J. Membrane Biol. 141, 231–237 (1994)

The Journal of
Membrane
Biology

Monovalent Amidiniums Block Calcium Channels in Chick Sensory Neurons

S.L. Mironov
Max-Planck-Institute for Psychiatry, Am Klopferspitz 18a, Planegg-Martinsried 82142, Germany

Received: 18 November 1993/Revised: 8 March 1994

Abstract. The effect of amidiniums on high-threshold Ca^{2+} channel currents (I_{Ca}) was studied in chick dorsal root ganglion neurons. Guanidinium reduced I_{Ca} in a dose-dependent fashion. The block was relieved by increasing the concentration of the permeant ions, Ba^{2+} or Ca^{2+}, suggesting a competition for a common binding site within the channel. Formamidinium and methyl-guanidinium suppressed I_{Ca} with similar potencies, whereas L-arginine had no effect. A neutral amidine, urea, increased I_{Ca}. In Ca^{2+}-free solutions guanidinium and Na^+ permeated through the Ca^{2+} channel equally well. Structure-activity relationship obtained for blocking efficacies of different amidiniums are used to discuss possible configurations of the selectivity filter in the Ca^{2+} channel.

Key words: Ca^{2+} channel — Amidiniums — Block and permeability — Selectivity filter

Introduction

Ca^{2+} channels demonstrate conduction properties that point to a mechanism in which ions bind to specific sites in the permeation pathway as they traverse the pore. Under physiologic conditions, Ca^{2+} channels are selective for divalent over monovalent cations when both are present [7, 13], but they can also carry large monovalent currents in the absence of divalent cations [2, 7, 11, 15].

Organic cations have been used to map the permeation pathways. Guanidinium ions were used as structural probes for K^+ channels from the sarcoplasmic reticulum [4], acetylcholine-activated channels [1, 23] and voltage-activated Na^+ channels [12]. McCleskey and Almers [18] have shown that guanidinium permeates across the muscle Ca^{2+} channel in a Ca^{2+}-free solution when the channel becomes nonselective, but no permeability value was reported. In this article, amidiniums are shown to block a neuronal Ca^{2+} channel current. This is the first observed instance of monovalent-cation interference with Ca^{2+} or Ba^{2+} permeation in Ca^{2+} channels. Further studies of structure-function relations of amidinium block may provide a new tool to gain more insight into the spatial and chemical organization of the Ca^{2+} channel's selectivity filter.

Materials and Methods

Isolation of neurons from chick dorsal root ganglia (DRG) and recording of whole-cell membrane currents were previously described in full [22]. Cultures were maintained at 37°C in BME medium enriched by 10% horse serum, and containing 100 U/ml penicillin, 100 mg/ml streptomycin and 5 ng/ml nerve growth factor. Cells were used in experiments 6–12 hr after plating, before they started to extend neurites.

Recording pipettes were made from Kimax glass (Witz Scientific, Maumee, OH) and had inner tip diameters of 1.5–2 μm and resistances between 1 and 2 MΩ when filled with the standard pipette solution. Whole-cell currents were measured using an EPC-7 amplifier (List Electronics, Germany); about 70% of the series resistance was compensated. Three to five minutes were allowed for equilibration of patch pipette contents with the cytoplasm before recordings started, well after interfering potassium currents had disappeared. Only cells that showed no substantial rundown of calcium current were used. Step depolarizations were applied from the holding potential of −60 mV, lasted 30–500 msec and were delivered every 3 sec. Data were stored and digitized at 10 kHz for computer analysis. Records were corrected for linear leak and capacitance currents by fitting a current elicited by a 20 mV hyperpolarizing pulse and subtracting the proportionally scaled smooth function from each record. The amplitude of tail currents was obtained from the amplitude of a two-exponential. Averages are given as mean ± SEM with the number of different cells indicated in parentheses. Experiments were carried out at room temperature (20–22°C).

Three types of voltage-activated Ca^{2+} channels are distinguish-

Correspondence to: S.L. Mironov, Salzburg University, Institute for Zoology, Department of Animal Physiology, Hellbrunnerstr. 34, A-5020, Salzburg, Austria

able in chick sensory neurons: low-threshold (T-type) and high-threshold channels (L- and N-types). In whole-cell recordings these components can be separated by their kinetics [6]. Currents mediated by T- and N-type channels are transient and inactivate within tens of milliseconds, whereas L-type channels inactivate much more slowly. In DRG neurons the three channel types are differently expressed. Cells used in this study were grown in the presence of nerve growth factor, which promotes the expression of L-type channels [6]. Only those cells were used that showed no changes in the time course of activation and inactivation following a change of holding potential from −60 to −100 mV, which indicated that any component of current flowing through transient Ca^{2+} channels was marginal. In the experiments done at low ionic strength or at submicromolar $[Ca]_o$, the holding potential was made more negative by −30 mV (*see legends* to Figs. 4 and 5) to compensate for changes in the surface potential [12, 14, 20].

The standard external solution contained (in mM): 1 $BaCl_2$, 126 NaCl, 20 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (adjusted to pH 7.5 with NaOH), 10 glucose. Patch electrodes were filled with a standard internal solution containing (in mM): 100 CsCl, 40 tetraethylammonium (TEA) chloride, 10 glucose, 10 ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 10 HEPES, adjusted to pH 7.5 with CsOH. Variations from the standard composition are listed in the respective figure legends. In calculations of permeability, the amount of base added to titrate HEPES and EGTA in external and internal solutions was taken into account. This gives 60 mM external Na^+ and 120 mM internal Cs^+ for the standard solutions. To all external solutions, 3 μM tetrodotoxin was added to block the fast sodium current. All chemicals were from Sigma (Deisenhofen, Germany). Solution exchanges were made using a large-bore six-barrelled pipette placed 50–100 μm from the patched cell.

Results

External Guanidinium Blocks Ca^{2+} Channel Current by Binding in the Pore

Changing the standard external recording medium to a solution containing guanidinium in place of Na^+ led to a decrease of Ca^{2+} channel current (Fig. 1). An equilibrium was attained within several seconds and subsequent washout with the standard recording solution restored the initial amplitude of the current. The fast development and the full reversibility of the block indicate an external site for guanidinium action. The shape of the *I-V* curve was not altered as guanidinium block manifested itself in a proportional reduction of peak current at each potential. By contrast, complete Na^+ replacement by $Tris^+$ did not change the Ca^{2+} channel current (n = 11, *data not shown*), which was in agreement with the results obtained for other cell types [7, 13].

Guanidinium block was relieved by both reduced external guanidinium concentration and increased Ba^{2+} concentration (Fig. 2), suggesting a pore blockade by guanidinium. Competition between guanidinium (G) and Ba^{2+} for a common binding site would modulate the Ca^{2+} channel current according to [8]

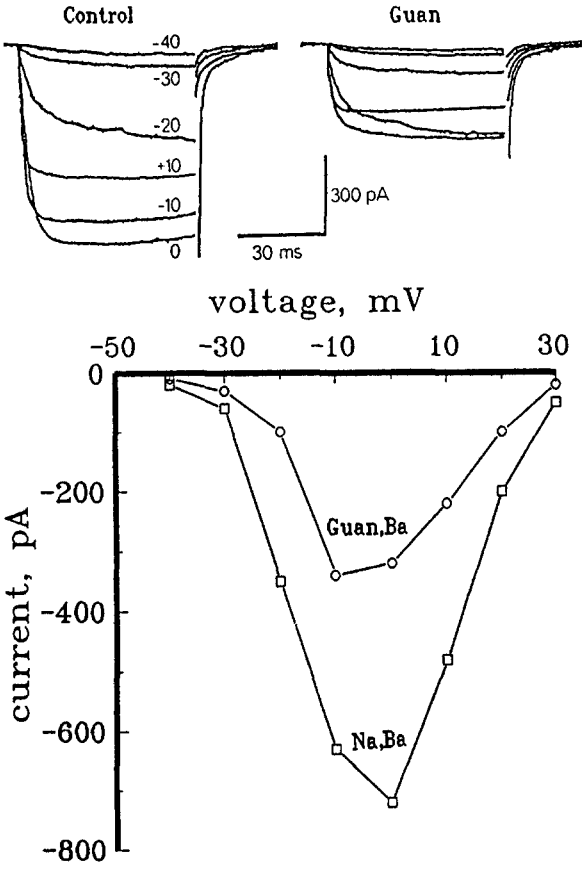


Fig. 1. Guanidinium blocks the Ca^{2+} channel current. Recordings were made in standard external solution containing 1 Ba^{2+} and 126 mM Na^+ or $guanidinium^+$. (*Top*) Currents recorded from holding potential of −60 mV with different test voltages (in mV) as indicated. (*Bottom*) Peak currents *vs.* applied voltage.

$$I = I_{max} \frac{[Ba]/K_{Ba}}{1 + [Ba]/K_{Ba} + [G]/K_G} \quad (1)$$

The ratio of the control current (I_{Ba}) to that recorded in the presence of guanidinium (I_G) then is

$$I_{Ba}/I_G = 1 + \frac{[G]/K_G}{(1 + [Ba]/K_{Ba})} \quad (2)$$

The slopes of straight lines corresponding to this linearized representation and their intersection with axes gave slightly different dissociation constants K_G = 110 and K_{Ba} = 6 mM (Fig. 2*B*) and K_G = 130 and K_{Ba} = 5.5 mM (Fig. 2*D*) for variations in guanidinium and Ba^{2+} concentrations, respectively. The K_{Ba} values agreed with the dissociation constant K_{Ba} = 4 ± 2 mM obtained from the dependence of I_{Ba} on Ba^{2+} concentration (varied from 1 to 50 mM, n = 8). This supports the interpretation that guanidinium and Ba^{2+} compete for the pore. In the voltage range from −20 to +30 mV K_{Ba}

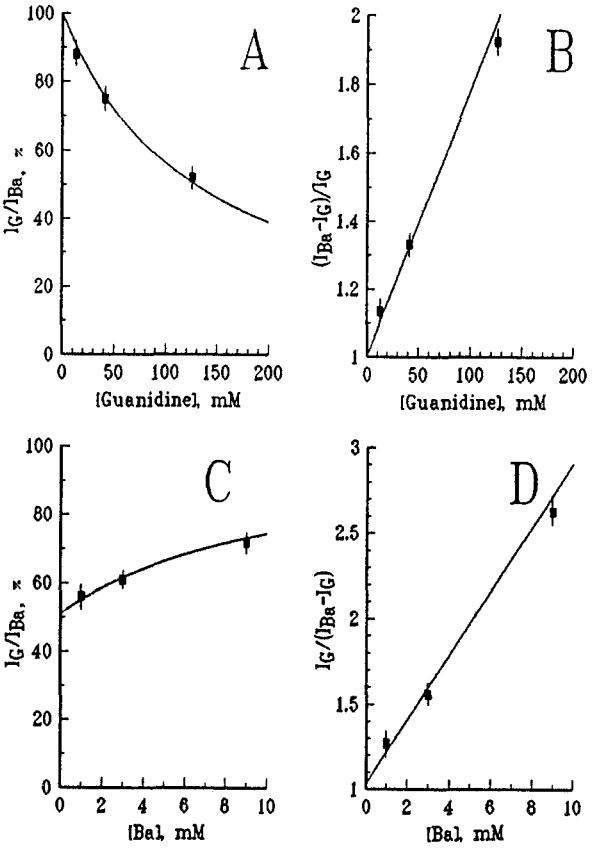


Fig. 2. Competition between Ba^{2+} and guanidinium at a common channel binding site. Isotonic substitution for NaCl was used to make solutions with different Ba^{2+} or guanidinium concentrations. Peak Ba^{2+} currents corresponding to the maximum of *I-V* curve were analyzed. Ba^{2+} current in the presence of guanidinium (I_G) was normalized to the control value (I_{Ba}) recorded in the standard external solution and different combinations of (I_G/I_{Ba}) are plotted as mean values with vertical bars denoting SEM (n = 8–11 for each point). Shown are the concentration dependence of guanidinium block (*A*) and of its relief with increased Ba^{2+} concentration (*C*). Linearized representations (*B,D*) are plotted using coordinates defined by Eq. (2). Unbroken lines were drawn according to Eq. (1) with the dissociation constants K_{Ba} = 6 mM and K_G = 110 mM (*A,B*), K_{Ba} = 5.5 mM and K_G = 130 mM (*C,D*).

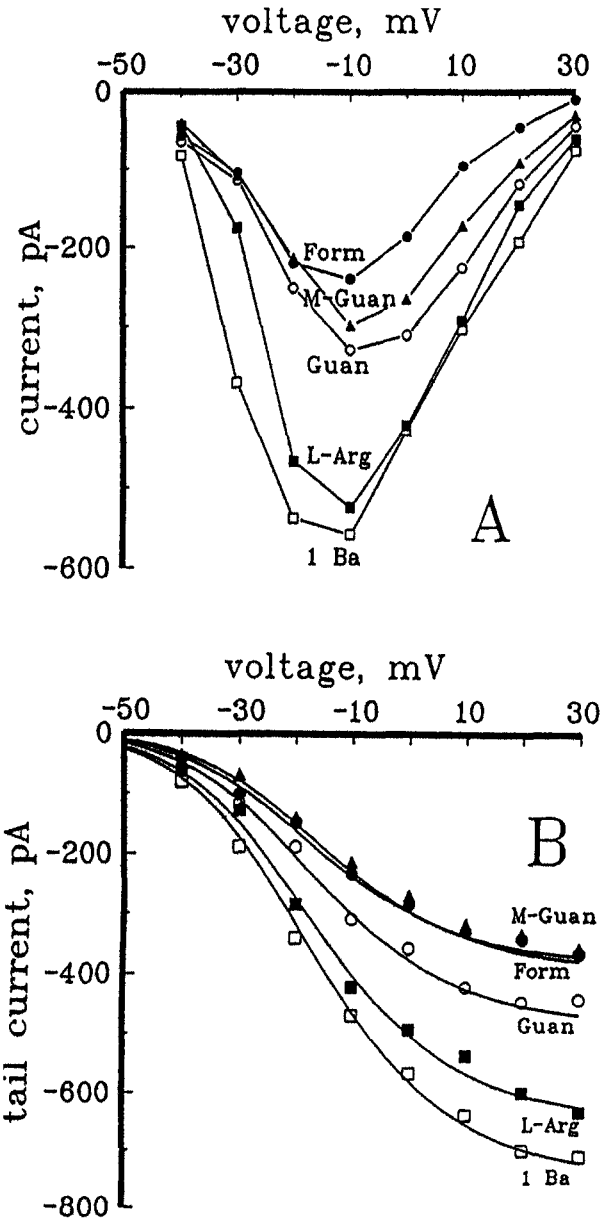


Fig. 3. Block of Ca^{2+} channel current by amidiniums. Currents were recorded using the standard external solution containing 1 mM Ba^{2+} and 126 mM X^+ (X = formamidinium, methylguanidinium, guanidinium, L-arginine, Na). Holding potential −60 mV. (*A*) Peak currents *vs.* applied voltage. (*B*) Amplitude of tail currents *vs.* voltage applied in the preceding voltage step. Unbroken lines are drawn according to $I_{tail} = I_{max} \times m_\infty^2$ where $m_\infty = 1/(1 + \exp(-a(V - V_o)))$ with a = 0.08 and V_o = −28 mV (−29 mV for formamidinium).

depended on voltage, corresponding to an "electric distance" of 0.15. If guanidinium block occurred at the same electrical location, its voltage dependence would be weak: over a 50 mV range of membrane voltage, K_G should vary by less than 15%.

Specificity of Ca^{2+} Current Block by Amidiniums

The guanidinium block represents a specific effect since other monovalent organic cations of similar structure had different blocking potency. Only methyl-guanidinium and formamidinium reduced the current, whereas L-arginine was ineffective (Fig. 3*A*). Tail currents (Fig. 3*B*) were changed in the same proportions as peak currents. For example, formamidinium reduced the peak current at 0 mV by 44 ± 5%, and the tail current at −60 mV by 41 ± 4% (n = 8).

Ca^{2+} channel current reduction by amidiniums could be due to amidinium binding to surface charges, causing a decrease in concentration of permeant ions at the external mouth of the channel. This current reduction would appear as a channel block and would have weak voltage dependence [20]. To test this possibility, activation curves for Ca^{2+} current were measured from

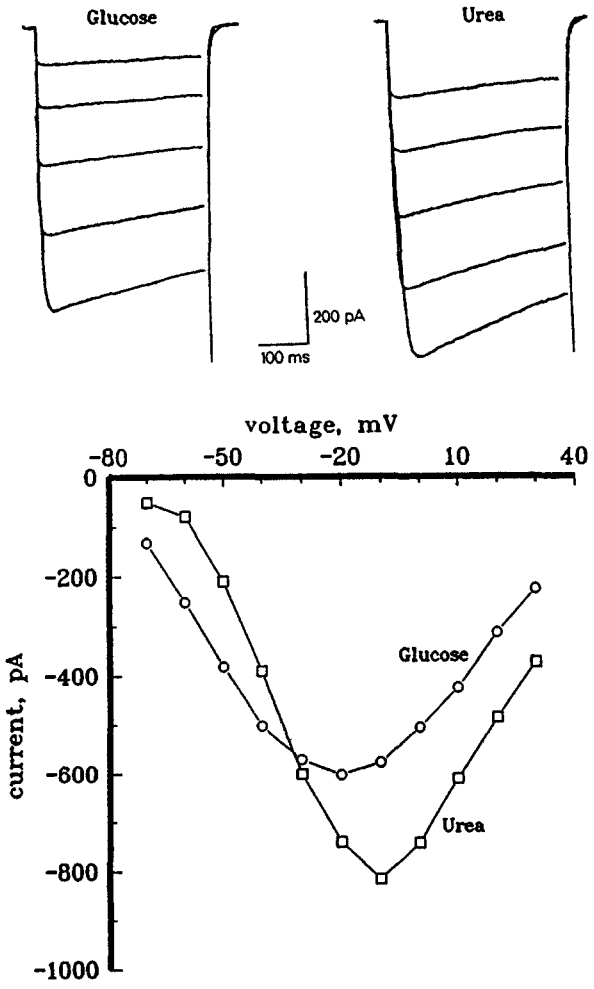


Fig. 4. The effects of urea on Ca^{2+} channel current. (*Top*) Membrane currents for test pulses taking the potential from −90 mV to different voltages, starting from −20 mV with an increment of +10 mV. The holding potential was shifted into the hyperpolarizing direction to compensate for low ionic strength. Currents were recorded using external solutions containing (in mM): 1 Ba^{2+} and 126 Na^+, 252 glucose or urea. (*Bottom*) Peak currents recorded in the presence of glucose (circles) or urea (squares) *vs.* applied voltage.

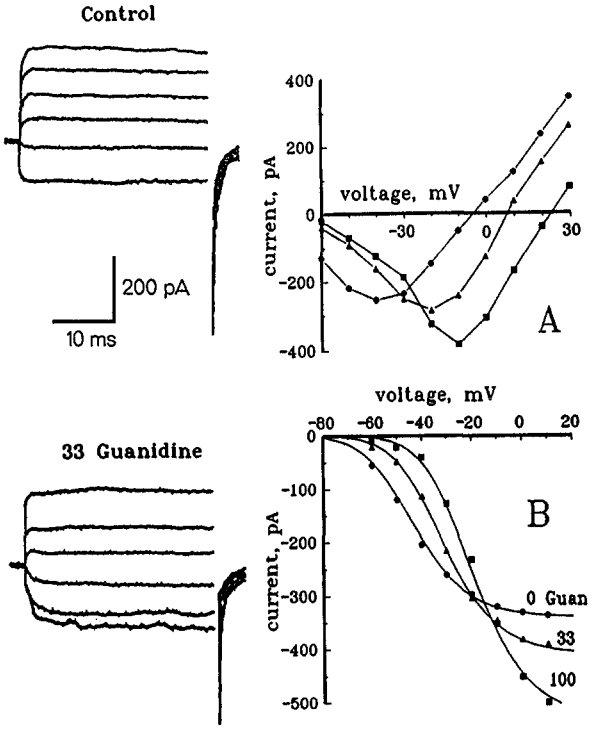


Fig. 5. Na^+ and guanidinium currents through the Ca^{2+} channel. The basic Ca^{2+}-free external solution contained (in mM): 30 NaCl, 100 N-methyl-glucamine (NMG), 10 EGTA, 10 HEPES, titrated to pH 7.5 with NaOH. In two other solutions, NMG^+ was replaced for 33 or 100 mM guanidinium. Left inset shows currents recorded by taking the potential from −90 mV to different test voltages, starting from −20 mV with an increment of +10 mV. (*Top*) Control records. (*Bottom*) Currents recorded in solution, containing 33 mM guanidinium. (*A*) Peak currents *vs.* applied voltage. (*B*) Amplitude of tail currents *vs.* test voltage. Unbroken lines were drawn according to $I_{tail} = I_{max} \times m_\infty^2$, where $m_\infty = 1/(1 + \exp(-a(V - V_o)))$ with $a = 0.08$ and $V_o = -54$, −42 and −30 mV at 0, 33 and 100 mM guanidinium, respectively.

the amplitudes of tail current at −60 mV following 30 msec test pulses to different voltages. Fit with the Boltzmann distribution (Fig. 3*B*) revealed no changes in the voltage dependence of activation, as expected if amidiniums do not specifically interact with surface charges.

Organic cations used to study Ca^{2+} channel pore blockade need to be strong bases. Amidiniums have $pK_a > 12$ and at neutral pH exist only in cationic form. In contrast, most amines are weak bases, and both charged and neutral species are present in aqueous solutions. Neutral amines permeate the cell membrane, thus increasing intracellular pH and concomitantly potentiating Ca^{2+} current [22]. For example, isotonic substitution of Na^+ for hydrazinium ($pK_a = 6.6$) increased the Ca^{2+} channel current by 100 ± 20% ($n = 7$).

Urea, a neutral amidine analogue, was also tested. Since replacement of NaCl by this non-ionized compound reduces the ionic strength, control experiments were done in which all NaCl was replaced by 252 mM glucose. Within seconds after solution exchange, the peak I_{Ba} increased by 50 ± 10% and the activation curve was shifted leftward by 12 ± 3 mV ($n = 7$). Both effects were consistent with a surface potential decrease in the low ionic strength solution. However, a subsequent change to a solution containing 252 mM urea increased peak currents further by 35 ± 8% ($n = 7$) and shifted the activation curve rightward by 10 ± 3 mV (Fig. 4).

Guanidinium Permeability in Ca^{2+} Channel in Ca^{2+}-Free Solutions

In Ca^{2+}-free solutions, Ca^{2+} channels can pass monovalent cations [2, 7, 11, 15]. Guanidinium permeability was assessed by substituting *N*-methyl-glucamine (NMG) for external guanidinium. NMG^+ is not a per-

meant cation because no change in the reversal potential was observed when in a Ca^{2+}-free external solution containing 30 mM NaCl and 100 mM NMG-Cl, the latter was replaced by 200 mM glucose ($n = 5$). In both cases, the Ca^{2+} channel current reversed at $E^0_{rev} = -5 \pm 3$ mV ($n = 7$). Exchanging NMG^+ for guanidinium shifted both the reversal potential and the activation curve rightward (Fig. 5). At constant external $[Na^+]$ and internal $[Cs^+]$, the Goldman-Hodgkin-Katz equation gives

$$E_{rev} = \frac{RT}{F}\log_{10}\frac{P_{Na}[Na]_o + P_G[G]_o}{P_{Cs}[Cs]_i} = E^0_{rev} + \frac{RT}{F}\log_{10}(1 + \frac{P_G[G]_o}{P_{Na}[Na]_o}) \quad (3)$$

At 33 and 100 mM external guanidinium, $E_{rev} = 7 \pm 2$ and 21 ± 3 mV ($n = 9$), yielding P_G/P_{Na} of 1.06 and 1.05, respectively.

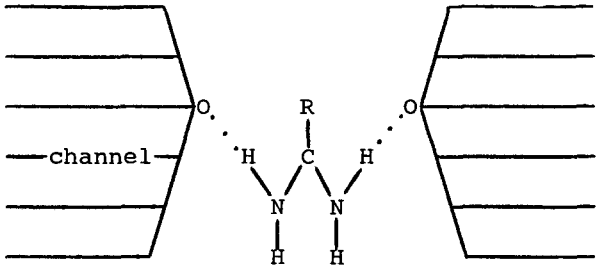
Changes in surface potential should not modify E_{rev} but can alter the channel conductance [20]. The concentration of permeant ions near the membrane, C^S, is related to the bulk concentration C via the Boltzmann equation $C^S = C(-z\Phi F/RT)$ where Φ is the surface potential. If for two different monovalent ion species the surface potential differs by $\Delta\Phi$, then the ratio of their surface concentration is $C^S_2/C^S_1 = C_2/C_1(-z\Delta\Phi F/RT)$. Na^+ and G^+ have nearly equal permeabilities, so they can be considered as one species. At 33 and 100 mM guanidinium, the bulk concentration of permeating monovalents increased by 55 and 166% and the activation curve shifted by $\Delta\Phi = 12 \pm 2$ and 24 ± 3 mV ($n = 9$). If the shift of the activation curve reflects variations in surface potential, C^S/C^S_0 should be equal to 98 and 101%, respectively. However, the maximal tail current and the slope conductance at E_{rev} showed a distinct increase. This means that either the voltage sensor and the pore vestibule sense the surface potential differently or that guanidinium modifies the voltage dependence of channel activation in ways not involving surface charges. The latter possibility seems more likely because all monovalents used in this study (Na^+, $Tris^+$, NMG^+ and amidiniums), were equivalent in terms of surface charge screening when the external solutions contained divalent cations. Since in the experiments with Ca^{2+}-free solutions the ionic strength was kept constant, the voltage shift of channel activation is likely due to direct guanidinium effects on Ca^{2+} channel gating, analogous to that observed in urea-containing solution (Fig. 4).

Discussion

The results suggest that the site of the guanidinium effect is the permeation pathway in the Ca^{2+} channel. Formamidinium and methyl-guanidinium were equally effective, indicating that only two of three guanidinium amino groups are sufficient for channel blockade. Thus, the basic chemical motive for interaction with the Ca^{2+} channel is the amidinium moiety $[H_2N - C = NH_2]^+$. The corresponding binding site can be confined to a relatively narrow space, possibly the selectivity filter. L-arginine, which possesses a long residue ($-(CH_2)_3CH(NH_2)COOH$) attached to the amidinium group, did not block the Ca^{2+} channel current.

Ion channels with relatively wide ion pores [12] do not possess a specificity to amidiniums as that observed for the Ca^{2+} channels. For example, the K^+-selective channel from the sarcoplasmic reticulum conducts monovalent organic compounds [4]. These bind in the middle of a channel with similar affinities for methyl-guanidinium ($K_d = 120$ mM) and L-arginine ($K_d = 108$ mM). Guanidinium permeates better than Na^+ through acetylcholine-activated channels [1], and ethyl-guanidinium and L-arginine bind with dissociation constants of 3 and 33 mM, respectively [23].

The geometry and chemical properties of the amidinium group suggest that its amino hydrogens might form two hydrogen bonds with ligands lining the Ca^{2+} channel pore. The hypothetical guanidinium complex with the channel can be visualized as follows:



The positive ionic charge is an absolute requirement for amidinium binding. A neutral analogue, urea ($H_2N - CO = NH_2$), which was applied in a twice larger concentration, only increased the Ca^{2+} channel current, likely involving another mechanism. The structure shown may be suggested as a prototype for complexes of metal cations with the selectivity filter of the Ca^{2+} channel. In the drawing, Ca^{2+} or other permeant ion could substitute for carbon, and water molecules could replace the amino groups. The formation of the complex would require only modest distortions in the positions of the channel ligands. This configuration also implies that ions permeating across the Ca^{2+} channel partially retain their hydration shell, as has been suggested for Na^+ and K^+ channels [12].

The distance between two hypothetical channel oxygens (probably carbonyls, $-C = O$) can be estimated, using requirements imposed by geometry of guanidinium and its hydrogen bonding complexes with oxygen donors [25]. Since guanidinium is planar and

all interbond angles are ≈120°, the distance between two oxygens is $1_{O\ldots O} = 2\sin 60°(1_{C-N} + 1_{N-H\ldots O}) = 1.732 \cdot (0.137 + 0.291) = 0.75$ nm. When this structure acts as molecular sieve, then oxygens should be represented by hard core atoms and twice the van-der-Waals radius for oxygen (0.135 nm [5]) should be subtracted from $1_{O\ldots O}$, leaving 0.48 nm. This is 0.12 nm smaller than that obtained for the Ca^{2+} channel in muscle in the monovalent-conducting mode [18]. The data are not conflicting since two different approaches were used. McCleskey and Almers [18] used alkylammoniums, which should not demonstrate specific interactions with ligands comprising the selectivity filter. Moreover, the filter likely possesses a dynamic structure and may become tighter in the presence of an amidinium moiety, which offers the possibility of hydrogen bond formation. This suggestion is supported by the observation of a high guanidinium permeability, which is equal to that of Na^+, although the two permeant ions have different dimensions.

The model suggested above incorporates previously suggested structural elements of channel selectivity [12, 19], which involves electronegative oxygen ligands located in the transmembrane pore and arranged to mimic hydration. Recently, it was suggested that cation-π interactions might be strong enough to solvate ion within a K^+ channel [9] and to govern its selectivity [16]. Amidiniums have a delocalized system of π-electrons capable of interacting with aromatic residues. If amidinium block of the Ca^{2+} channel involves π-π interactions, the narrowest pore width would correspond to the van-der-Waals diameter for carbon or nitrogen, which is about 0.3 nm. This is much smaller than the values obtained by McCleskey and Almers [18] and in the present study.

Voltage-activated ion channels, named according to the most permeable cation, are selective among similar ions and do not allow ions of other charge to pass. Thus, Na^+ channels have low permeability to Ca^{2+} [3], but they can be blocked by external Ca^{2+} [24]. Potassium channels do not possess the measurable permeability to divalent ions but could be blocked by Ba^{2+} [17]. Similarly, monovalent cations do not interfere with Ca^{2+} channel selectivity unless external Ca^{2+} concentration is decreased down to micromolar levels. Amidiniums comprise the first example of monovalent cations that are able to interact with the permeation pathway of the Ca^{2+} channel in its normal mode when it conducts divalent ions.

The different potencies of amidiniums to block the calcium channel clearly indicate the importance of charge interactions and formation of hydrogen bonds with channel carbonyls. They may belong to glutamates supposed to coordinate Ca^{2+} in high-affinity binding sites [15]. A recent discovery [10] showed that after mutation of two aminoacids into glutamates in the putative Na^+ channel, the latter acquires the ion permeation properties of the Ca^{2+} channel. The use of a similar approach for the cardiac high-threshold Ca^{2+} channel also revealed the importance of these glutamates in regulating the conductance and ion permeation properties [26]. These findings necessitated modification of the multi-ion mechanism of Ca^{2+} channel permeation with two high-affinity Ca^{2+}-binding sites [2, 11]. It was suggested [26] that a single high-affinity site gives rise to the two closely spaced low-affinity sites, each providing two glutamates for chelation of two Ca^{2+} ions. However, as in a previous version [11], this mechanism has no parallels with properties of Ca^{2+}-binding proteins or synthetic macrocyclic compounds.

The alternative is the conformational model [21], assuming that the single intrachannel Ca^{2+}-binding site modifies dynamically its affinity to reflect the history of the use. The predictions of this model are shown to be equivalent to those of the multi-ion model, reproducing such anomalies as voltage and concentration dependence of ion block, mole-fraction relationship, and the Ussing exponent. The deviations from the independency principle observed using these protocols are being traditionally considered as indicators of the simultaneous presence of several ions within the channel. Although the multi-ion model may operate for the K^+ channel, for the Ca^{2+} channel it appears difficult to reconcile such features as the single channel conductance and the concentration and the voltage dependence of ion block [21]. In the multi-ion model of Ca^{2+} channel permeation, the alkaline earth metal cations are assumed to bind to the channel in the nonselective mode with high affinity and to the Ca^{2+}-conducting mode with low affinity due to ion-ion repulsion. However, the channel affinity to transition metal cations (Cd^{2+}, Mn^{2+}, La^{3+}, etc.) is similar in both conducting states [2]. Likewise, amidiniums do not increase their blocking potency in Ca^{2+}-free solutions but are permeating cations. Synthetic chemistry can provide compounds with different separation and orientation of amino groups capable of forming hydrogen bonds with glutamates lining the channel pore. Therefore, further exploration of amidinium complexation in the Ca^{2+} channel is important to gain more insight on the structure of the selectivity filter.

The author wishes to thank Ms. S. Engers for the cell isolation and making of the electrodes.

References

1. Adams, D., Nonner, W., Dwyer, T., Hille, B. 1981. Block of end-plate channels by permeant cations in frog skeletal muscle. *J. Gen. Physiol.* **78**:593–615
2. Almers, W., McCleskey, E. 1984. Non-selective conductance in Ca channels of frog muscle: Ca selectivity in a single channel pore. *J. Physiol.* **307**:585–608

3. Baker, P.F., Hodgkin, A.L., Ridgway, E.B. 1971. Depolarization and Ca entry in squid giant axons. *J. Physiol.* **218:**709–755
4. Coronado, R., Miller, C. 1980. Conduction and block by organic cations in a K^+-selective channel for sarcoplasmic reticulum incorporated into planar phospholipid bilayers. *J. Gen. Physiol.* **79:**529–547
5. Fasman, G.D. 1978. Bond angles and distances. *In:* CRC Handbook of Biochemistry and Molecular Biology. G.D. Fasman, editor. Section D, Volume II, p. 221. J. Wiley, Chichester.
6. Fox, A.P., Nowycky, M., Tsien, R.W. 1987. Kinetic and pharmacological properties distinguishing three types of calcium current in chick sensory neurons. *J. Physiol.* **394:**149–172
7. Fukushima, Y., Hagiwara, S. 1985. Currents carried by monovalent cations through Ca channels in mouse neoplastic B lymphocytes. *J. Physiol.* **358:**255–284
8. Hagiwara, S. 1973. Ca spike. *Adv. Biophys.* **4:**71–102
9. Heginbotham, L., MacKinnon, R. 1992. The aromatic binding site for tetraethylammonium ion on potassium channel. *Neuron* **8:** 483–491
10. Heinemann, S., Terlau, H., Stühmer, W., Impto, K., Numa, S. 1992. Calcium channel characteristics conferred on the sodium channel by single mutations. *Nature* **356:**441–444
11. Hess, P., Tsien, R. 1984. Mechanism of ion permeation through Ca channels. *Nature* **309:**453–456
12. Hille, B. 1991. Ionic Channels of Excitable Membranes. Sinauer, Sunderland, MA
13. Isenberg, G., Klöckner, U. 1982. Calcium currents in isolated bovine ventricular myocytes are fast and of large amplitude. *Pfluegers Arch.* **395:**30–41
14. Kostyuk, P.G., Mironov, S.L., Doroshenko, P.A., Ponomarev, V.N. 1982. Surface charges on the outer surface of mollusc neuron membrane. *J. Membrane Biol.* **70:**171–179
15. Kostyuk, P.G., Mironov, S.L., Shuba, Ya.M. 1983. Two ion-selecting filters in the calcium channel of the somatic membrane of mollusc neurons. *J. Membrane Biol.* **76:**83–93
16. Kumpf, R.A., Dougherty, D.A. 1993. A mechanism for ion selectivity in potassium channels: computational studies of cation-π interactions. *Science* **261:**1708–1710
17. Latorre, R., Miller, C. 1983. Conduction and selectivity in potassium channels. *J. Membrane Biol.* **71:**11–30
18. McCleskey, E., Almers, W. 1985. The Ca channel in skeletal muscle is a large pore. *Proc. Natl. Acad. Sci. USA* **82:**7149–7153
19. Miller, C. 1993. Potassium selectivity in proteins: Oxygen cage or in the F ace? *Science* **261:**1692–1693
20. Mironov, S.L. 1984. Is there a carboxylic group in the sodium channel of excitable membranes? *Bioelectrochem. Bioenerg.* **12:** 235–242
21. Mironov, S.L. 1992. Conformational model for ion permeation in membrane channels: A comparison with multi-ion models and applications to Ca channel permeability. *Biophys. J.* **63:**485–496
22. Mironov, S.L., Lux, H.D. 1991. Cytoplasmic alkalinization increases high-threshold calcium current in chick sensory neurons. *Pfluegers Arch.* **418:**138–143
23. Sanchez, J.A., Dani, J., Siemen, D., Hille, B. 1986. Slow permeation of organic cations acetylcholine receptor channels. *J. Gen. Physiol.* **87:**985–1001
24. Yamamoto, D., Yeh, J.Z., Narahashi, T. 1984. Voltage-dependent Ca block of normal and tetramethrin-modified single Na^+ channel. *Biophys. J.* **45:**337–343
25. Yamamoto, Y., Kojima, S. 1991. Synthesis and chemistry of guanidine derivatives. *In:* The Chemistry of Amidines and Imidates. S. Patai, Z. Rappoport, editors. pp. 485–527. J. Wiley, Chichester
26. Yang, J., Ellinor, P.T., Sather, W.A., Zhang, J., Tsien, R.Y. Molecular determinants of Ca^{2+} selectivity and ion permeation in L-type calcium channel. *Nature* **366:**158–162