carcinomas reveal

that CACNA1G, the gene encoding alpha1G, is frequently aberrantly methylated (Shen *et al.*, 2002; Toyota *et al.*, 1999; Ueki *et al.*, 2000). The methylation of *CACNA1G* occurs in a region 300–800 bp upstream of the translation initiation site, and results in suppression of expression (Toyota *et al.*, 1999).

Finally, altered expression of T-type channels have also been implicated in prostate cancer. Analysis of undifferentiated human prostate epithelial cancer cells demonstrated that some cells had functional T-type channels. Differentiation increases both T-type current density and the number of cells with T-type current (Mariot *et al.*, 2002). These currents are probably conducted by alpha1H, as alpha1H mRNA is the only LVA alpha1 molecule detected by RT-PCR (Mariot *et al.*, 2002). It remains to be determined if differentiation-induced expression of alpha1H contributes to the invasiveness and poor prognosis of differentiated prostate cancers (Mariot *et al.*, 2002), or if expression of T-type channels has positive ramifications as suggested by analysis of both transformed fibroblasts and colon cancers.

Diabetes

Like many endocrine cells, pancreatic beta cells express both L- and T-type calcium currents. Electrophysiological studies of diabetes models including streptozocin-induced diabetes (Kato *et al.*, 1994), nonobese diabetic mice (Wang *et al.*, 1996a), and the genetically occurring non-insulin-dependent diabetes mellitus (NIDDM) rat model (Kato *et al.*, 1996) reveal that T-type calcium current density is increased in diabetes. The increased T-type current results in a significantly increased intracellular calcium concentration compared to control beta cells (Wang *et al.*, 1996a). Although the mechanism for increased T-type current is unclear, treatment of rodent islet cells with a combination of cytokines induces T-type current (Wang *et al.*, 1999). Finally, antagonists of T-type calcium channels prevent cytokine-induced DNA fragmentation, a measure of apoptosis (Wang *et al.*, 1999), suggesting T-type calcium channels may be a useful therapeutic target in preventing destruction of pancreatic beta cells.

CONCLUSIONS

Our understanding of T-type channels has been greatly advanced over the past 5 years by the discovery of the genes that encode alpha1G, alpha1H, and alpha1I subunits that form LVA T-type channels. T-type channels are expressed throughout the body and appear to have roles in a multitude of neural and non-neural processes as discussed in the previous sections. It is anticipated that future studies of T-type VDCC expression in both normal and diseased cells will continue to yield many interesting and surprising discoveries, including their molecular composition; mechanisms that coordinate their spatial and temporal expression with other LVAs and HVAs; and their roles in nonexcitable tissues. Furthermore, we anticipate that in years to come the heterogeneity of endogenous T-type VDCC currents will be better appreciated relative to the variations in T-type VDCC structure via splice variants, posttranslational modifications, and auxiliary subunits. Finally, it is likely that the discovery of new and selective T-type channel antagonists coupled with the appreciation of distinct T-type VDCC complexes will aid in both the study of endogenous T-type channels and the development of therapeutic avenues to manage neurological, cardiovascular, and endocrine diseases.

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REFERENCES

Ahmmed, G. U., Dong, P. H., Song, G., Ball, N. A., Xu, Y., Walsh, R. A., and Chiamvimonvat, N. (2000). *Circ. Res.* **86**, 558–570.
Aizenman, C. D., and Linden, D. J. (1999). *J. Neurophysiol.* **82**, 1697–1709.
Akaike, N. (1991). *Comp. Biochem. Physiol. C* **98**, 31–40.
Akaike, N., Kanaide, H., Kuga, T., Nakamura, M., Sadoshima, J., and Tomoike, H. (1989a). *J. Physiol.* **416**, 141–160.
Akaike, N., Kostyuk, P. G., and Osipchuk, Y. V. (1989b). *J. Physiol.* **412**, 181–195.
Akasu, T., Nishimura, T., and Tokimasa, T. (1990). *J. Physiol.* **422**, 303–320.
Akopian, A., Krizaj, D., and Witkovsky, P. (1997). *Brain Res.* **1997**, 121–130.
Akopian, G., Kressin, K., Derouiche, A., and Steinhauser, C. (1996). *Glia* **17**, 181–194.
Alivizatos, P. A., Maxa, J., Lucio, S., and Chen, J. (1993). *Transplantation* **56**, 1604–1605.
Amagai, Y., and Kasai, S. (1989). *Jpn. J. Physiol.* **39**, 773–777.
Andersen, P., Eccles, J. C., and Sears, T. A. (1964). *J. Physiol.* **174**, 370–399.
Andreasen, D., Jensen, B. L., Hansen, P. B., Kwon, T.-H., Nielsen, S., and Skott, O. (2000). *Am. J. Physiol. Renal. Physiol.* **279**, F997–F1005.
Angus, J. A., Sarsero, D., Fujiwara, T., Molenaar, P., and Xi, Q. (2000). *Clin. Exp. Pharmacol. Physiol.* **27**, 1019–1021.
Aradi, I., and Holmes, W. R. (1999). *J. Comput. Neurosci.* **6**, 215–235.

Armstrong, C. M., and Matteson, D. R. (1985). *Science* **227**, 65–67.
Arnoult, C., Cardullo, R. A., Lemos, J. R., and Florman, H. M. (1996). *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13004–13009.
Arnoult, C., Lemos, J. R., and Florman, H. M. (1997). *EMBO J.* **16**, 1593–1599.
Asem, E. K., Qin, W., and Rane, S. G. (2002). *Am. J. Physiol. Endocrinol. Metab.* **282**, E184–E196.
Ashcroft, F. M., Kelly, R. P., and Smith, P. A. (1990). *Pflugers Arch.* **415**, 504–506.
Assandri, R., Egger, M., Gassmann, M., Niggli, E., Bauer, C., Forster, I., and Gorlach, A. (1999). *J. Physiol.* **516**, 343–352.
Audesirk, G., Audesirk, T., Ferguson, C., Lomme, M., Shugarts, D., Rosack, J., Caracciolo, P., Caracciolo, P., Gisi, T., and Nichols, P. (1990). *Brain Res. Dev. Brain. Res.* **1990**, 109–120.
Avanzini, G., de Curtis, M., Panzica, F., and Spreafico, R. (1993). *J. Physiol.* **416**, 111–122.
Avery, R. B., and Johnston, D. (1996). *J. Neurosci.* **16**, 5567–5582.
Avoli, M., Rogawski, M. A., and Avanzini, G. (2001). *Epilepsia* **42**, 445–457.
Babitch, J. (1990). *Nature* **346**, 321–322.
Bading, H. (1999). *Epilepsy Res.* **36**, 225–231.
Bai, J., and Shi, Y. (2002). *Eur. J. Pharmacol.* **440**, 1–6.
Bal, T., and McCormick, D. A. (1993). *J. Physiol.* **468**, 669–691.
Balyis, C., Qiu, C., and Engels, K. (2001). *Am. J. Kidney Dis.* **38**, 1292–1297.
Barbara, J. G., and Takeda, K. (1995). *J. Physiol.* **488**, 609–622.
Barish, M. E. (1991a). *J. Physiol.* **444**, 545–565.
Barish, M. E. (1991b). *J. Physiol.* **444**, 523–543.
Barnett, D. W., Pressel, D. W., and Misler, S. (1996). *Pflugers Arch.* **430**, 272–282.
Barres, B. A., Chun, L. L., and Corey, D. P. (1989). *J. Neurosci.* **9**, 3169–3175.
Barres, B. A., Chun, L. L. Y., and Corey, D. P. (1990a). *Annu. Rev. Neurosci.* **13**, 441–474.
Barres, B. A., Koroshetz, W. J., Chun, L. L., and Corey, D. P. (1990b). *Neuron* **5**, 527–544.
Barrett, P. Q., Isales, C. M., Bollag, W. B., and McCarthy, R. T. (1991a). *Am. J. Physiol.* **261**, F706–F719.
Barrett, P. Q., Isales, C. M., Bollag, W. B., and McCarthy, R. T. (1991b). *Can. J. Physiol. Pharmacol.* **69**, 1553–1560.
Bean, B. P. (1985). *J. Gen. Physiol.* **86**, 1–30.
Bean, B. P., and McDonough, S. I. (1998). *Neuron* **20**, 825–828.
Bean, B. P., Sturek, M., Puga, A., and Hermsmeyer, K. (1986). *Circ. Res.* **59**, 229–235.
Beam, K. G., and Knudson, C. M. (1988). *J. Gen. Physiol.* **91**, 799–815.
Beaudu-Lange, C., Despeyroux, S., Marcaggi, P., Coles, J. A., and Amedee, T. (1998). *Eur. J. Neurosci.* **10**, 1796–1809.
Beck, H., Steffens, R., Elger, C. E., and Heinemann, U. (1998). *Epilepsy Res.* **32**, 321–332.
Beck, H., Steffens, R., Heinemann, U., and Elger, C. E. (1997). *J. Neurophysiol.* **77**, 1526–1537.
Bénardeau, A., Weissenburger, J., Hondeghem, L., and Ertel, E. A. (2000). *J. Pharmacol. Exp. Ther.* **292**, 561–575.
Benham, C. D., Hess, P., and Tsien, R. W. (1987). *Circ. Res.* **61**, 110–116.
Berger, A. J., Bayliss, D. A., Bellingham, M. C., Umemiya, M., and Viana, F. (1995). *Adv. Exp. Med. Biol.* **81**, 63–71.
Berger, A. J., Bayliss, D. A., and Viana, F. (1996). *J. Appl. Physiol.* **81**,1039–1048.
Berger, T., Schnitzer, J., Orkand, P. M., and Kettenmann, H. (1992). *Eur. J. Neurosci.* **4**, 1277–1284.
Bernheim, L., and Bader, C. R. (2002). *News Physiol. Sci.* **17**, 22–26.
Berthier, C., Monteil, A., Lory, P., and Strube, C. (2002). *J. Physiol.* **539**, 681–691.
Bertolesi, B. E., Shi, C., Elbaum, L., Jollimore, C., Rozenberg, G., Barnes, S., and Kelly, M. E. M. (2002). *Mol. Pharmacol.* **62**, 210–219.
Beuckelmann, D. J., Nabauer, M., and Erdmann, E. (1991). *J. Mol. Cell. Cardiol.* **23**, 929–937.
Bhattacharjee, A., Whitehurst, R. M., Jr., Zhang, M., Wang, L., and Li, M. (1997). *Endocrinology* **138**, 3735–3740.
Biagi, B. A., and Enyeart, J. J. (1991). *Am. J. Physiol.* **260**, C1253–C1263.
Biagi, B. A., Mlinar, B., and Enyeart, J. J. (1992). *Am. J. Physiol.* **263**, C986–C994.
Bijlenga, P., Liu, J.-H., Espinos, E., Haenggeli, C.-A., Fischer-Lougheed, J., Bader, C. R., and and Bernheim, L. (2000). *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7627–7632.
Bilici, D., Akpinar, E., Gursan, N., Dengiz, G. O., Bilici, S., and Altas, S. (2001). *Pharmacol. Res.* **44**, 527–531.
Billups, S. J., and Carter, B. L. (1998). *Ann. Pharmacother.* **32**, 659–671.
Birnbaumer, L., Qin, N., Olcese, R., Tareilus, E., Platano, D., Costantin, J. L., and Stefani, E. (1998). *J. Bioenerg. Biomembr.* **30**, 357–375.
Black, J. L., and Lennon, V. A. (1999). *Mayo Clin. Proc.* **74**, 357–361.
Blackburn, K., and Highsmith, R. F. (1990). *Am. J. Physiol.* **258**, C1025–C1030.
Bocker-Meffert, S., Rosenstiel, P., Rohl, C., Warneke, N., Held-Feindt, J., Sievers, J., and Lucius, R. (2002). *Invest. Ophthalmol. Vis. Sci.* **43**, 2021–2026.
Bohn, G., Moosmang, S., Conrad, H., Ludwig, A., Hofmann, F., and Klugbauer, N. (2000). *FEBS Lett.* **8**, 73–76.
Bonvallet, R. (1987). *Pflugers Arch.* **408**, 540–542.
Bootman, M. D., Lipp, P., and Berridge, M. J. (2001). *J. Cell Science* **114**, 2213–2222.
Bossu, J. L., Elhamdani, A., and Feltz, A. (1992a). *FEBS Lett.* **16**, 239–242.
Bossu, J. L., Elhamdani, A., Feltz, A., Tanzi, F., Aunis, D., and Theirse, D. (1992b). *Pflügers Arch.* **420**, 200–207.
Bossu, J. L., and Feltz, A. (1986). *J. Physiol.* **376**, 341–357.
Bossu, J. L., Feltz, A., Rodeau, J. L., and Tanzi, F. (1989). *FEBS Lett.* **25**, 377–380.
Boyden, P. A., and Pinto, J. M. (1994). *Circulation* **89**, 2747–2759.
Brice, N. L., Berrow, N. S., Campbell, V., Page, K. M., Brickley, K., Tedder, I., and Dolphin, A. C. (1997). *Eur. J. Neurosci.* **9**, 749–759.
Briede, J., Daija, D., Bisenieks, E., Makarova, N., Uldriks, J., Poikans, J., and Duburs, G. (1999). *Cell Biochem. Funct.* 97–105.
Bringmann, A., Schopf, S., and Reichenbach, A. (2000). *J. Neurophysiol.* **84**, 2975–2983.
Brixius, K., Mohr, V., Muller-Ehmsen, J., Hoischen, S., Munch, G., and Schwinger, R. H. (1998). *Br. J. Pharmacol.* **125**, 41–48.
Brotto, M. A., and Creazzo, T. L. (1996). *Am. J. Physiol.* **270**, H518–H525.
Buhler, F. R. (1997). *Hypertens. Suppl.* **15**, S3–S7.
Burgess, D. L., Gefrides, L. A., Foreman, P. J., and Noebels, J. L. (2001). *Genomics* **71**, 338–350.
Burgess, D. L., Jones, J. M., Meisler, M. H., and Noebels, J. L. (1997). *Cell* **88**, 385–392.
Burgess, D. L., and Noebels, J. L. (1999). *Epilepsy Res.* **36**, 111–122.
Burlhis, T. M., and Aghajanian, G. K. (1987). *Synapse* **1**, 582–588.
Caffrey, J. M., and Farach-Carson, M. C. (1989). *J. Biol. Chem.* **264**, 20265–20271.
Canti, C., Page, K. M., Stephens, G. J., and Dolphin, A. C. (1999). *J. Neurosci.* **19**, 6855–6964.
Carbone, E., Clementi, F., Formenti, A., Pollo, A., and Sher, E. (1989). *Cell. Biol. Int. Rep.* **13**, 1155–1164.
Carbone, E., and Lux, H. D. (1984a). *Biophys. J.* **46**, 413–418.
Carbone, E., and Lux, H. D. (1984b). *Nature* **310**, 501–502.
Carbone, E., and Lux, H. D. (1987). *J. Physiol.* **386**, 547–570.
Carbone, E., Sher, E., and Clementi, F. (1990). *Pflugers Arch.* **416**, 170–179.
Cardanas, C. G., Del Mar, L. P., and Scroggs, R. S. (1995). *J. Neurophysiol.* **74**, 1870–1879.
Catterall, W. A. (2000). *Annu. Rev. Cell. Dev. Biol.* **16**, 521–555.
Cavelier, P., Pouille, F., Desplantez, T., Beekenkamp, H., and Bossu, J.-L. (2002). *J. Physiol.* **540**, 57–72.
Chameau, P., Lucas, P., Melliti, K., Bournaud, R., and Shimahara, T. (1999). *Neuroscience* **90**, 383–388.

Charpak, S., Mertz, J., Beaurepaire, E., Moreaux, L., and Delaney, K. (2001). *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1230–1234.

Chemin, J., Monteil, A., Bourinet, E., Nargeot, J., and Lory, P. (2001a). *Biophysical Journal* **80**, 1238–1250.

Chemin, J., Monteil, A., Briquaire, C., Richard, S., Perez-Reyes, E., Nargeot, J., and Lory, P. (2000). *FEBS Lett.* **478**, 166–172.

Chemin, J., Monteil, A., Dubel, S., Nargeot, J., and Lory, P. (2001b). *Eur. J. Neurosci.* **14**, 1678–1686.

Chemin, J., Monteil, A., Perez-Reyes, E., Bourinet, E., Nargeot, J., and Lory, P. (2002a). *J. Physiol.* **540**, 3–14.

Chemin, J., Nargeot, J., and Lory, P. (2002b). *J. Neurosci.* **22**, 6856–6862.

Chen, C.-C., Wang, Z., Barresi, R., Hill, J. A., Hrska, R. F., Williamson, R., and Campbell, K. P. (2002). *Biophys. J.* 572A.

Chen, C. F., Corbley, M. J., Roberts, T. M., and Hess, P. (1988). *Science* **239**, 1024–1026.

Chesnoy-Marchais, D., and Fritsch, J. (1988). *J. Physiol.* **398**, 291–311.

Chien, A. J., Zhao, X., Shirokov, R. E., Puri, T. S., Chang, C. F., Sun, D., Rios, E., and Hosey, M. M. (1995). *J. Biol. Chem.* **270**, 30036–30044.

Cibulsky, S. M., and Sather, W. A. (2000). *J. Gen. Physiol.* **116**, 349–362.

Clozel, J. P., Ertel, E. A., and Ertel, S. I. (1999). *Proc. Assoc. Am. Physicians* **1999**, 429–437.

Cohen, C. J., McCarthy, R. T., Barrett, P. Q., and Rasmussen, H. (1988). *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2412–2416.

Cohen, E. D. (2001). *Vis. Neurosci.* **2001**, 799–809.

Coombs, J. S., Curtis, D. R., and Eccles, J. C. (1959). *J. Physiol.* **145**, 505–528.

Corvalan, V., Cole, R., Vellis, J. D., and Hagiwara, S. (1990). *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4345–4348.

Cota, G. (1986). *J. Gen. Physiol.* **88**, 83–105.

Couderc, B., Dufy-Barbe, L., and Sartor, P. (1995). *Placenta* **16**, 527–537.

Coulter, D. A. (1997). *J. Child Neurol.* **12**, S2–S9.

Coulter, D. A. Huguenard, J. R., and Prince, D. A. (1989a). *J. Physiol.* **414**, 587–604.

Coulter, D. A., Huguenard, J. R., and Prince, D. A. (1989b). *Neurosci. Lett.* **98**, 74–78.

Coulter, D. A., Huguenard, J. R., and Prince, D. A. (1990). *Br. J. Pharmacol.* **100**, 800–806.

Coulter, D. A., Huguenard, J. R., and Prince, D. A. (1991). *Exp. Brain Res.* **20**, 201–204.

Coulter, D. A., and Lee, C. J. (1993). *Brain Res.* **631**, 137–142.

Craig, P. J., Beattie, R. E., Folly, E. A., Banerjee, M. D., Reeves, M. B., Priestley, J. V., Carney, S. L., Sher, E., Perez-Reyes, E., and Volsen, S. G. (1999). *Eur. J. Neurosci.* **11**, 2949–2964.

Cribbs, L. L., Gomora, J. C., and Daud, A. S., Lee, J.-H., and Perez-Reyes, E. (2000). *FEBS Lett.* **466**, 54–58.

Cribbs, L. L. Lee, J.-H., Yang, J., Satin, J., Zhang, Y., Daud, A., Barclay, J., Williamson, M. P., Fox, M., Rees, M., and Perez-Ryeys, E. (1998). *Circ. Res.* **83**, 103–109.

Cribbs, L. L., Martin, B. L., Schroder, E. A., Keller, B. B., Delisle, B. P., and Satin, J. (2001). *Circ. Res.* **2001**, 403–407.

Crunelli, V., Lightowler, S., and Pollard, C. E. (1989). *J. Physiol.* **413**, 543–561.

Curtis, B. M., and Catterall, W. A. (1984). *Biochemistry* **23**, 2113–2118.

Daniels, R. J., Peden, J. F., Lloyd, C., Horsley, S. W., Clark, K., Turfarelli, C., Kearney, L., Buckle, V. J., Doggett, N. A., Flint, J., and Higgs, D. R. (2001). *Hum. Mol. Gen.* **10**, 339–352.

Danisova, A., Scsukova, S., Matulova, L., Orlicky, J., and Kolena, J. (1995). *Physiol. Res.* **44**, 185–192.

Davies, M. P., An, R. H., Doevendans, P., Kubalak, S., Chien, K. R., and Kass, R. S. (1996). *Circ. Res.* **78**, 15–25.

Day, M. L., Johnson, M. H., and Cook, D. I. (1998). *Pflugers Arch.* **436**, 834–842.

deBustros, A., Baylin, S. B., Levine, M. A., and Nelikin, B. D. (1986). *J. Biol. Chem.* **261**, 8036–8041.

deBustros, A., Lee, R. Y., Compton, D., Tsong, T. Y., Baylin, S. B., and Nelkin, B. D. (1990). *Mol. Cell. Biol.* **10**, 1773–1778.

Deitmer, J. W., Verkhratsky, A. J., and Lohr, C. (1998). *Cell Calcium* **24**, 405–416.

De Jongh, K. S., Merrick, D. K., and Catterall, W. A. (1989). *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8585–8589.

De Jongh, K. S., Warner, C., and Catterall, W. A. (1990). *J. Biol. Chem.* **265**, 14738–14741.

De Jongh, K. S., Warner, C., Colvin, A. A., and Catterall, W. A. (1991). *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10778–10782.

de la Villa, P., Vaquero, C. F., and Kaneko, A. (1998). *Eur. J. Neurosci.* **10**, 317–323.

De Leon, M., Wang, Y., Jones, L., Perez-Reyes, E., Wei, X., Soong, T. W., Snutch, T. P., and Yue, D. T. (1995). *Science* **270**, 1502–1506.

Desmadryl, G., Hilaire, C., Vigues, S., Dichot, S., and Valmier, J. (1998). *Eur. J. Neurosci.* **10**, 545–552.

Destexhe, A., Contreras, D., Steriade, M., Sejnowski, T. J., and Huguenard, J. R. (1996). *J. Neurosci.* **18**, 3574–3588.

Destexhe, A., McCormick, D. A., and Sejnowski, T. J. (1993). *Biophys. J.* **65**, 3574–3588.

Destexhe, A., Neubig, M., Ulrich, D., and Huguenard, J. (1998). *J. Neurosci.* **18**, 3574–3588.

De Waard, M., Liu, H. Y., Walker, D., Scott, V. E. S., Gurnett, C. A., and Campell, K. P. (1997). *Nature* **385**, 446–450.

Dogrul, A., Yesilyurt, O., Isimer, A., and Guzeldemir, M. E. (2001). *Pain* **93**, 61–68.

Dogrul, A., Zagli, U., and Tulunay, F. C. (2002). *Life Sci.* **71**, 725–734.

Dolphin, A. C., Wyatt, C. N., Richards, J., Beattie, R. E., Criag, P. J., Lee, J.-H., Cribbs, L. L., Volsen, S. G., and Perez-Reyes, E. (1999). *J. Physiol.* **519**, 35–45.

Draheim, H. J., Repp. H., and Dreyer, F. (1995). *Biochim. Biophys. Acta* **1269**, 57–63.

Droogmans, G., and Nilius, B. (1989). *J. Physiol.* **419**, 627–650.

Elvan, A. (2000). *Cardiovasc. Res.* **46**, 361–363.

Emanuel, K., Mackiewicz, U., Pythkowski, B., and Lewartowski, B. (1998). *J. Physiol. Pharmacol.* **49**, 577–590.

Endoh, T., and Suzuki, T. (1998). *Arch. Oral Blol.* **43**, 221–233.

Enyeart, J. J., Biagi, B. A., and Mlinar, B. (1992). *Mol. Pharmacol.* **42**, 364–372.

Ertel, E. A., Campbell, K. P., Harpold, M. M., Hofmann, F., Mori, Y., Perez-Reyes, E., Schwartz, A., Snutch, T. P., Tanabe, T., Birnbaumer, L., Tsien, R. W., and Catterall, W. A. (2000). *Neuron* **25**, 533–535.

Ertel, S. I., Ertel, E. A., and Clozel, J.-P. (1997). *Cardiovasc. Drugs Ther.* **11**, 723–739.

Espinosa, F., Lopez-Gonzalez, I., Serrano, C. J., Gasque, G., De la Vega-Beltran, J. L., Trevino, C. L., and Darszon, A. (1999). *Dev. Genet.* **25**, 103–114.

Estacion, M. (1991). *J. Physiol.* **436**, 579–601.

Estacion, M., and Mordan, L. J. (1993). *Cell Calcium* **14**, 161–171.

Fan, Y. P., Horn, E. M., and Waldrop, T. G. (2000). *J. Neurophysiol.* 2896–2903.

Fareh, S., Bénardeau, A., Thibault, B., and Nattel, S. (1999). *Circulation* **100**, 2191–2197.

Fares, N., Gomez, J. P., and Potreau, D. (1996). *C. R. Acad. Sci. III* **319**, 569–576.

Fedulova, S. A., Kostyuk, P. G., and Veselovsky, N. S. (1985). *J. Physiol.* **359**, 431–446.

Fedulova, S. A., Kostyuk, P. G., and Veselovsky, N. S. (1991). *Neuroscience* **41**, 303–309.

Fedulova, S. A., Kostyuk, P. G., and Veselovsky, N. S. (1994). *Neuroscience* **58**, 341–346.

Fern, R. (2002). *J. Neurosci.* **18**, 7232–7243.

Ferron, L., Capuano, V., Droubaix, E., Coulombe, A., and Renaud, J. F. (2002). *J. Mol. Cell. Cardiol.* **34**, 533–546.

Fisher, A. B., Al-Mehdi, A. B., and Manevich, Y. (2002). *Crit. Care Med.* **30**, S192–S197.

Fisher, R. P., Gray, R., and Johnson, D. (1990). *J. Neurophysiol.* **64**, 91–104.

Fletcher, C. F., Copeland, N. G., and Jenkins, N. A. (1998). *J. Bioenerg. Biomembr.* **30**, 387–398.

Fletcher, C. F., Tottene, A., Lennon, V. A., Wilson, S. M. Dubel, S. J., Paylor, R., Hosford, D. A., Tessarollo, L., McEnery, M. W., Peitrobon, D., Copeland, N. G., and Jenkins, N. A. (2001). *FASEB J.* **15**, 1288–1290.

Frankel, W. N. (1999). *Epilepsy Res.* **36**, 97–110.

Fraser, D. D., and MacVicar, B. A. (1991). *J. Neurosci.* **11**, 117–122.

Fratacci, M. D., Shimahara, T., Bournaud, R., and Atlan, G. (1996). *Respir. Physiol.* **104**, 1–9.

Freise, D., Himmerkus, N., Schroth, G., Trost, C., WeiBgerber, P., Freichel, M., and Flockzerzi, V. (1999). *Biol. Chem.* **380**, 897–902.

Friedman, A., and Gutnick, M. J. (1987). *Neurosci. Lett.* **81**, 117–122.

Friedman, M. E., Suarez-Kurtz, G., Kaczorowski, G. J., Katz, G. M., and Reuben, J. P. (1986). *Am. J. Physiol.* **250**, H699–H703.

Frischkrecht, F., and Randall, A. D. (1998). *Neuroscience.* **85**, 1135–1149.

Fritsch, J., and Chesnoy-Marchais, D. (1994). *Cell Sig.* **6**, 645–655.

Furukawa, T., Ito, H., Nitta, J., Tsujino, M., Adachi, S., Hiroe, M., Marumo, F., Sawanobori, T., and Hiraoka, M. (1992). *Circ. Res.* **71**, 1242–1253.

Furukawa, T., Miura, R., Mori, Y., Stobeck, M., Suzuki, K., Ogihara, Y., Asano, T., Morishita, R., Hashii, M., Higashida, H., Yoshii, M., and Nukada, T. (1998). *J. Biol. Chem.* **273**, 17595–17603.

Ganitkevich, V., and Isenberg, G. (1991). *J. Physiol.* **443**, 703–725.

Ganitkevich, V. Y., and Isenberg, G. (1990). *J. Physiol.* **426**, 19–42.

Gao, B., Sekido, Y., Maximov, A., Saad, M., Forgacs, E., Latif, F., Wei, M. H., Lerman, M., Lee, J.-H., Perez-Reyes, E., Bezprozvanny, I., and Minna, J. D. (2000). *J. Biol. Chem.* **275**, 12237–12242.

Garber, S. S., Hoshi, T., and Aldrich, R. W. (1989). *J. Neurosci.* **9**, 3976–3987.

Garcia, J., and Beam, K. G. (1994). *J. Gen. Physiol.* **104**, 1113–1128.

Gauck, V., Thomann, M., Jaeger, D., and Borst, A. (2001). *J. Neurosci.* **21**, 1–4.

Ghosh, A., and Greeberg, M. E. (1995). *Science* **268**, 239–247.

Giffin, K., Solomon, J. S., Burkhalter, A., and Nerbonne, J. M. (1991). *Neuron* **6**, 321–332.

Goette, A., Honeycutt, C., and Langberg, J. L. (1996). *Circulation* **94**, 2968–2974.

Gomez-Mancilla, B., Latulippe, J. F., Boucher, R., and Bedard, P. J. (1992). *Mov. Disord.* **7**, 137–141.

Gomora, J. C., Murbartian, J., Arias, J. M. Lee, J.-H., and Perez-Reyes, E. (2002). *Biophys. J.* **83**, 229–241.

Gonoi, T., and Hasegawa, S. (1988). *J. Physiol.* **401**, 617–637.

Gordienko, D. V., Clausen, C., and Goligorsky, M. S. (1994). *Am. J. Physiol.* **266**, F325–F341.

Gottmann, K. Dietzel, I. D., Lux, H. D., Huck, S., and Rohrer, H. (1988). *J. Neurosci.* **8**, 3722–3732.

Gottmann, K., and Lux, H. D. (1990). *Neurosci. Ltt.* **110**, 34–39.

Gottmann, K., and Lux, H. D. (1995). *Perspect. Dev. Neurobiol.* **2**, 371–377.

Gottmann, K., Rohrer, H., and Lux, H. D. (1991). *J. Neurosci.* **11**, 3371–3378.

Green, P. G., Warre, R., Hayes, P. D., McNaughton, N. C. L., Medhurst, A. D., Pangalos, M., Duckworth, D. M., and Randall, A. D. (2001). *J. Physiol.* **533**(2), 467–478.

Greene, R. W., Haase, H. L., and McCarley, R. W. (1986). *Science* **234**, 738–740.

Gregg, R. G., Messing, A., Strube, C., Beurg, M., Moss, R., Behan, M., Manana, S., Haynes, S., Powell, J. A., Coronado, R., and Powers, P. A. (2001). *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13961–13966.

Griffin, K. A., Picken, M., Bakris, G. L., and Bidani, A. K. (2001). *Hypertension* **37**, 1268–1272.

Gu, X., Olson, E. C., and Spitzer, N. C. (1994). *J. Neurosci.* **14**, 6325–6335.

Gu, X., and Spitzer, N. C. (2001). *J. Neurosci.* **13**, 4936–4948.

Gu, Y., Preston, M. R., El Haj, A. J., Hamid, J., Zamponi, G. W., Howl, J., and Publicover, S. J. (1999). *Pflügers Arch. Eur. J. Physiol.* **438**, 553–560.

Guo, W., Kamiya, K., Kodama, I., and Toyama, J. (1998). *J. Mol. Cell. Cardiol.* **30**, 1095–1103.

Gurnett, C. A., De Waard, M., and Campbell, K. P. (1996). *Neuron* **16**, 431–440.

Gustafsson, F., Andreasen, D., Salomonsson, M., Jensen, B. L., and Holstein-Rathlou, N.-H. (2001). *Am. J. Physiol. Heart Circ. Physiol.* **280**, H582–H590.

Gutnick, M. J., and Yarom, Y. (1989). *J. Neurosci. Methods* **28**, 93–99.

Hagiwara, N., Irisawa, H., and Kameyama, M. (1988). *J. Physiol.* **395**, 233–253.

Hagiwara, S., and Kawa, K. (1984). *J. Physiol.* **356**, 135–149.

Hagiwara, S., Ozawa, S., and Sand, O. (1975). *J. Gen. Physiol.* **65**, 617–644.

Hamill, O. P., Huguenard, J. R., and Prince, D. A. (1991). *Cereb. Cortex* **1**, 48–61.

Han, W., Chartier, D., Li, D., and Nattel, S. (2001). *Circulation* **104**, 2095–2100.

Hansen, P. B., Jensen, B. L., Andreasen. D., and Skott, O. (2001). *Circ. Res.* **89**, 630–638.

Hayashi, K., Endoh, T., Shibukawa, Y., Yamamoto, T., and Suzuki, T. (2002). *Bull. Tokyo Dent. Coll.* **43**, 31–39.

Heinemann, U., Lux, H. D., and Gutnick, M. J. (1977). *Exp. Brain Res.* **27**, 237–243.

Hell, J. W., Westenbroek, R. E., Breeze, L. J., Wang, K. K. W., Chavkin, C., and Catterall, W. A. (1996). *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3362–3367.

Hering, S., Berjukow, S., Sokolov, S., Marksteiner, R., Weiβ, R. G., Kraus, R., and Timin, E. N. (2000). *J. Physiol.* **528**, 237–249.

Herlitze, S., Hockerman, G. H., Scheuer, T., and Catterall, W. A. (1997). *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1512–1516.

Hermsmeyer, K. (1991). *Am. J. Hypertens.* **4**, 412S–415S.

Hernandez-Crus, A., and Pape, H.-C. (1989). *J. Neurophysiol.* **61**, 1270–1283.

Herrington, J., and Lingle, C. J. (1992). *J. Neurophysiol.* **68**, 213–231.

Hirano, Y., Fozzard, H. A., and January, C. T. (1989). *Am. J. Physiol.* **256**, H1478–H1492.

Hirooka, K., Bertolesi, G. E., Kelly, M. E. M., Denovan-Wright, E. M., Sun, X., Hamid, J. Zamponi, G. W., Juhasz, A. E., Haynes, L. W., and Barnes, S. (2002). *J. Neurophysiol.* **88**, 196–205.

Hockerman, G. H., Johnson, B. D., Abbott, M. R., Scheuer, T., and Catternall, W. A. (1997). *J. Biol. Chem.* **272**, 18759–18765.

Hoehn, K., Watson, T. W., and MacVicar, B. A. (1993). *J. Neurosci.* **13**, 1244–1257.

Hoischen, S., Brixius, K., and Schwinger, R. H. (1998). *J. Cardiovasc. Pharmacol.* **32**, 323–330.

Honda, M., Hayashi, K., Matsuda, H., Kubota, E., Tokyuama, H., Okubo, K., Takamatsu, I., Ozawa, Y., and Saruta, T. (2001). *J. Hypertens.* **19**, 2031–2037.

Horvath, A., Szabadkai, G., Varnai, P., Aranyi, T., Wollheim, C. B., Spat, A., and Enyedi, P. (1998). *Cell Calcium* **1998**, 33–42.

Hosey, M. M., Barhanin, J., Schmid, A., Vandaele, S., Ptasienski, J., O'Callahan, C., Cooper, C., and Lazdunski, M. (1987). *Biochem. Biophys. Res. Commun.* **147**, 1137–1145.

Huang, B., Qin, D., Deng, L., Boutjdir, M., and El-Sherif, N. (2000). *Cardiovasc. Res.* **2000**, 442–449.

Huguenard, J. R. (1996). *Annu. Rev. Physiol.* **58**, 329–348.

Huguenard, J. R., Gutnick, M. J., and Prince, D. A. (1993). *J. Neurophysiol.* **70**, 1373–1383.

Huguenard, J. R., and McCormick, D. A. (1992). *J. Neurophysiol.* **68**, 1373–1383.

Huguenard, J. R., and Prince, D. A. (1992). *J. Neurosci.* **12**, 3804–3817.

Huguenard, J. R., and Prince, D. A. (1994). *J. Neurosci.* **14**, 5485–5502.

Huizinga, J. D., Farraway, L., and Den Hertog, A. (1991). *J. Physiol.* **442**, 15–29.

Hüser, J., Blatter, L. A., and Lipsius, S. L. (2000). *J. Physiol.* **524**, 415–422.

Imbert, N., Vandebrouck, C., Duport, G., Raymond, G., Hassoni, A. A., Constantin, B., Cullen, M. J., and Cognard, C. (2001). *J. Physiol.* **534**, 343–355.

Ishige, K., Aizawa, M., Ito, Y., and Fukuda, H. (1996). *Neuropharmacology* **35**, 45–55.

Ishige, K., Endo, H., Saito, H., and Ito, Y. (2001). *Neurosci. Lett.* **297**, 207–210.

Ishige, K., Ito, Y., and Fukuda, H. (1998). *Jpn. J. Psychopharmacol.* **18**, 117–122.

Ishige, K., Ito, Y., and Fukuda, H. (1999). *Neurosci. Lett.* **262**, 53–56.

Ito, H., Hirata, Y., Hiroe M., Tsujino, M., Adachi, S., Takamoto, T., Nitta, M., Taniguchi, K., and Marumo, F. (1991). *Circ. Res.* **69**, 209–215.

Ito, H., Hiroe, M., Hirata, Y., Fujisaki, H., Adachi, S., Akimoto, H., Ohta, Y., and Marumo, F. (1994). *Circulation* **89**, 2198–2203.

Ito, M. (1957). *Jp. J. Physiol.* **7**, 297–323.

Jacob, T. J. C. (1991). *Am. J. Physiol.* **261**, C808–C813.

Jagannathan, S., Publicover, S. J., and Barratt, C. L. R. (2002a). *Reproduction* **123**, 203–215.

Jagannathan, S., Pun, E. L., Gu, Y., Arnoult, C., Sakkas, D., Baratt, C. L. R., and Publicover, S. J. (2002b). *J. Biol. Chem.* **277**, 8449–8456.

Jahnsen, H., and Llinás, R. (1982). *J. Physiol.* **315**, 569–584.

Jahnsen, H., and Llinás, R. (1984a). *J. Physiol.* **349**, 205–226.

Jahnsen, H., and Llinás, R. (1984b). *J. Physiol.* **349**, 227–247.

Janssen, L. J. (1997). *Am. J. Physiol.* **272**, C1757–C1765.

Jay, S. D., Sharp, A. H., Kahl, S. D., Vedvick, T. S., Harpold, M. M., *et al.* (1991). *J. Biol. Chem.* **266**, 3287–3293.

Jones, S. W. (1998). *J. Bioenerg. Biomembr.* **30**, 299–318.

Kandel, E. R., and Spencer, W. A. (1961). *J. Neurophysiol.* **24**, 243–259.

Kaneda, M., and Akaike, N. (1989). *Brain Res.* **497**, 187–190.

Kaneko, A., Pinto, L. H., and Tachibana, M. (1989). *J. Physiol.* **410**, 613–629.

Kang, M.-G., Chen, C.-C., Felix, R., Letts, V. A., Frankel, W. N., Mori, Y., and Campbell, K. P. (2001). *J. Biolog. Chem.* **276**, 32917–32924.

Kano, M., Satoh, R., and Nakabayashi, Y. (1992). *Neurosci. Lett.* ***144****, 161–164.*

Karschin, A., and Lipton, S. A. (1989). J. Physiol. **418**, 379–396.

Karst, H., Joëls, M., and Wadman, W. J. (1993). *Neurosci. Lett.* **164**, 154–158.

Kase, M., Kakimoto, S., Sakuma, S., Houtani, T., Ohishi, H., Ueyama, T., and Sugimoto, T. (1999). *Neurosci. Lett.* **268**, 77–80.

Kato, S., Ishida, H., Okamoto, Y., Tsuji, K., Horie, M., Okada, Y., and Seino, Y. (1994). *Metabolism* **43**, 1395–1400.

Kato, S., Ishida, H., Tsuura, Y., Tsuji, K., Nishimura, M., Horie, M., Taminato, T., Ikehara, S., Odaka, H., Ikeda, I., Okada, Y., and Seino, Y. (1996). *J. Clin. Invest.* **97**, 2417–2425.

Kato, T., Otsu, Y., Furune, Y., and Yamamoto, T. (1992). *Neurochem. Int.* **21**, 99–107.

Kavalali, E. T., Zhuo, M., Bito, H., and Tsien, R. W. (1997). *Neuron* **18**, 651–663.

Kawai, F. (1999). *Neurosci. Res.* **35**, 253–263.

Kawai, F., Kurahashi, T., and Kaneko, A. (1996). *J. Gen. Physiol.* **108**, 525–535.

Kawai, F., Kurahashi, T., and Kaneko, A. (1997). *J. Gen. Physiol.* **109**, 265–272.

Kawai, F., Kurahahsi, T., and Kaneko, A. (1999). *Nat. Neurosci.* **2**, 133–138.

Kawai, F., and Miyachi, E. (2001a). *J. Neurosci.* **21**, 1–5.

Kawai, F., and **Miyachi**, E. (2001b). *Neurosci. Res.* **39**, 327–337.

Kawano, S., and DeHaan, R. L. (1989). *Am. J. Physiol.* **256**, H1505–H1508.

Kawano, S., and DeHaan, R. L. (1990). *J. Membr. Biol.* **116**, 9–17.

Kawano, S., and DeHaan, R. L. (1991). *J. Membr. Biol.* **120**, 17–28.

Kim, D., Song, I., Keum, S., Lee, T., Jeong, M. J., Kim, S. S., McEnery, M. W., and Shin, H. S. (2001). *Neuron* **31**, 35–45.

Kirischuk, S., Scherer, J., Möller, T., Verkhratsky, A., and Kettenmann, H. (1995). *Glia* **13**, 1–12.

Kitasato, H., Kai, R., Ding, W. G., and Omatsu-Kanbe, M. (1996). *Jpn. J. Physiol.* **46**, 363–373.

Kito, M., Maehara, M., and Watanabe, K. (1999). *Najoya. N. Med. Sci.* **62**, 39–45.

Klöckner, U., Lee, J.-H., Cribbs, L. L., Daud, A., Hescheler, J., Pereverzev, A., Perez-Reyes, E., and Schneider, T. (1999). *Eur. J. Neurosci.* **11**, 4171–4178.

Klugbauer, N., Dai, S., Specht, V., Lacinová, L., Marais, E., Bohn, G., and Hofmann, F. (2000). *FEBS Lett.* **470**, 189–197.

Klugbauer, N., Marais, E., Lacinová, L., and Hofmann, F. (1999). *Pflugers Arch.* **437**, 710–715.

Ko, F.-N., Huang, S.-Y., and Teng, C.-M. (1997). *Br. J. Pharmacol.* **120**, 565–570.

Kobrinsky, E. M., Pearson, H. A., and Dolphin, A. C. (1994). *Neuroscience* **58**, 539–552.

Koizumi, A., Watanabe, S.-I., and Kaneko, A. (2001). *J. Neurophysiol.* **86**, 1006–1016.

Komatsu, Y., and Iwakiri, M. (1992). *J. Neurophysiol.* **67**, 401–410.

Kostyuk, P., Pronchuk, N., Savchenko, A., and Verkhratsky, A. (1993). *J. Physiol.* **461**, 467–483.

Kostyuk, P. G., Molokanova, E. A., Pronchuk, N. F., Savchenko, A. N., and Verhratsky, A. N. (1992). *Neuroscience* **51**, 755–758.

Kostyuk, P. G., Shuba, Y., and Savchenko, A. N. (1988). *Pflugers Arch.* **411**, 661–669.

Kozlov, A. S., McKenna, F., Lee, J.-H., Cribbs, L. L., Perez-Reyes, E., Feltz, A., and Lambert, R. C. (1999). *Eur. J. Neurosci.* **11**, 4149–4158.

Kuga, T., Kobayashi, S., Hirakawa, Y., Kanaide, H., and Takeshita, A. (1996). *Circ. Res.* **79**, 14–19.

Kuga, T., Sadoshima, J., Tomoike, H., Kanaide, H., Akaike, N., and Nakamura, M. (1990). *Circ. Res.* **67**, 469–480.

Kunert-Radek, J., Stepien, H., Lyson, K., and Pawlikowski, M. (1990). *Agents Act.* **29**, 254–258.

Kusaka, M., Tohse, N., Nakaya, H., Tanaka, T., Kanno, M., and Fujimoto, S. (1993). *Biol. Reprod.* **49**, 95–103.

Kushmerick, C., Romano-Silva, M. A., Gomez, M. V., and Prado, M. A. M. (2001). *Brain Res.* **916**, 199–210.

Lacerda, A. E., Perez-Reyes, E., Wei, X., Castellano, A., and Brown, A. M. (1994). *Biophys. J.* **66**, 1833–1843.

Lacinová, L., Klugbauer, N., and Hofmann, F. (1999). *J. Physiol.* **516**, 639–645.

Lam, E., Skarsgard, P., and Laher, I. (1998). *Eur. J. Pharmacol.* **358**, 165–168.

Lambert, R. C., Maulet, Y., Mouton, J., Beattie, R., Volsen, S., De Waard, M., and Feltz, A. (1997). *J. Neurosci.* **17**, 6621–6628.

Lambert, R. C., McKenna, F., Maulet, Y., Talley, E. M., Bayliss, D. A., Cribbs, L. L., Lee, J.-H., Perez-Reyes, E., and Feltz, A. (1998). *J. Neurosci.* **18**, 8605–8613.

Lee, J.-H., Daud, A. N., Cribbs, L. L., Lacerda, A. E., Pereverzev, A., Klöckner, U., Schneider, T., and Perez-Reyes, E. (1999). *J. Neurosci.* **19**, 1912–1921.

Lee, J. H., Kim, E. G., Park, B. G., Kim, K. H., Cha, S. K., Kong, I. D., Lee, J. W., and Jeong, S. W. (2002). *J. Neurophysiol.* **87**, 2844–2850.

Letts, V. A., Felix, R., Biddlecome, G. H., Arikkath, J., Mahaffey, C. L., Valenzuela, A., Bartlett, F. S., Mori, Y., Campell, K. P., and Frankel, W. N. (1998). *Nat. Genet.* **19**, 340–347.

Leuranguer, V., Bourinet, E., Lory, P., and Nargeot, J. (1998). *Neuropharmacology* **37**, 701–708.

Leuranguer, V., Monteil, A., Bourninet, E., Dayanithi, G., and Nargeot, J. (2000). *Am. J. Physiol.* **279**, H2540–H2548.

Lewis, R. S., and Cahalan, R. S. (1995). *Annu. Rev. Immunol.* **13**, 623–653.

Li, D., Melnyk, P., Feng, J., Wang, Z., Petrecca, K., Shrier, A., and Nattel, S. (2000). *Circulation* **101**, 2631–2638.

Li, J., Qu, J., and Nathan, R. D. (1997). *Am. J. Physiol.* **273**, H2481–H2489.

Lieberherr, M., Grosse, B., and Machelon, V. (1999). *J. Cell Biochem.* **74**, 50–60.

Liévano, A., Santi, C. M., Serrano, J., Treviêno, C. L., Bellvé, A. R., Hernández-Cruz, A., and Darszon, A. (1996). *FEBS Lett.* **388**, 150–154.

Lingle, C. J., Sombati, S., and Freeman, M. E. (1986). *J. Neurosci.* **6**, 2995–3005.

Lipkind, G. M., and Fozzard, H. A. (2001). *Biochemistry* **40**, 6786–6794.

Lipsius, S. L., Hüser, J., and Blatter, L. A. (2001). *News Physiol. Sci.* **16**, 101–106.

Liu, H., De Waard, M., Scott, V. E., Gurnett, C. A., Lennon, V. A., and Campbell, K. P. (1996). *J. Biol. Chem.* **271**, 13804–13810.

Liu, J. H., Bijlenga, P., Occhiodoro, T., Fischer-Lougheed, J., Bader, C. R., and Bernheim, L. (1999). *Br. J. Pharmacol.* **126**, 245–250.

Liu, Y., and Lasater, E. M. (1994). *J. Neurophysiol.* **71**, 733–742.

Llinás, R. (1988). *Science* **242**, 1654–1664.

Llinás, R., and Jahnsen, H. (1982). *Nature* **297**, 406–408.

Llinás, R., and Yarom, Y. (1981a). *J. Physiol.* **315**, 549–567.

Llinás, R., and Yarom, Y. (1981b). *J. Physiol.* **315**, 569–584.

Lovinger, D. M., and White, G. (1989). *Neurosci. Lett.* **102**, 50–57.

Lux, H. D. (1988). *PR Health Sci.* **7**, 116–121.

Lüthi, A., and McCormick, D. A. (1998). *Neuron* **21**, 9–12.

MacVicar, B. A. (1984). *Science* **226**, 1345–1347.

Magee, J. C., and Johnston, D. (1995). *Science* **268**, 301–304.

Magistretti, J., and de Curtis, M. (1998). *J. Neurophysiol.* **79**, 808–816.

Maguire, G., Maple, B., Lukasiewicz, P., and Werblin, F. (1992). *Proc. Natl. Acad. Sci. U.S.A.* **86**, 10144–10147.

Mariot, P., Vanoverberghe, K., Lalevée, N., Rossier, M. F., and Prevarskaya, N. (2002). *J. Biol. Chem.* **277**, 10824–10838.

Markram, H., and Sakmann, B. (1994). *Proc. Natl. Acad. Sci. U.S.A.* **91**, 5207–5211.

Martin-Caraballo, M., and Greer, J. J. (2001). *J. Neurobiol.* **46**, 231–248.

Martin-Moutot, N., Charvin, N., Leveque, C., Sato, K., Nishi, T., *et al.* (1996). *J. Biol. Chem.* **271**, 6567–6570.

Martin-Moutot, N., Leveque, C., Sato, K., Kato, R., Takahashi, M. *et al.* (1995). *FEBS Lett.* **366**, 21–25.

Martinez, M. L., Heredia, M. P., and Delgado, C. (1999). *J. Mol. Cell. Cardiol.* **31**, 1617–1625.

Massie, B. M. (1998). *Am. J. Hypertens*, **11**, 95S–102S.

Matsuda, J. J., Volk, K. A., and Shibata, E. F. (1990). *J. Physiol.* **427**, 657–680.

Matsuura, T., Yamagat, T., Burgess, D. L., Rasmussen, A., Grewel, R. P., Watase, K., Khajavi, M., McCall, A. E., Davis, C. F., Zu, L., Achari, M., Pulst, S. M., Alonso, E., Noebels, J. L., Nelson, D. L., Zoghbi, H. Y., and Ashizawa, T. (2000). *Nat. Genet.* **26**, 191–194.

Matteson, D. R., and Armstrong, C. M. (1986). *J. Gen. Physiol.* **87**, 161–182.

Matthews, E. A., and Dickenson, A. H. (2001). *Eur. J. Pharmacol.* **415**, 141–149.

Maylie, J., and Morad, M. (1995). *Am. J. Physiol.* **269**, H1695–H1703.

McCarthy, R. T., Isales, C., and Rasmussen, H. (1993). *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3260–3264.

McCobb, D. P., and **Beam**, K. G. (1991). *Neuron* **7**, 119–127.

McCobb, D. P., Best, P. M., and Beam, K. G. (1989). *Neuron* **2**, 1633–1643.

McCormick, D. A., and Bal, T. (1997). *Annu. Rev. Neurosci.* **20**, 185–215.

McCormick, D. A., and Huguenard, J. R. (1992). *J. Neurophysiol.* **68**, 1384–1400.

McCormick, D. A., and Pape, H.-C. (1990). *J. Physiol.* **431**, 291–318.

McEnery, M. W., Copeland, T. D., and Vance, C. L. (1998a). *J. Biol. Chem.* **273**, 21435–21438.

McEnery, M. W., Snowman, A. M., Sharp, A. H., Adams, M. E., and Snyder, S. H. (1991). *Proc. Natl. Acad. Sci. U.S.A.* **88**, 11095–11099.

McEnery, M. W., Vance, C. L., Begg, C. M., Lee, W. L., Choi, Y., and Dubel, S. J. (1998b). *J. Bioenerg. Biomembr.* **30**, 409–418.

McRory, J. E., Santi, C. M., Hamming, K. S., Mezeyova, J., Sutton, K. G., Baillie, D. L., Stea, A., and Snutch, T. P. (2001). *J. Biol. Chem.* **276**, 3999–4011.

Mealing, G., Morley, P., Whitfield, J. F., Tsang, B. K., and Schwartz, J. L. (1994). *Pflugers Arch.* **428**, 307–314.

Meier, H. (1967). *Arch. Neurol.* **16**, 59–66.

Meir, A., and Dolphin, A. C. (1998). *Neuron* **20**, 341–351.

Miftakhov, R. N., and Abdusheva, G. R. (1996). *Biol. Cybern.* **74**, 455–467.

Miftakhov, R. N., and Abdusheva, G. R., and Christensen, J. (1999a). *J. Theor. Biol.* **197**, 89–112.

Miftakhov, R. N., Abdusheva, G. R., and Christensen, J. (1999b). *J. Theor. Biol.* **200**, 261–290.

Miftakhov, R. N., Abdusheva, G. R., and Wingate, D. L. (1996). *Biol. Cybern.* **74**, 167–179.

Mishra, S. K., and Hermsmeyer, K. (1994). *Circ. Res.* **75**, 144–148.

Misler, S., Barnett, D. W., Gills, K. D., and Pressel, D. M. (1992). *Diabetes* **41**, 1221–1228.

Mitra, R., and Morad, M. (1986). *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5340–5344.

Mittman, S., Guo, J., and Angew, W. S. (1999a). *Neurosci. Lett.* **274**, 143–146.

Mittman, S., Guo, J., Emerick, M. C., and Agnew, W. S. (1999b). *Neurosci. Lett.* **269**, 121–124.

Mlinar, B., Biagi, B. A., and Enyeart, J. J. (1993). *J. Gen. Physiol.* **102**, 217–237.

Mochizuki, T., Wu, G., Hayashi, T., Xenophontos, S. L., Veldhuisen, B., Saris, J. J., Reynolds, D. M., Cai, Y., Gabow, P. A., Pierides, A., Kimberling, W. J., Breuning, M. H., Deltas, C. C., Peters, D. J., and Somlo, S. (1996). *Science.* **272**, 1339–1342.

Monteil, A., Chemin, J., Bourinet, E., Mennessier, G., Lory, P., and Nargeot, J. (2000a). *J. Biol. Chem.* **275**, 6090–6100.

Monteil, A., Chemin, J., Leurangeur, V., Altier, C., Mennessier, G., Bourinet, E., Lory, P., and Nargeot, J. (2000b). *J. Biol. Chem.* **275**, 16530–16535.

Montgomery, J. C., Silverman, K. A., Buchberg, A. M. (1997). *Mamm. Genome.* **7**, S190–S208.

Montiminy, M. R., Gonzalez, G. A., and Yamamoto, K. K. (1990). *Trends Neurosci.* **13**, 184–188.

Morley, P., Tsang, B. K., Whitfield, J. F., and Schwartz, J. L. (1992). *Endocrinology* **130**, 663–670.

Morley, P., Vanderhyden, B. C., Tremblay, R., Mealing, G. A., Durkin, J. P., and Whitfield, J. F. (1994). *Endocrinology* **134**, 1269–1276.

Mouginot, D., Bossu, J. L., and Gähwiler, B. H. (1997). *J. Neurosci.* **17**, 160–170.

Mulder, P., Richard, V., Compagnon, P., Henry, J. P., Lallemand, F., Clozel, J. P., Koen, R., Mace, B., and Thuillez, C. (1997). *J. Am. Coll. Cardiol.* **29**, 416–421.

Muramatsu, M., Tyler, R. C., Rodman, D. M., and McMurtry, I. F. (1997). *Am. J. Physiol.* **272**, H2616–H2621.

Murchison, D., and Griffith, W. H. (1995). *J. Neurophysiol.* **74**, 876–887.

Nair, R. R., and Nair, P. (2001). *Int. J. Biochem. Cell Biol.* **33**, 119–125.

Nakamura, Y., Ono, H., and Frohlich, E. D. (1999). *Hypertension* **34**, 273–278.

Narahashi, T., Tsunoo, A., and Yoshii, M. (1987). *J. Physiol.* **383**, 231–249.

Nattel, S. (1999). *Circ. Res.* **85**, 473–476.

Nattel, S., and Li, D. (2000). *Circ. Res.* **87**, 440–447.

Neely, A., Wei, X., Olcese, R., Birnbaumer, L., and Stefani, E. (1993). *Science* **262**, 575–578.

Neveu, D., Nargeot, J., and Richard, S. (1993). *Pflugers Arch.* **424**, 45–53.

Neveu, D., Quignard, J. F., Fernandez, A., Richard, S., and Nargeot, J. (1994). *J. Physiol.* **479**, 171–182.

Nilius, B., Hess, P., Lansman, J. B., and Tsien, R. W. (1985). *Nature* **316**, 443–446.

Nilius, B., Prenen, J., Kamouchi, M., Viana, F., Voets, T., and Droogmans, G. (1997). *Br. J. Pharmacol.* **121**, 547–555.

Nowycky, M. C., Fox, A. P., and Tsien, R. W. (1985). *Nature* **316**, 440–443.

Nuss, H. B., and Houser, S. R. (1993). *Circ. Res.* **73**, 777–782.

Nuss, H. B., and Marbàn, E. (1994). *J. Physiol.* **479**, 265–279.

Officer, T. M., and Ewert, D. L. (1998). *Biomed. Instrum. Technol.* **32**, 155–163.

Ohya, Y., Abe, I., Fujii, K., Takata, Y., and Fujishima, M. (1993). *Circ. Res.* **73**, 1090–1099.

Oparil, S. (1998). *Am. J. Hypertens.* **11**, 88S–94S.

Osaka, T., and Joyner, R. W. (1991). *Circ. Res.* **68**, 788–796.

Ouadid, H., Séguin, S., Chaptal, P. A., and Nargeot, J. (1991). *Circ. Res.* **23**, 41–54.

Ozawa, Y., Hayashi, K., Nagahama, T., Fujiwara, K., and Saruta, T. (2001). *Hypertension* **38**, 343–347.

Page, K. M., Stephens, G. M., Berrows, N. S., and Dolphin, A. C. (1997). *J. Neurosci.* **17**, 1330–1338.

Pan, Z.-H., Hu, H.-J., Perring, P., and Andrade, R. (2001). *Neuron* **32**, 89–98.

Pan, Z.-H., and Lipton, S. A. (1995). *J. Neurosci.* **15**, 2668–2679.

Paquette, F., Jasmin, G., and Dumont, L. (1999). *Cardiovasc. Drugs Ther.* **13**, 525–530.

Pare, D., Curro'Dossi, R., and Steriade, M. (1990). *Neuroscience* **35**, 217–226.

Park, K. S., Jeong, S. W., Cha, S. K., Lee, B. S., Kong, I. D., Ikeda, S. R., and Lee, J. W. (2001). *J. Pharmacol. Exp. Ther.* **299**, 501–508.

Pellegrini, A., Dossi, R. C., Dal Pos, F., Ermani, M., Zanotto, L., and Testa, G. (1989). *Brain Res.* **497**, 344–360.

Perchenet, L., Benardeau, A., and Ertel, E. A. (2000). *Naunyn Schmiedebergs Arch. Pharmacol.* **361**, 590–599.

Perez-Reyes, E. (1998). *J. Bioenerg. Biomembr.* **30**, 313–318.

Perez-Reyes, E., Cribbs, L. L., Daud, A., Lacerda, A. E., Barclay, J., Williamson, M. P., Fox, M., Rees, M., and Lee, J.-H. (1998). *Nature* **391**, 896–900.

Perez-Reyes, E., Lee, J.-H., and Cribbs, L. L. (1999). *Ann. N.Y. Acad. Sci.* **868**, 131–143.

Perez-Reyes, E., Wei, X., Castellano, A., and Birnbaumer, L. (1990). *J. Biol. Chem.* **265**, 20430–20436.

Peterson, B. Z., DeMaria, C. D., Adelman, J., and Yue, D. T. (1999). *Neuron* **22**, 549–558.

Pfeiffer-Linn, C., and Lasater, E. M. (1993). *J. Gen. Physiol.* **102**, 277–294.

Pfrieger, F. W., Veselovsky, N. S., Gottmann, K., and Lux, H. D. (1992). *J. Neurosci.* **12**, 4347–4357.

Pinto, J. M., Sosunov, E. A., Gainullin, R. Z., Rosen, M. R., and Boyden, P. A. (1999). *J. Cardiovasc. Electrophysiol.* **10**, 1224–1235.

Pirchio, M., Lightowler, S., and Crunelli, V. (1990). *Neurosciene* **38**, 39–45.

Pouille, F., Cavelier, P., Desplantez, T., Beekenkamp, H., Craig, P. J., Beattie, R. E., Volsen, S. G., and Bossu, J. L. (2000). *J. Physiol.* **527**, 265–282.

Pragnell, M., De Waard, M., Mori, Y., Tanabe, T., Snutch, T. P., and Campbell, K. P. (1994). *Nature* **368**, 67–70.

Pulver, A. E., Karayiorgou, M., Wolyniec, P. S., Lasseter, V. K., Kasch, L., Nestadt, G., Antonarakis, S., Housman, D., Kazazian, H. H., and Meyers, D. (1994). *Am. J. Med. Genet.* **54**, 36–43.

Qin, N., Platano, D., Olcese, R., Costantin, J. L., Stefani, E., and Birnbaumer, L. (1998). *Proc. Natl. Acad. Sci. U.S.A.* **95**, 4690–4695.

Qin, N., Platano, D., Olcese, R., Stefani, E., and Birnbaumer, L. (1997). *Proc. Natl. Acad. Sci. U.S.A.* **94**, 8866–8861.

Qu, Y., and Boutjdir, M. (2002). *Pediatr. Res.* **50**, 569–574.

Quignard, J. F., Harricane, M. C., Lory, P., Nargeot, J., Capron, L., Mornet, D., and Richard, S. (2001). *Cardiovasc. Res.* **49**, 177–188.

Raman, I. M., and Bean, B. P. (1999). *J. Neurosci.* **19**, 1663–1674.

Randall, A. D. (1998). *J. Membr. Biol.* **161**, 207–213.

Randall, A. D., and Tsien, R. W. (1997). *Neuropharmacology* **36**, 879–893.

Ravichandra, B., and Joshi, P. G. (1999). *J. Neurochem.* **73**, 557–567.

Rettig, J., Sheng, Z. H., Kim, D. K., Hodson, C. D., Snutch, T. P., and Catterall, W. A. (1996). *Proc. Natl. Acad. Sci. U.S.A.* **93**, 7363–7368.

Richard, S., Leclercq, F., Lemaire, S., Piot, C., and Nargeot, J. (1998). *Cardiovasc. Res.* **37**, 300–311.

Richard, S., Neveu, D., Carnac, G., Bodin, P., Travo, P., and Nargeot, J. (1992). *Biochim. Biophys.* **Acta 1160**, 95–104.

Richard, S., Tiaho, F., Charnet, P., Nargeot, J., and Nerbonne, J. M. (1990). *Am. J. Physiol.* **258**, H1872–H1881.

Ritchie, K. K. (1993). *Endocrinology* **132**, 1621–1629.

Rossier, M. F., Ertel, E. A., Vallotton, M. B., and Capponi, A. M. (1998). *J. Pharmacol. Exp. Ther.* **1988**, 824–831.

Rossier, M. F., Python, C. P., Capponi, A. M., Schlegel, W., Kwan, C. Y., and Valloton, M. B. (1993). *Endocrinology* **132**, 1035–1043.

Rothe, T., Juttner, R., Bahring, R., and Grantyn, R. (1999). *J. Neurobiol.* **38**, 191–206.

Roussier, M. F., Pyton, C. P., Capponi, A. M., Schlegel, w., Kwan, C. Y., and Vallotton, M. B. (1993). *Endocrinology* **76**, 859–885.

Rohwedel, J., Maltsev, V., Bober, E., Arnold, H. H., Hescheler, J., Wobus, A. M. (1994). *Dev. Biol.* **164**, 87–101.

Sala, S., and Matteson, D. R. (1990). *Biophys. J.* **1990**, 567–571.

Sandmann, S., Bohle, R. M., Dreyer, T., and Unger, T. (2000). *Virchows Arch.* **436**, 147–157.

Sandmann, S., Min, J. Y., Meisser, A., and Unger, T. (1999). *Cardiovasc. Res.* **44**, 67–80.

Sandmann, S., Spitznagel, H., Chung, O., Xia, Q. G., Illner, S., Janichen, G., Rossius, B., Daemen, M. J., and Unger, T. (1998). *Cardiovasc. Res.* **39**, 339–350.

Santi, C. M., Darszon, A., and Hrnandez-Cruz, A. (1996). *Am. J. Anat.* **271**, C1583–C1593.

Sarsero, D., Fujiwara, T., Molenaar, P., and Angus, J. A. (1998). *Br. J. Pharmacol.* **125**, 109–119.

Sartor, P., Couderc, B., Corcuff, J. B., Odessa, M. F., Mayer, G., and Dufy, B. (1992). *Endocrinology* **130**, 3433–3440.

Satin, J., and Cribbs, L. L. (1999). *Circ. Res.* **86**, 636–642.

Satin, L. S. (2000). *Endocrine* **13**, 251–262.

Satin, L. S., and Cook, D. L. (1988). *Pflugers Arch.* **411**, 401–409.

Satoh, R., Nakabayashi, Y., and Kano, M. (1991). *Neurosci. Lett.* **122**, 233–236.

Sayer, R. J., Brown, A. M., Schwindt, P. C., and Crill, W. E. (1993). *J. Neurophysiol.* **69**, 1596–1606.

Schmitt, R., Clozel, J.-P., Iberg, N., and Buhler, F. (1995). *Arterioscler. Thromb. Vasc. Biol.* **15**, 1161–1165.

Schmitt, R., Clozel, J.-P., Iberg, N., and Buhler, F. R. (1996). *Cardiovasc. Drugs Ther.* **10**, 101–105.

Schrier, A. D., Wang, H., Talley, E. M., Perez-Reyes, E., and Barrett, P. Q. (2001). *Am. J. Physiol. Cell Physiol.* **280**, C265–C272.

Schroeder, J. E., Fischbach, P. S., and McCleskey, E. W. (1990). *J. Neurosci.* **10**, 947–951.

Schulptoreanu, A., Abramovici, H., Abdllah, A. A. R., Bibikova, A., Panet-Raymond, V., Frankel, D., Schipper, H. M., Pinsky, L., and Trifiro, M. A. (2000). *Mol. Cell. Biochem.* **203**, 23–31.

Schwartz, J. L., Asem, E. K., Mealing, G. A., Tsang, B. K., Rousseau, E. C., Whitfield, J. F., and Payet, M. D. (1989). *Endocrinology* **125**, 1973–1982.

Schwartz, J. L., Mealing, G. A. R., Asem, E. K., Tsang, B. K., and Whitfield, J. F. (1988). *FEBS Lett.* **241**, 169–172.

Self, D. A., Bian, K., Mishra, S. K., and Hermsmeyer, K. (1994). *J. Vasc. Res.* **31**, 359–366.

Sen, L., and Smith, T. W. (1994). *Circ. Res.* **75**, 149–155.

Sharp, A. H., Black, J. L., Dubel, S. J., Sundarraj, S., Shen, J.-P., Yunker, A. M. R., Copeland, T. D., and McEnery, M. W. (2001). *Neuroscience* **105**, 599–617.

Shen, L., Ahuja, N., Shen, Y., Habib, N. A., Toyata, M., Rashid, A., and Issa, J. P. J. (2002). *J. Natl. Cancer Inst.* **94**, 755–761.

Shimahara, T., and Bournaud, R. (1991). *Cell Calcium* **12**, 727–733.

Shoimi, H., Yamano, S., and Anono, T. (1996). *Arch. Androl.* **37**, 79–86.

Silve, R. A., and Bolsover, S. R. (1991). *J. Physiol. Paris* **85**, 79–83.

Simen, A. A., and Miller, R. J. (1998). *J. Neurosci.* **18**, 3689–3698.

Simen, A. A., and Miller, R. J. (2000). *Mol. Pharm.* **57**, 1064–1074.

Sipido, K. R., Carmeliet, E., and Van de Werf, F. (1998). *J. Physiol.* **508**, 439–451.

Smirnov, S., Zholos, A., and Shuba, M. (1992a). *J. Physiol.* **454**, 549–571.

Smirnov, S. V., and Aaronson, P. l. (1992). *J. Physiol.* **457**, 455–475.

Smirnov, S. V., Zholos, A. V., and Shuba, M. F. (1992b). *J. Physiol.* **454**, 549–571.

Smith, M. R., Nelson, A. B., and DuLuc, S. (2002). *J. Neurophysiol.* **87**, 2031–2042.

Snutch, T. P., and Reiner, P. B. (1992). *Curr. Neurobiol.* **2**, 247–253.

Snutch, T. P., Tomilson, W. J., Leonard, J. P., and Gilbert, M. M. (1991). *Neuron* **7**, 45–57.

Soltesz, I., Lightowler, S., Leresche, N., Jassik-Gerschenfeld, D., Pollard, C. E., and Crunelli, V. (2001). *J. Physiol.* **441**, 175–197.

Son, W. Y., Han, C. T., Lee, J. H., Jung, K. Y., Lee, H. M., and Choo, Y. K. (2002). *Dev. Growth Differ.* **44**, 181–190.

Son, W. Y., Lee, J. H., Lee, J. H., and Han, C. T. (2000). *Mol. Hum. Reprod.* **6**, 893–897.

Song, D., Xie, X., Wang, Z., and Berger, T. W. (2001). *Neurobiol. Learn Mem.* **76**, 375–387.

Sontheimer, H. (1994). *Glia* **11**, 156–172.

Soong, T., Stea, A., Hodson, C. D., Dubel, S. J., Vincent, S. R., and Snutch, T. P. (1993). *Science* **260**, 1133–1136.

Stea, A., Soong, T. W., and Snutch, T. P. (1995). *Neuron* **15**, 929–940.

Stephens, G. J., Morris, N. P., Fyffe, R. E., and Robertson, B. (2001). *Eur. J. Neurosci.* **13**, 1902–1912.

Steriade, M., Contreras, D., and Amzica, F. (1994). *Trends Neurosci.* **17**, 199–208.

Steriade, M., and Deschenes, M. (1984). *Brain Res. Rev.* **8**, 1–63.

Steriade, M., and Llinás, R. (1988). *Physiol. Rev.* **68**, 649–742.

Steriade, M., Nunez, A., and Amzica, F. (1993a). *J. Neurosci.* **13**, 3252–3265.

Steriade, M., Nunez, A., and Amzica, F. (1993b). *J. Neurosci.* **13**, 266–283.

Strobeck, M. W., Okuda, M., Yamaguchi, H., Schwartz, A., and Fukasawa, K. (1999). *J. Biol. Chem.* **274**, 15694–15700.

Struass, O., and Wienrich, M. (1994). *J. Cell. Physiol.* **160**, 89–96.

Strube, C., Beurg, M., Powers, P. A., Gregg, R. G., and Coronado, R. (1996). *Biophys. J.* **71**, 2531–2543.

Strube, C., Tourneur, Y., and Ojeda, C. (2000). *Biophys. J.* **78**, 1282–1292.

Su, H., Sochivko, D., Becker, A., Chen, J., Jiang, Y., Yaari, Y., and Beck, H. (2002). *J. Neurosci.* **22**, 3645–3655.

Sui, G. P., Wu, C., and Fry, C. H. (2001). *J. Urol.* **165**, 621–626.

Sullivan, J. M., and Lasater, E. M. (1992). *J. Gen. Physiol.* **99**, 84–107.

Sutor, B., and Zieglgansberger, W. (1987). *Pflugers Arch.* **410**, 102–111.

Suzuki, S., and Rogawski, M. A. (1989). *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7228–7232.

Swandulla, D., and Armstrong, C. M. (1988). *J. Gen. Physiol.* **92**, 197–218.

Takahashi, H., Tateishi, N., Kaneda, M., and Akaike, N. (1989a). *Neurosci. Lett.* **103**, 29–33.

Takahashi, K., and Akaike, N. (1991). *J. Pharmacol. Exp. Ther.* **256**, 169–175.

Takahashi, K., Ueno, S., and Akaike, N. (1991). *J. Neurophysiol.* **65**, 148–155.

Takahashi, K., Wakamori, M., and Akaike, N. (1989b). *Neurosci. Lett.* **104**, 229–234.

Talavera, K., Staes, M., Janssens, A., Klugbauer, N., Droogmans, G., Hofmann, F., and Nilius, B. (2001). *J. Biol. Chem.* **276**, 45628–25635.

Talley, E. M., Cribbs, L. L., Lee, J.-H., Daud, A., Perez-Reyes, E., and Bayliss, D. A. (1999). *J. Neurosci.* **19**, 1895–1911.

Talley, E. M., Solorzano, G., Depaulis, A., Perez-Reyes, E., and Bayliss, D. A. (2000). *Brain Res. Mol. Brain Res.* **75**, 159–165.

Tang, C. M., Presser, F., and Morad, M. (1988). *Science* **240**, 213–215.

Tarasenko, A. N., Isaev, D. S., Eremin, A. V., and Kostyuk, P. G. (1998). *J. Physiol.* **509**, 385–394.

Tarasenko, A. N., Kostyuk, P. G., Eremin, A. V., and Isaev, D. S. (1997). *J. Physiol.* **499**, 77–86.

Tareilus, E., Roux, M., Qin, N., Olcese, R., Zhou, J., Stefani, E., and Birnbaumer, L. (1997). *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1703–1708.

T. E. P. K. D. Consortium (1994). *Cell* **77**, 881–894.

Terrian, D. M., Damron, D. S., Dorman, R. V., and Gannon, R. L. (1989). *Neurosci. Lett.* **106**, 322–327.

Thompson, S. M., and Wong, R. S. (1991). *J. Physiol.* **439**, 671–689.

Thornbury, K. D. (1999). *Ir. J. Med. Sci.* **1999**, 201–207.

Todorovic, S. M., Jevtovic-Todorovic, V., Mennerick, S., Perez-Reyes, E., and Zorumski, C. F. (2001). *Mol. Pharm.* **60**, 603–610.

Todorovic, S. M., Perez-Reyes, E., and Lingle, C. J. (2000). *Mol. Pharm.* **58**, 98–108.

Toyota, M., Ho, C., Ohe-Toyota, M., Baylin, S. B., and Issa, J. P. (1999). *Cancer Res.* **59**, 4535–4541.

Triggle, D. J. (1998). *AJH* **11**, 80S–87S.

Trombley, P. Q., and Westbrook, G. L. (1991). *J. Neurosci.* **11**, 435–444.

Tsakiridou, E., Bertollini, L., de Curtis, M., Avanzini, G., and Pape, H. C. (1995). *J. Neurosci.* **15**, 3110–3117.

Tseng, G.-N., and Boyden, P. (2002). *Circ. Res.* **65**, 1735–1750.

Uchida, K., Yuzki, R., and Kamikawa, Y. (1998). *Br. J. Pharmacol.* **125**, 849–857.

Ueda, Y., and Steinberg, R. H. (1993). *Invest. Ophthalmol. Vis. Sci.* **34**, 3408–3418.

Ueki, T., Toyota, M., Sohn, T., Yeo, C. J., Issa, J. P., Hruban, R. H., and Goggins, M. (2000). *Cancer* **Res. 60**, 1835–1839.

Vacher, E., Richer, C., Fornes, P., Clozel, J. P., and Giudicelli, J. F. (1996). *J. Cardiovasc. Pharmacol.* **27**, 686–694.

van der Lee, R., Pfaffendorf, M., and van Zwieten, P. A. (1999). *Fundam. Clin. Pharmacol.* **13**, 198–203.

van Luijtelaar, G., Wiaderna, D., Elants, C., and Scheenen, W. (2000). *Eur. J. Pharmacol.* **406**, 381–389.

Vance, C. L., Begg, C. M., Lee, W. L., Haase, H., Copeland, T. D., and McEnery, M. W. (1998). *J. Biol. Chem.* **273**, 14495–14502.

Vandebrouck, C., Imbert, N., Constantin, B., Duport, G., Raymond, G., and Cognard, C. (2002). *Neuromuscul. Disord.* **12**, 266–272.

Verkhratsky, A., Orkand, R. K., and Kettenmann, H. (1998). *Physiol. Rev.* **78**, 99–141.

Villame, J., Massicotte, J., Jasmin, G., and Dumont, L. (2001). *Cardiovasc. Drugs Ther.* **15**, 41–48.

Vinet, R., and Vargas, F. F. (1999). *Am. J. Physiol.* **276**, H1313–H1322.

Vivaudou, M., Clapp, L., Walsh, J., Jr., and Singer, J. (1988). *FASEB J.* **2**, 2497–2504.

Walker, D., Bichet, D., Campbell, K. P., and De Waard, M. (1998). *J. Biol. Chem.* **273**, 2361–2367.

Walker, D., and DeWaard, M. (1998). *Trends. Neurosci.* **21**, 148–154.

Wang, L., Bhattacharjee, A., Fu, J., and Li, M. (1996a). *Diabetes* **45**, 1678–1683.

Wang, L., Bhattacharjee, A., Zuo, Z., Hu, F., Honkanen, R. E., Berggren, P.-O., and Li, M. (1999). *Endocrinology* **140**, 1200–1204.

Wang, X., McKenzie, J. S., and Kemm, R. E. (1996b). *J. Neurophysiol.* **75**, 1138–1151.

Wang, Z., Estacion, M., and Mordan, L. J. (1993). *Am. J. Physiol.* **265**, C1239–C1246.

Weisman, H. F. (1993). *Clin. Immunol. Immunopathol.* **68**, 170–174.

Wetzel, G. T., Chen, F., and Klitzner, T. S. (1991). *Pediatr. Res.* **30**, 89–94.

White, G., Lovinger, D. M., and Weight, F. F. (1989). *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6802–6806.

Wilcox, K. S., Gutnick, M. J., and Christoph, G. R. (1988). *J. Neurophysiol.* **59**, 212–225.

Williams, M. E., Marubio, L. M., Deal, C. R., Hans, M., Brust, P. F., Phillipson, L. H., Miller, R. J., Johnson, E. C., Harpold, M. M., and Ellis, S. B. (1994). *J. Biol. Chem.* **269**, 22347–22357.

Williams, M. E., Washburn, M. S., Hans, M., Urrutia, A., Brust, B. F., Prodanovich, P., Harpold, M. M., and Stauderman, K. A. (1999). *J. Neurochem.* **72**, 791–799.

Williams, S. R., Töth, T. I., Turner, J. P., Hughes, S. W., and Crunelli, V. (1997). *J. Physiol.* **505**, 689–705.

Wingertzahn, M. A., and Ochs, R. S. (2001). *Exp. Biol. Med. (Maywood)* **226**, 119–126.

Witcher, D. R., De Waard, M., and Campbell, K. P. (1993). *Neuropharmacology* **32**, 1127–1139.

Wyatt, C. N., Page, K. M., Berrow, N. S., Brice, N. L., and Dolphin, A. C. (1998). *J. Physiol.* **510**, 347–360.

Xiao, G.-Q., Keli, H., and Boutjdir, M. (2000). *Circulation* **103**, 1599–1604.

Xiong, Z., Sperelakis, N., Noffsinger, A., and Fenoglio-Preiser, C. (1993). *Am. J. Physiol.* **265**, C617–C625.
Xiong, Z., Sperelakis, N., Noffsinger, A., and Fenoglio-Preiser, C. (1995). *J. Physiol.* **269**, G378–G385.
Xu, X. P., and Best, P. M. (1990). *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4655–4659.
Xu, X., and Best, P. M. (1992). *J. Physiol.* **454**, 657–672.
Yaari, Y., Hamon, B., and Lux, H. D. (1987). *Science* **235**, 680–682.
Yang, L., Ellinor, P. T., Sather, W. A., Zhang, J.-F., and Tsien, R. (1993). *Nature* **366**, 158–161.
Yokokawa, K., Kohno, M., Murakawa, K., Yasunari, K., and Takeda, T. (1990). *J. Hypertens.* **8**, 843–849.
Young, R. C., Smith, L. H., and McLaren, M. D. (1993). *Am. J. Obstet. Gynecol.* **169**, 785–792.
Yue, L., Feng, J., Gaspo, R., Li, G. R., Wang, Z., and Nattel, S. (1997). *Circ. Res.* **81**, 512–525.
Yunker, A. M. R. (2003). *Bioenerg. Biomembr.* **35**, 577–598.
Yunker, A. M. R., Sharp, A. H., Sundarraj, S., Ranganathan, V., Copeland, T. D., and McEnery, M. W. (2003). *Neuroscience* **117**, 321–335.
Zamponi, G. W., Bourinet, E., Nelson, D., Nargeot, J., Snutch, T. P. *et al.* (1997). *Nature* **385**, 442–446.
Zhang, F., Ram, J. L., Standley, P. R., and Sowers, J. R. (1994). *Am. J. Physiol.* **266**, C975–C980.
Zhang, J.-F., Randall, A. D., Ellinor, P. T., Hornes, W. A., Sather, W. A., Tanabe, T., Schwarz, T. L., and Tsien, R. W. (1993a). *Neuropharmacology* **32**, 1075–1088.
Zhang, L., Valiante, T. A., and Carlen, P. L. (1993b). *J. Neurophysiol.* **70**, 223–231.
Zhang, Y., Cribbs, L. L., and Satin, J. (2000). *Am. J. Physiol.* **278**, H184–H193.
Zhang, Y., Mori, M., Burgess, D. L., and Noebels, J. L. (2002). *J. Neurosci.* **22**, 6362–6371.
Zhang, Y. F., and Coulter, D. A. (1996). *Epilepsy Res.* **23**, 55–70.
Zhou, Z., and January, C. T. (1998). *Biophys. J.* **74**, 1830–1839.
Zhou, Z., and Lipsius, S. L. (1994). *Mol. Cell. Cardiol.* **26**, 1211–1219.
Zhu, Y., Zboran, E. L., and Ikeda, S. R. (1995). *J. Physiol.* **489**, 363–375.
Zhuang, H., Bhattacharjee, A., Hu, F., Zhang, M., Goswami, T., Wang, L., Wu, S., Berggren, P. O., and Li, M. (2000). *Diabetes* **49**, 59–64.
Zu, L., Figueroa, K. P., Grewal, R., and Pulst, S. M. (1999). *Am. J. Hum. Genet.* **64**, 594–549.