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 amine, 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-
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$^{2+}$], 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, 1 ethyleneeglycol-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]

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

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

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

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. 2B) and $K_G = 130$ and $K_{Ba} = 5.5$ mM (Fig. 2D) for variations in guanidinium and Ba$^{2+}$ concentrations, respectively. The $K_{Ba}$ values agreed with the dissociation constant $K_{Ba} = 4 \pm 2$ 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. 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.](image-url)