Differences in Apparent Pore Sizes of Low and High Voltage-activated Ca\(^{2+}\) Channels*

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Pore size is of considerable interest in voltage-gated Ca\(^{2+}\) channels because they exemplify a fundamental ability of certain ion channels; to display large pore diameter, but also great selectivity for their ion of choice. We determined the pore size of several voltage-dependent Ca\(^{2+}\) channels of known molecular composition with large organic cations as probes. T-type channels supported by the Ca\(_{a}\)3.1, Ca\(_{a}\)3.2, and Ca\(_{a}\)3.3 subunits; L-type channels encoded by the Ca\(_{a}\)1.2, β\(_{1}\), and α\(_{2}β_{1}\) subunits; and R-type channels encoded by the Ca\(_{a}\)2.3 and β\(_{3}\) subunits were each studied using a Xenopus oocyte expression system. The weak permeabilities to organic cations were resolved by looking at inward tails generated upon repolarization after a large depolarizing pulse. Large inward NH\(_{4}\)\(^{+}\) currents and sizable methylammonium and dimethylammonium currents were observed in all of the channels tested, whereas trimethylammonium permeated only through L- and R-type channels, and tetramethylammonium currents were observed only in L-type channels. Thus, our experiments revealed an unexpected heterogeneity in pore size among different Ca\(^{2+}\) channels, with L-type channels having the largest pore (effective diameter = 6.2 Å), T-type channels having the tiniest pore (effective diameter = 5.1 Å), and R-type channels having a pore size intermediate between these extremes. These findings ran counter to first-order expectations for these channels based simply on their degree of selectivity among inorganic cations or on the bulkiness of their acidic side chains at the locus of selectivity.

T-type Ca\(^{2+}\) channels are found in many cell types and are important for cellular functions as diverse as cell proliferation, cardiac pacemaker activity, and rhythmic firing of neural networks (1, 2). In contrast to the better studied high voltage-activated (HVA) Ca\(^{2+}\) channels (L-, N-, P/Q-, and R-type), T-type channels activate at relatively negative membrane potentials and are often referred to as “low voltage-activated” (LVA). The distinctive permeation properties of T-type Ca\(^{2+}\) channels were critical for their original identification as distinct entities (3–16). In native preparations, T-type channels are equally permeable to Ca\(^{2+}\) and Ba\(^{2+}\) ions, unlike all known HVA channels, for which unitary Ba\(^{2+}\) fluxes are larger than those supported by Ca\(^{2+}\) (17). Despite general agreement about these differences (1), relatively little is known about the underlying basis of ion selectivity and permeation in T-type channels. New impetus for approaching such questions is provided by the molecular cloning of three different pore-forming Ca\(_{a}\) subunits with biophysical properties that clearly identify them as T-type channels (18–20), Ca\(_{a}\)3.1, Ca\(_{a}\)3.2, and Ca\(_{a}\)3.3 (α\(_{1C}\), α\(_{1D}\), and α\(_{1F}\), respectively, in the former voltage-dependent calcium channel nomenclature). These subunits form a closely related family with only limited sequence homology to the subfamilies of HVA channels (~28%). In the regions thought to be important for permeation, the Ca\(_{a}\)3 subunits and HVA channel are more similar (40%). The Ca\(_{a}\)3 subunits also have “P-loops,” each containing an amino acid with an acidic side chain thought to be important for permeation. However, instead of a set of four glutamates as in HVA channels (EEEE locus), the corresponding amino acids in the three T-type channel α\(_{1}\) subunits are glutamates in domains I and II and aspartates in domains III and IV, forming a putative EEDD locus (21–23).

Knowledge about the molecular differences between the pore-forming regions of T-type channels and HVA channels has redirected attention to differences in their permeation properties. Because current models of ion permeation through voltage-dependent Ca\(^{2+}\) channels are based almost entirely on studies of L-type channels (24–26), characterizing permeation and selectivity in various T-type channels offers broader perspectives about mechanisms. A promising start was provided by Talavera et al. (27), who showed that the aspartates at the EEDD locus of a T-type α\(_{1}\) subunit contribute to its distinctive selectivity properties (see also Ref. 28).

Here, we focus on pore size, a matter of fundamental importance to understanding interactions between the pore-forming groups and permeant ions. Even in the absence of direct evidence about channel structure from x-ray crystallography, valuable information on minimal pore size can be gathered by studies on the permeation of large organic cations (17, 29–34). Early studies on the frog skeletal L-type Ca\(^{2+}\) channel (35, 36) showed that the pore is permeable to tetramethylammonium and thus has a minimal internal diameter of ~6 Å. There was also a brief report that T-type channels in neoplastic B-lymphocytes are impermeable to tetramethylammonium (37). By contrast, no systematic study of the pore size of Ca\(^{2+}\) channels of known molecular composition has yet been reported.

Here, we used the Xenopus oocyte expression system to determine the apparent pore size of calcium channels encoded by...
cloned CaV subunits. CaV1.2 (X15539), the subject of the vast majority of recent permeation experiments, was a logical starting point for comparison with the three T-type channel subunits, CaV3.1, CaV3.2, and CaV3.3. We also studied the pore size of channels encoded by the CaV3.3 subunit (X15539), which supports the R-type current (38), of interest because this channel resembles T-type channels in permeation and selectivity properties, although its amino acid sequence is much closer to L-type than T-type α1 subunits (39, 40).

MATERIALS AND METHODS

Molecular Biology—For RNA preparation, the human CaV3.2 (19) and rat CaV3.1 subunits (GenBank™/EBI accession numbers NM_021098 and AF027984, respectively) were cloned in the pGEM-HEA vector were linearized with ApalI (18, 19), whereas the rat CaV3.3 subunit (NM_020084) subcloned in the pSP73 vector (28) and the human CaV2.3 (L27745) subunit subcloned in the pHBE vector (41) were digested with EcoRI and HindIII, respectively. All experiments using the CaV1.2 subunit (X15539) of L-type voltage-dependent calcium channels were performed using the CARD5 isoform (42), a deletion mutant of the CARD3 isoform subcloned in the pSP72 vector and digested with XhoI. The rat β3 (43) β4 (44) accessory subunits (M25817 and NM_012828) were linearized with XhoI and NotI, respectively, whereas the rabbit α1D subunit (M21948) (42) was digested with XhoI. Linearized DNAs were cleaned with phenol/chloroform, and RNA was prepared in vitro with commercial kits (Ambion Inc., Austin, TX) using the SP6 polymerase for the β3 subunit or the T7 polymerase for all other channels.

CaV3.1 Channel Expression in Xenopus Oocytes and Electrophysiology—Xenopus laevis females (NASCO, Fort Atkinson, WI) were anesthetized by immersion in 0.2% ice-cold Tricaine solution for 30–45 min, and then three to four ovarian lobes were removed by abdominal incision, minced into small fragments with sterile scissors, and digested for 1–1.3 h with 1 mg/ml type I collagenase (Inovotest) in nominally “calcium-free” solution (82.5 mM NaCl, 2.5 mM KCl, 1 mM MgCl2, and 5 mM HEPS) (pH 7.6) with NaOH. Stage V and VI oocytes were selected in vitro and kept overnight in calcium-containing solutions used during the experiments. To minimize changes in the recording conditions, the duration of the experiments was kept short by switching from one solution to another.

In calculating this permeability ratio, we assumed the mean current density at the peak of the inward current was 2.20 ± 0.31 nA/pF. The recovery from inactivation (τ,1) for each CaV channel was determined from the peak current density, and the recovery from inactivation (τ,rev) among the CaV3.1, CaV3.2, and CaV3.3, or the HVA channel subunits, CaV1.2, the putative R-type channel CaV2.3; and the three members of the T-type family, CaV3.1, CaV3.2, and CaV3.3, with Li+ as the charge carrier in divalent-free solutions. The currents were elicited by 75-ms depolarizing pulses from a holding potential of −80 mV, ranging up to +50 mV in 10-mV increments (frequency of 0.6 Hz). Horizontal bar, 15 ms; vertical bar, 0.5 μA. B, current-voltage plot for Li+ currents in CaV1.2 (41), CaV3.2 (40), CaV3.1 (41), CaV3.2 (41), and CaV3.3 (41). To make the comparison among different channels easier, the currents were normalized as a percentage of the maximal inward current. Each point is the mean ± S.E. of the data obtained in at least six different oocytes.

RESULTS

To estimate the minimal dimensions of the permeation pore, we pursued the classical approach of recording the Ca2+ channel currents supported by a series of monovalent cations of increasing size (17, 35). The experiments were carried out in buffered Ca2+-free solutions to avoid the potent blocking effects of Ca2+ ions on monovalent cation currents (35, 46). When currents were evoked by a series of increasingly large depolarizing steps, inward currents first grew in size as the channels activated, but became progressively smaller and eventually reversed when the depolarization became strong enough to drive a net positive charge out of the oocytes, presumably supported by K+ efflux. Fig. 1A compares currents carried by Li+ ions in oocytes expressing the T-type channel subunits, CaV3.1, CaV3.2, and CaV3.3, or the HVA channel subunits, CaV1.2 (L-type) and CaV2.3 (R-type). As expected, each of these channels became highly permeable to monovalent ions once divalent cations were removed from the bath, and large inward currents were recorded with Li+ as the external charge carrier (Fig. 1). Despite obvious differences in activation and inactivation kinetics from one channel to the next, Li+ current reversed at around +5 mV in both L- and R-type channels and in each of the T-type channel isoforms. This value is in good agreement with previous determinations of Li+ current reversal in oocytes expressing CaV1.2 (47) and leads to an estimated Ppeak/Prev of 1.46 using the Hodgkin-Goldman-Katz formalism for bi-ionic permeation. In calculating this permeability ratio, we assumed that the intracellular potassium concentration in Xenopus oocytes is 120 mM (48) and does not change significantly upon recording with KCl-filled pipettes because of the large volume of the oocytes. Posed results from many oocytes displayed no significant difference in reversal potential (Erev) among the

![Fig. 1. Li+ currents carried by LVA and HVA channels. A, representative traces of the currents carried by the L-type channel CaV1.2; the putative R-type channel CaV2.3; and the three members of the T-type family, CaV3.1, CaV3.2, and CaV3.3, with Li+ as the charge carrier in divalent-free solutions. The currents were elicited by 75-ms depolarizing pulses from a holding potential of −80 mV, ranging up to +50 mV in 10-mV increments (frequency of 0.6 Hz). Horizontal bar, 15 ms; vertical bar, 0.5 μA. B, current-voltage plot for Li+ currents in CaV1.2 (41), CaV3.2 (40), CaV3.1 (41), CaV3.2 (41), and CaV3.3 (41). To make the comparison among different channels easier, the currents were normalized as a percentage of the maximal inward current. Each point is the mean ± S.E. of the data obtained in at least six different oocytes.](http://www.jbc.org/doi/10.1074/jbc.M111.271534)
various channel types (Fig. 1B and Table I).

**CaV3.2 and CaV1.2 Differ in Their Permeability to Organic Cations**—To determine whether there is any difference in the pore size of LVA and HVA Ca²⁺ channels, we compared the relative permeabilities of L- and T-type channels to organic cations (results with R-type channels are deferred to the end of “Results”). Ammonium and its di-, tri-, and tetramethyl substituents were chosen for two main reasons (49). First of all, their pKₐ values, all of these compounds are >95% ionized at pH 7.4, allowing recordings at physiological pH without complications due to pH-induced changes in the channel itself. Second and more importantly, these compounds are expected to offer reliable reagents to determine channel pore size because the progressive addition of methyl groups produces a gradual increase in the size of these molecules without introducing gross modifications in their overall three-dimensional structure. For reference (49), the maximal diameters and the volumes of these ions, respectively, are as follows: NH₄⁺, 3.6 Å and 20.1 Å³; methylammonium (MA), 3.8 Å and 35.1 Å³; dimethylammonium (DMA), 4.6 Å and 49.7 Å³; trimethylammonium (TriMA), 6.0 Å and 64.2 Å³; and tetramethylammonium (TMA), 6.0 Å and 78.8 Å³. Interesting differences were found in CaV1.2- and CaV3.2-expressing oocytes when these ions were used as charge carriers for inward currents evoked by depolarizing steps (Fig. 2). NH₄⁺ and MA clearly permeated through both types of channels, but only CaV1.2-expressing oocytes displayed clear inward DMA currents. This provided an initial clue that channels supported by CaV3.2 and CaV1.2 differ in their permeability.

We looked more closely at the currents with TriMA and TMA as external cations to develop a more quantitative picture of the cutoff sizes of T- and L-type channels. The failure of these ions to support significant inward currents during step depolarizations must be cautiously interpreted because of the possibility that T- and L-type channels are permeable to these cations, but not detectably so at voltage levels sufficiently depolarized to activate the various Ca²⁺ channels. As an indication that this was of concern for CaV1.2, both TriMA and TMA supported clear inward current tails after the depolarizing pulses upon repolarization to −80 mV, where the driving force was larger (Fig. 2, lower row). Accordingly, to improve the resolution of weak permeabilities, we carried out additional experiments to look systematically at inward current tails over a wide range of potentials. This approach is illustrated in Fig. 3, with tail currents recorded in the presence of 100 mM Li⁺. Ca²⁺ channels were strongly activated by application of a 5-ms depolarizing pulse to +70 mV, and then the inward driving force was sharply increased by sudden repolarizations to a range of voltage levels to generate inward tail currents through the open channels. The large tail currents that resulted were outward at repolarization levels at or positive to +10 mV, but increasingly negative at levels at or below 0 mV. The values of reversal potential determined with the tail protocol agreed with Eᵣᵣᵥ values obtained with step depolarizations to within 2–3 mV (Table I). In no case were the differences significant, validating the tail current procedure.

Next, we proceeded to use the same tail current protocol to explore the permeation of organic cations through the various voltage-dependent Ca²⁺ channels, focusing first on the CaV1.2 and CaV3.2 subunits. In oocytes expressing either of these subunits, very large inward NH₄⁺ currents and sizable MA and DMA currents were observed (Fig. 4 and Table II). The contrast became more dramatic in considering even larger organic cations. In CaV3.2-expressing oocytes, no inward tails were detected with either external TriMA or TMA. In contrast, in CaV1.2-expressing oocytes, TriMA carried small but unmistakable inward currents that reversed around −30 mV, and TMA supported extremely small tails negative to −60 mV (Fig. 4).

Measurements of reversal potential provided a more quantitative assessment of the differences between channels. For NH₄⁺, no significant difference was observed in Eᵣᵣᵥ between CaV1.2 and CaV3.2 (Table II), suggesting that pore size was not critical in allowing permeation of these small ions. In the case of MA and DMA, Eᵣᵣᵥ was −20 mV more negative for CaV3.2 than for CaV1.2. This difference corresponds to a lower relative permeability of the T-type channels for the intermediate-sized ions.

**CaV3.1 and CaV3.3 Show Organic Cation Permeability Similar to That of CaV3.2**—All of the data reported in above point to the conclusion that the channel pore is smaller in CaV3.2 than in CaV1.2. To establish whether a smaller pore size is a general feature of T-type channels, we extended our studies to the two other members of the T-type channel family, CaV3.1 and CaV3.3, using the tail protocol to study their permeability to NH₄⁺ and its methyl-substituted derivatives (Fig. 5). Despite clear differences in the kinetic properties of tail currents carried by these two channels, their permeability to organic cations was remarkably similar. Very large tails were observed when ammonium was the charge carrier, and these currents reversed at approximately +20 mV, as in the case of CaV3.2 and CaV1.2. Clear tails were observed with MA and DMA, and, once again, the reversal potentials for these currents were
Indicates 0.5 frequency. The in repetitive episodes (10-mV increments, 50-ms pulse duration, 0.6-Hz stepping down the potential to values increasing from 5 ms to maximally open the voltage-dependent calcium channels and by stepping down the potential to values increasing from −30 to +30 mV in repetitive episodes (10-mV increments, 50-ms pulse duration, 0.6-Hz frequency). The horizontal bar is set at 15 ms, whereas the vertical bar indicates 0.5 μA. B, current-voltage plot derived from the data obtained in CaV1.2 (○), CaV3.1(△), CaV3.2 (□), and CaV3.3 (○) using this tail protocol. To make the comparison among different channel types easier, current measurements were normalized to the value obtained when the potential was stepped down to −20 mV. Each point represents the mean ± S.E. of the data obtained in at least five different oocytes.

Remarking similar to that observed in CaV3.2-expressing oocytes, ranging at around −20 mV for MA and −60 mV for DMA (Table II). No clear inward tails were observed with TriMA and TMA as charge carriers. Indeed, with either of these external cations, outward currents flowed through the channel at potentials as negative as −80 mV. All of these data support the conclusion that T-type channels constitute a homogeneous family with regard to pore size, significantly different from L-type channels.

Unusual Permeation Properties of CaV2.3—As mentioned in the Introduction, a large body of experimental evidence suggests that CaV2.3-encoded channels may differ from the other members of the HVA channel subfamily in displaying peculiar permeation properties typical of T-type channels. However, no information is available on the pore size of channels supported by CaV2.3. To explore this, we used the same approach that allowed us to establish a small pore size in T-type channels, we used the same approach in CaV3.3. Their consistent behavior stands in clear contrast to that of CaV1.2, as assessed by clear differences in the relative permeability ratios for MA and DMA as indicated in oocytes expressing the CaV3.3 LVA channels (upper row) and the CaV1.2 LVA channels (lower row). Tail currents were elicited by increasing the membrane potential from a holding potential of −80 mV to +70 mV for 5 ms to maximally open the voltage-dependent calcium channels and by stepping down the potential to values increasing from −30 to +30 mV in repetitive episodes (10-mV increments, 0.6-Hz frequency). The tail currents carried by MA, DMA, TriMA, and TMA as indicated in oocytes expressing the CaV3.3 LVA channels (upper row) and the CaV1.2 LVA channels (lower row). Tail currents were elicited by increasing the membrane potential from a holding potential of −80 mV to +70 mV for 5 ms to maximally open the voltage-dependent calcium channels and by stepping down the potential to values increasing from −30 to +30 mV in repetitive episodes (10-mV increments, 0.6-Hz frequency). The vertical bar indicates 2 μA in the case of CaV3.2 and 0.5 μA in the case of CaV1.2.
Erev and permeability ratios obtained in cloned HVA and LVA channels using ammonium methyl-substituted organic cations as charge carriers

The Erev and P(NH4) versus Li+ values for NH4+, MA, DMA, TriMA, and TMA in CaV1.2, CaV3.1, CaV3.2, CaV3.3, and CaV3.2 are shown. Erev values were determined by linear fitting using the current determinations obtained with the tail protocol reported in Figs. 3–6. Only three points comprising the closest to 0 nA and the ones immediately before and immediately after were used to calculate the fit. Permeability ratios (P(NH4)/P(Li)) were determined by linear fitting using the current determinations obtained with the tail protocol reported in Figs. 3–6.

Permeability ratios obtained in cloned HVA and LVA channels using ammonium methyl-substituted organic cations as charge carriers

|        | NH4+ | MA  | DMA | TriMA | TMA  |
|--------|------|-----|-----|-------|------|
| CaV1.2 | 19.1±1.2 | −2.3±1.2 | −15.8±2.0 | −29.8±1.2 | −58.3±3.5 |
| CaV2.3 | 19.7±0.02 | 0.76±0.06 | 0.50±0.03 | 0.29±0.02 | 0.08±0.01 |
| CaV3.1 | 12.9±1.5 | −26.1±1.2 | −37.4±2.9 | −47.2±3.4 | NP   |
| CaV3.2 | 14.9±0.09 | 0.32±0.02 | 0.25±0.04 | 0.20±0.02 | NP   |
| CaV3.3 | 15.4±0.8 | −22.1±1.6 | −54.3±2.7 | NP   | NP   |
| CaV2.3 | 14.5±0.8 | −19.2±0.6 | −58.8±0.3 | NP   | NP   |
| CaV3.2 | 14.0±0.04 | 0.39±0.02 | 0.12±0.02 | NP   | NP   |
| CaV3.3 | 17.4±1.2 | −17.4±0.8 | −52.1±0.4 | NP   | NP   |
|        | 1.7±0.2 | 0.40±0.01 | 0.100±0.003 | NP   | NP   |

Fig. 5. Tail currents carried by NH4+ and its methyl-substituted derivatives in CaV3.1 and CaV3.3. A, representative traces of tail currents carried by NH4+, MA, DMA, TriMA, and TMA in oocytes expressing CaV3.1 (upper row) or CaV3.3 (lower row). Tail currents were elicited using the protocol described in the legend to Fig. 4 and are depicted in the insets. Each set of traces was obtained in a different oocyte and is representative of the behavior of a group of at least six different cells. The vertical bar is set at 1 μA for CaV3.1 and at 0.5 μA for CaV3.3. B, current-voltage plot derived from the data obtained using this tail protocol in CaV3.1- and CaV3.3-expressing oocytes for ammonium and each of the cations of its methyl-substituted series. Using the same approach described in the legend to Fig. 4, the currents were normalized to Li+ currents measured at −20 mV. Each point represents the mean ± S.E. of the data obtained in at least five different oocytes.

or by differences in the cutoff size: the estimated cutoff diameter for CaV3.2 was 6.16 Å. The plot of relative permeability also shows that CaV2.3 occupies an intermediate position between L- and T-type channels, inasmuch as the relative permeability to DMA, TriMA, and TMA is larger in CaV2.3 than in T-type channels, but smaller than in L-type channels. Although the data for CaV2.3 are not readily fitted by the same theoretical function as used for the other channel types, the intermediate nature of the behavior is clear.

DISCUSSION

T-type Channels Differ from L-type Channels in Pore Size—This study demonstrates a striking heterogeneity between various subfamilies of voltage-gated Ca2+ channel with respect to their minimal pore size, estimated by passage of large organic cations in the absence of external Ca2+ within the T-type channel subfamily, each of the pore-forming subunits (CaV3.1, CaV3.2, and CaV3.3) showed the same orderly behavior, a monotonically increasing relationship between the size of organic cations and their relative permeability. The effective diameter of the permeation pathways of CaV3.1, CaV3.2, and CaV3.3 was estimated as 5.1 Å based on the ability of these subunits to support the permeation of DMA, but not TriMA. This stands in contrast with 6.2 Å, the estimated minimal pore size of CaV1.2, an L-type channel subunit originally derived from cardiac muscle. The very small amplitude of the currents carried by CaV1.2 when expressed without any accessory subunit precluded the analysis of the pore size of CaV1.2 in isolation, but our results for CaV1.2 coexpressed in oocytes with the β1a and α2δ1a accessory subunits are in good agreement with earlier estimates for L-type channels in situ both in cardiac myocytes (CaV1.2 in a native environment) (50) and in skeletal muscle fibers (CaV1.1) (35, 36). Thus, although the properties within each of the subfamilies appear homogeneous, T- and L-type channel classes show consistent differences in pore dimensions.

It was unexpected to find that T-type channels are smaller in their minimal pore than L-type channels, given the known properties of T-type channels: they do not show strong selectivity for Ca2+ relative to Ba2+; their selectivity for divalent cations over monovalent cations is weaker than for HVA channels; and their selectivity filter includes two aspartate residues, whose side chains are less bulky than the corresponding glutamates in HVA channels. If anything, these features would lead to the hypothesis that T-type channels possess larger diameter pores than L-type channels. Likewise, the logical expectation for R-type channels was that their pore diameter would be larger than that of other HVA channels (because
they show little discrimination among divalent cations) or at least equal in size (because CaV2.3 is highly homologous to other HVA α1 subunits). Once again, our observations of a smaller cutoff size for organic cation permeation through R-type channels ran counter to what might have been expected a priori, although they do reinforce previous evidence that R-type channels differ significantly from other HVA channels in their selectivity properties but share some characteristics of T-type channels (39).

Possible Determinants of Pore Size—We have considered various explanations for the differences in pore size (see also Ref. 27). One possibility is that the minimal pore diameter is dependent on residues at the EEDD locus itself. This would follow the precedent of the selectivity filter of sodium channels, where alterations of individual amino acid side chains of the DEKA locus have a strong influence on pore size (49). At the EEEE locus of L-type calcium channels (51), like the DEKA locus have a strong influence on pore size (49). At the EEEE locus of L-type calcium channels (51), like the DEKA locus in each repeat (termed the “position 0”), based on analysis of CaV1.2, side chains at position −1 also appear to project into the pore lumen (51) and clearly participate in controlling permeation (55). If this is the case for the T-type channels, one may consider the involvement of a conserved lysine at position −1 in domain III. Once again, the Na+ channel provides precedent for narrowing of a pore by a protruding lysine side chain (49).

A second possibility is that the minimal pore diameter might be determined by residues neighboring those at the EEEE or EEDD locus. The size of the ion-binding pocket is critical in determining the Ca2+ selectivity of organic chelators and Ca2+-binding proteins (60). To maintain a similar configuration of carboxylate oxygen groups in T- and L-type channels and similar Ca2+-coordinating capabilities, the smaller chain lengths of the aspartates may have required a narrower stabilized in a configuration that allows passage of large organic cations.

A third alternative is that the minimal pore diameter of T-type channels arises from a different tertiary structure, possibly reflecting an adaptation in the rest of the channel to compensate for the difference between an EEDD locus and an EEEE locus. The size of the ion-binding pocket is critical in determining the Ca2+ selectivity of organic chelators and Ca2+-binding proteins (60). To maintain a similar configuration of carboxylate oxygen groups in T- and L-type channels and similar Ca2+-coordinating capabilities, the smaller chain lengths of the aspartates may have required a narrower
spacing between the opposing pore walls. If this were the case, it would not be a coincidence that the -1.2 Å difference in chain length between glutamate and aspartate agrees closely with the difference in pore diameters. Although this line of thinking is both simplistic and speculative, it illustrates how differences in pore size might have physiological significance for divergent cation selectivity in various types of Ca^{2+} channel.

**Structural Clues Set Limits on Possible Models of Ca^{2+} Channel Permeation**—Our results establish that a relatively large pore size is a general feature of voltage-gated Ca^{2+} channels. Even including the T-type subfamily, the estimated minimal diameter of any of the Ca^{2+} channels is still much greater than the 1.9 Å diameter of the dehydrated Ca^{2+} ion. It is notable that this holds for T-type channels as well as L-type channels (35, 36, 50) because T-type Ca^{2+} channels, like HVA channels, are highly selective for their ion of choice under physiological conditions. Taken together, these findings are relevant to current efforts at approaching Ca^{2+} channel permeation with experiments (55, 61, 62) and theoretical calculations (63–67).

In the absence of crystal structures or any other direct structural information about Ca^{2+} channel pores, it is natural to ask whether Ca^{2+} selectivity and permeation could be explained by analogy to current thinking about K+ channel selectivity filters using, as a springboard, the elegant molecular structure of the bacterial KcsA K+ channel (68). That structure has directly supported earlier hypotheses that selectivity for K+ ions is generated by a narrow passageway lined with oxygen groups acting as ligands for the dehydrated ion (30, 69, 70). Turning back to Ca^{2+} channels, the minimal diameter of the pore is much too large to allow a snug fit with a completely dehydrated Ca^{2+} ion, even in the case of T-type channels. This precludes a strict extrapolation from K+ and KcsA channels to Ca^{2+} and Ca^{2+} channels. In considering how a rigid cage and a snug fit might hold for Ca^{2+} channels, McCleskey and Almers (35) suggested that the discrepancy between the minimal pore diameter of an L-type channel and the diameter of an unhydrated Ca^{2+} ion might be made up by a closely associated water molecule. This hypothesis was reminiscent of classical proposals that Na+ ions pass through the sodium channel selectivity filter along with a single H2O (17, 29, 49) and still remains worthy of consideration for L-type channels. However, the same combination of Ca^{2+} ion + water molecule would exceed the minimal aperture of T-type channels. As an alternative to such scenarios, we suggest that, across the spectrum of Ca^{2+} channels, Ca^{2+} permeation can be accommodated within the prevailing model for L-type channels (21). A generalized model might propose the following: 1) that the ion permeation pathway in all Ca^{2+} channels is generally much wider than the minimal pore diameter; 2) that acidic side chains, be they glutamates or aspartates, protrude into the pore and provide a flexible complex to closely coordinate one or more Ca^{2+} ions when these are present; and 3) that, in the absence of permselective divalent ions, the acidic side chains swing partly out of the way, allowing passage of large organic monovalent cations to varying degrees.

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