Low-voltage-activated (T-type) calcium-channel genes identified

Tremendous advances have been made in recent years regarding the molecular biology of voltage-dependent Ca^{2+} channels[1]. These macromolecules are composed of a pore-forming α1 subunit with four homologous domains (I–IV), each with six transmembrane segments (S1–S6). Accessory subunits, which modify functional expression and gating, include β, α2–δ and γ. Various neuronal α1 subunits have been cloned, although until recently they all seemed to encode channels activated by relatively strong membrane depolarization, the so-called high-voltage-activated Ca^{2+} channels. In general, members of this functional class, which includes L, N, P/Q and R types, are thought to mediate calcium entry, especially that triggered by action potentials. This leads to increments in intracellular Ca^{2+} concentration and thus to secondary actions, such as neurotransmitter release or excitation–contraction coupling. The other major class of Ca^{2+} channels consists of low-voltage-activated (LVA), or T-type (for transient or tiny[2]), channels. These channels can be activated by membrane-potential changes that are subthreshold for action-potential generation. When expressed at high levels in neurons, burst-discharge (see below) and some forms of intrinsic rhythm generation are promoted[3]. A number of other putative T-channel functions in neuronal and non-neuronal cells have been identified[2,3]. Many attempts have been made to identify the molecular basis of this important Ca^{2+} channel family, but they have been met with little success. Recently, Perez-Reyes and colleagues described three genes encoding new members of the family of α1 calcium-channel subunits, including α1G (Ref. 4) and two related genes (α1H and α1I). High levels of mRNA for the α1G subunit are found in the brain, especially in some regions noted for neuronal burst firing, such as the thalamus and amygdala, but also in the cerebellum, where a subpopulation of neurons, the Purkinje cells, demonstrate phenotypical burst firing. When expressed in *Xenopus* oocytes these channels demonstrate all the properties of the classical T-type current[2,5]. Thus, α1G can be identified unambiguously as a new member of the LVA or T-channel family.

Calcium-dependent burst firing: functional significance

One of the most striking neuronal spiking patterns observed by neurophysiologists is the Ca^{2+}-dependent burst response that is prominent in a subset of neurons from many brain regions such as the cerebellum, inferior olive and thalamus. In an elegant set of brain slice experiments in the early 1980s, Rodolfo Llinás and colleagues demonstrated that the generation of this particular response, which consists of a phasic discharge of action potentials (Fig. 1, c.f. Fig 1B with Fig. 1A), depended on extracellular Ca^{2+} concentration[6,7]. They correctly predicted that a specialized form of voltage-dependent Ca^{2+} current was responsible for the response. This was later confirmed in voltage-clamp experiments, where it was shown, for example, that thalamic relay neurons expressed high levels of T-type current[8], consistent with their important role in burst generation in these cells.

The voltage-dependent properties of the T current[2,5,8] tend to be different from those for high-voltage-activated Ca^{2+} currents, but similar to those of the classical voltage-gated Na^{+} channel, first analysed in detail in the squid giant axon by Hodgkin and Huxley[9]. That is, like Na^{+} currents, T-current kinetics at the macroscopic level (microscopic gating is more complex[10–12]) can be described by two independent processes, activation and inactivation, which conspire to produce the complex-spike waveform (Fig. 1C and Fig. 1D). First, voltage-dependent activation, or opening, of channels leads to the regenerative response that is the rising limb of the spike – small depolarizations can increase channel openings leading to cation entry and thus further depolarization with incremental opening of channels, and so on. Second, a similar, but slower, voltage-dependent inactivation helps promote spike repolarization that results from channels closing. Thus T channels promote the generation of self-contained, low-threshold Ca^{2+} spikes that are quantitatively, but not qualitatively, different from fast Na^{+}-dependent action potentials (Fig. 1C versus Fig. 1D). Aside from the difference in threshold (straight arrows in Fig. 1C versus Fig. 1D), low-threshold spikes tend to be much smaller and slower than Na^{+}-dependent action potentials.

As indicated in Fig. 1, a functionally important consequence of T-channel inactivation properties is that low-threshold spikes can be generated only when the membrane potential is steadily, or even transiently, hyperpolarized (that is, made more negative than the normal resting potential, Fig. 1B versus Fig. 1A). Thus, T-channel expression confers a form of paradoxical excitability onto neurons. In this way, hyperpolarizing synaptic potentials, which would under other circumstances be inhibitory, can actually lead to rebound excitation, as is prominently seen in thalamic relay neurons[13]. The robust, reciprocally connected excitatory–inhibitory thalamic circuit can then, under the right conditions, maintain synchronized, repetitive neuronal activity that is highly dependent on T-channel function[14,15]. This type of synchronous network activity is a characteristic of certain stages of sleep[16], and a related, but pathological and hypersynchronous network response seems to be associated with generalized absence epilepsy[17,18]. Consistent with the latter observation is the finding that antiepileptic drugs with specific efficacy for generalized absence epilepsy are selective antagonists of T-type Ca^{2+} channels[19,20]. However, some studies suggest that other, non-T channel, mechanisms might also contribute to the action of these drugs[21,22].

Newly identified voltage-dependent calcium channel genes

Perez-Reyes *et al.* used a novel text-based search of Genbank to probe for a putative T-channel gene[4]. They simply searched for the terms 'calcium' and 'channel'. This returned hundreds of matches, with less than 30 being 'similar to' a calcium channel. These fragments were cloned and compared to known sequences, and one human brain clone (H06096) showed 45% sequence identity to the gene encoding domain III S1 of carp α1S. A full-length rat cDNA was obtained, α1G, as were homologous mouse and human sequences. Northern-blot analysis revealed two transcripts, with strong signals in the brain, especially in the amygdala, diencephalon and cerebellum. Lower levels were found in the heart.

Sequence identity with other α1 subunits is highest in the transmembrane regions, about 30%. The voltage sensor in S4 is conserved, as are the negative charges surrounding the pore. However, in this subunit two glutamates are replaced by aspartates in α1G, which might partially explain the altered selectivity of T channels compared with high-voltage-activated channels. High-voltage-activated channels exhibit a higher Ba^{2+}:Ca^{2+} permeability ratio[3], and the aspartate substitution might also explain the 'tiny' single-channel conductance. Intact binding sites for both Ca^{2+} (EF hand) and β subunits are conspicuously absent from α1G. These

John R. Huguenard
Dept of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305-5122, USA.

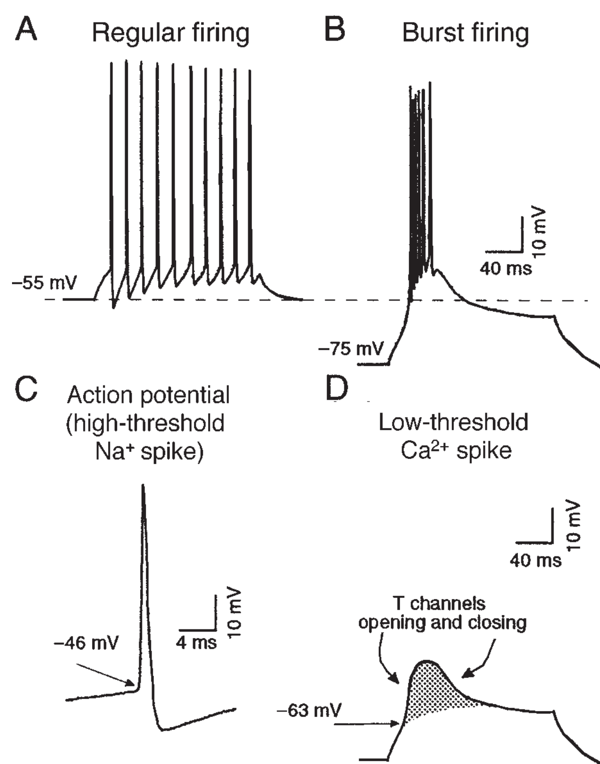

Fig. 1. *Patterns of action potential firing in thalamic relay neurons: underlying mechanisms.* **(A)** *When activated from a basal state (with a resting membrane potential near –55 mV; dashed line), a relay neuron responds with a regular, non-adapting, train of action potentials, which is maintained throughout the duration of the applied stimulus.* **(B)** *In contrast, when the neuron is conditioned by intracellular current injection to bring the membrane potential to –75 mV, the response changes to a group of high-frequency spikes clustered near the beginning of the stimulus. This type of response is a prominent behaviour of relay neurons during periods of sleep and drowsiness (see text). Application of tetrodotoxin, the voltage-dependent* Na^+ *channel antagonist, blocks fast spike generation to reveal an underlying low-threshold spike* **(D)**, *which can be abolished by inorganic* Ca^{2+} *channel blockers (not shown). The low threshold spike is similar to the* Na^+ *spike* **(C)** *in that it is a regenerative, self-limiting, depolarizing response, yet it is vastly different in terms of its amplitude, duration [time base in* (C) *is expanded tenfold from that in* (D)*] and threshold [–46 mV versus −63 mV; arrows in* (C) *and* (D)*, respectively]. The regenerative low-threshold spike serves to bring the membrane potential to the threshold for fast spike generation, and keep it near there for the duration of the burst* (B)*. The recently identified α1G* Ca^{2+} *channel is the likely mediator of such thalamic burst responses. All records were obtained via whole-cell-current clamp recordings in rat somatosensory thalamic slices maintained* in vitro[14].

findings might somehow be related to the observed lack of Ca^{2+}-dependent inactivation for T channels[23] (although the presence of the EF hand does not always confer such inactivation to other members of the α1 family), and the robust Ca^{2+} currents obtained when α1G is expressed in the absence of accessory Ca^{2+} channel subunits in either *Xenopus* oocytes[4] or HEK-293 cells (E. Perez-Reyes, pers. commun.).

Whole-cell and single-channel-current properties of α1G expressed in oocytes are as expected for a T channel. For example, macroscopic rates of activation and inactivation are voltage-dependent, with activation being particularly slow. This is consistent with the T current recorded in thalamic neurons, but contrasts with the typical properties of high-voltage-activated channels. Especially notable is the slow deactivation[24] (or tail currents) observed with α1G, which differs from the very rapid deactivation seen with most non-T channels. Conductance of single-channels is 7.5 pS with 115 mM Ba^{2+} as the charge carrier – similar to the value obtained with native T channels.

Two other members of this class which have already been identified are α1H and α1I (Ref. 25). The levels of α1H mRNA are higher in the heart than in the brain and this subunit has very similar voltage-dependent properties to α1G. In addition, α1H is similar to α1G with regard to absence of binding sites for Ca^{2+} and β subunits.

It was initially reported that α1E, when co-expressed with appropriate β subunits, had some properties expected for a T channel[26]. Indeed, α1E can be activated at low voltages, is blocked by Ni^{2+} (a characteristic of T channels in some preparations[3]), and displays a Ca^{2+}:Ba^{2+} permeability ratio near unity[27]. However, the single-channel conductance, relative metabolic stability in whole-cell recordings, and activation–deactivation kinetics all differ from classic T-channel properties. Small conductance LVA currents can be observed in cell-attached recordings from COS cells transfected with α1E or other α1 subunits[28], but these seem to contribute only a minor LVA component to the total whole-cell Ca^{2+} current[29]. Therefore, while α1E might form Ca^{2+} channels that are activated by weak depolarizations, they are unlikely to contribute in a major way to the regenerative burst response as observed in thalamic neurons.

Future directions

Given the putative importance of the T channel in thalamocortical rhythm generation during sleep and absence epilepsy, the race will now be on to screen for specific antagonists or toxins that could have important neurological actions. Also, considering the heterogeneity among T-channel properties in various neuronal populations[30,31], and the putative roles of different central nuclei in physiological and pathological brain activities[32], it will be important to test for agents that interact selectively with subsets of T-channel proteins. Finally, Ca^{2+}-channel defects have been demonstrated in some animal models of epilepsy[33,34], and rats of the Strasbourg inbred strain, which have a generalized absence phenotype[35], overexpress T channels in their thalamic reticular neurons[36], suggesting that defects in α1G function might be responsible for human absence epilepsies.

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