Distribution of K⁺-Dependent Na⁺/Ca²⁺ Exchangers in the Rat Supraoptic Magnocellular Neuron Is Polarized to Axon Terminals

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Neurons are polarized into compartments such as the soma, dendrites, and axon terminals, each of which has highly specialized functions. To test whether Ca²⁺ is differently handled in different compartments of a neuron, we investigated Ca²⁺ clearance mechanisms in somata of supraoptic magnocellular neurosecretory cells (MNCs) and in their axon terminals located in neurohypophyses. Using patch-clamp and microfluorometry techniques, Ca²⁺ transients were evoked by depolarizing pulses. Endogenous Ca²⁺ binding ratios (κs) and Ca²⁺ clearance rates were calculated from the decay phases of Ca²⁺ transients according to the single compartment model. Mean values of κs were 79 ± 2.6 in somata of MNCs and 187 ± 19 in axon terminals. Ca²⁺ clearance rate in axon terminals, which were calculated from time derivative of Ca²⁺ decay and the κs values, were approximately threefold higher than in soma. In response to external Na⁺ reduction, Ca²⁺ clearance rates were reduced by 65% in axon terminals, but did not change in soma. Immunohistochemical assays confirmed that K⁺-dependent Na⁺/Ca²⁺ exchanger (NCKX2) was specifically localized to neurohypophysial axon terminals and was not found in somata. In somata, inhibition of sarcoplasmic reticulum Ca²⁺-ATPase (SERCA) pumps, mitochondrial Ca²⁺-uniporter, and plasma membrane Ca²⁺-ATPase (PMCA) pumps decreased Ca²⁺ clearance rate by 48, 27, and 21%, respectively. These results suggest that neurohypophysial axon terminals have greater Ca²⁺ clearance power than somata because of the specific localization of NCKX2, and that Ca²⁺ clearance in somata of MNCs is mediated by SERCA pumps, mitochondrial uniporter, and PMCA pumps.

Key words: calcium clearance; supraoptic nucleus; neurohypophysis; NCKX; SERCA; mitochondria

Introduction

Neurons are polarized into compartments such as the soma, dendrites and axon terminals, each of which has highly specialized functions. Calcium, the most universal intracellular messenger, may be differently handled in different compartments of a neuron. Various Ca²⁺ clearance mechanisms, such as plasma membrane Ca²⁺-ATPase (PMCA), sarcoplasmic reticulum Ca²⁺-ATPase (SERCA), Na⁺/Ca²⁺ exchanger, and mitochondrial Ca²⁺ uniporter, have been described in neurons (for review, see Thayer et al., 2002), but most information stems from somata or axon terminals independently.

SERCA is thought to be the major mechanism of Ca²⁺ clearance in somata of cerebellar Purkinje neurons (Fierro et al., 1998) and the dendrites of cortical pyramidal neurons (Markram et al., 1995). Mitochondria have been reported to shape Ca²⁺ transients by buffering Ca²⁺ upstroke in dorsal root ganglion cells (Werth and Thayer, 1994). In axon terminals, contribution of Na⁺/Ca²⁺ exchange (Reuter and Porzig, 1995), mitochondria (Billups and Forsythe, 2002), and PMCA (Zenisek and Matthews, 2000; Usachev et al., 2002) have been described in various neuronal cells. Taken together, these studies suggest that the relative contribution of each of these mechanisms to Ca²⁺ clearance varies widely among different mammalian neurons. It has, however, not yet been studied whether any of these mechanisms is preferentially localized to a specific region of a neuron, probably because the opportunities for direct comparison of Ca²⁺ clearance between the axon terminals and somata of the same cell are limited. In this respect, magnocellular neurosecretory cells (MNCs) in the supraoptic nucleus and axon terminals in the neurohypophysis (NHP) allow a unique opportunity to investigate the subcellular localization of Ca²⁺ clearance mechanisms.

We previously reported that the K⁺-dependent Na⁺/Ca²⁺ exchanger NCKX plays a dominant role (~60%) in Ca²⁺ clearance in the axon terminals of rat neurohypophyses (Lee et al., 2002). NCKX is thought to be an efficient Ca²⁺ clearance mechanism in photoreceptors, in which constant Na⁺ influx threatens the transmembrane [Na⁺] gradient that is necessary for driving Ca²⁺ extrusion (Cervetto et al., 1989). Accordingly, we hypothesized that NCKX would be a suitable mechanism of Ca²⁺ clearance occurring in small compartments such as axon terminals that are often invaded by high-frequency Na⁺ spikes. This hypothesis led us to quantify the contribution of NCKX to Ca²⁺ clearance in the somata of MNCs, which have low surface-to-volume ratio. To our surprise, no Na⁺/Ca²⁺ exchange activity was found in somata. As expected from the absence of NCKX, the Ca²⁺ clearance rates in somata were much slower than in axon...
terminals. We also quantified the contribution of other Ca\(^{2+}\) clearance mechanisms in somata, showing that the SERCA pump plays a dominant role. This study is the first clear demonstration of the polarized distribution of Na\(^+\)/Ca\(^{2+}\) exchanger in single neuronal cells.

**Materials and Methods**

**Preparation of isolated nerve endings and brain slice.** Isolated nerve endings and brain slices were prepared from male Sprague Dawley rats (11–12 weeks old; 325 ± 25 gm) using previously described methods with small modification (Li and Hatton, 1997; Lee et al., 2002). Briefly, animals were killed by decapitation after being anesthetized with diethyl ether. For isolation of nerve endings, neural lobes were carefully removed from pituitary gland and cut into 9 or 12 pieces with a razor blade in artificial CSF (aCSF). Small pieces of the neural lobe were kept in HEPES-buffered F-10 culture media at room temperature until next use. Just before electrophysiological recording, a piece of neural lobe was transferred to a recording chamber on the stage of an inverted microscope (IX70; Olympus, Tokyo, Japan), in which the neural lobe piece was triturated in culture medium with fire-polished pipettes (300–500 μm in diameter).

For preparation of brain slices, the whole brain was rapidly removed from the skull and chilled in aCSF at 4°C. After trimming a tissue block containing the hypothalamus, coronal brain slices (300-μm-thick) were cut on a vibratome (Vibratome 1000; TPI, St. Louis, MO). Slices were incubated at 37°C for 30 min and thereafter maintained at room temperature (23–25°C) until next use. Supraptic nuclei were visualized with an upright microscope (BX50WI; Olympus).

**Electrophysiological recordings.** Calcium currents were evoked from axon terminals or somata of MNGCs by conventional whole-cell patch clamp technique. Patch pipettes with a resistance of 3–6 and 2–3 MΩ were used for recording from axon terminals and somata, respectively. The pipette solution contained (in μM): 140 K-glucuron, 5 KCl, 15 HEPES, 4 MgATP, and 4 Na-ascorbate with the pH adjusted to 7.3 with KOH. For measurement of Ca\(^{2+}\)-binding ratios in axon terminals, we used Cs\(^+\)-based pipette solution, which contained 130 Cs-aspartate, 10 NaCl, 5 Na-aspartate, and 4 Na-ascorbate, pH 7.3 with CsOH. Bath solution for axon terminals contained (in μM): 150 NaCl, 5.4 KCl, 0.5 MgCl\(_2\), 1.8 CaCl\(_2\), 5 HEPES, and 10 glucose, pH 7.4 with NaOH. Bath solution for somata contained (in μM): 125 NaCl, 5 KCl, 25 NaHCO\(_3\), 2 CaCl\(_2\), 1 MgCl\(_2\), 1.25 NaH\(_2\)PO\(_4\), 10 glucose, 5 sucrose, and 0.4 Na-ascorbate, pH 7.4 when saturated with carbogen (95% O\(_2\) and 5% CO\(_2\)). The superfusion rate and volume of the recording chamber for slices were 1.8 ml/min and 1.2 ml, respectively. Recordings were made in axon terminals with EPC-9 amplifier and somata with EPC-8 amplifier (HEKA, Lambrecht, Germany). Experiments were performed at 35 ± 1°C. All chemicals were obtained from Sigma (St. Louis, MO), except ruthenium red and 5(6)-carboxyeosin diacetate from Fluka (Buchs, Switzerland), Ru360 from Calbiochem (Nottingham, UK), and fura-2 (HEKA, Lambrecht, Germany). Experiments were performed at 35 ± 1°C. All chemicals were obtained from Sigma (St. Louis, MO), except ruthenium red and 5(6)-carboxyeosin diacetate from Fluka (Buchs, Switzerland), Ru360 from Calbiochem (Nottingham, UK), and fura-2 (HEKA, Lambrecht, Germany).

**Cytoplasmic Ca\(^{2+}\) measurement.** The procedures for cytosolic Ca\(^{2+}\) measurement in isolated nerve ending have been described in detail in our recent publication (Lee et al., 2002). Somatic Ca\(^{2+}\) concentration was measured using fluorescence imaging. Cells were loaded with fura-2 (pentapotassium salt) via patch electrodes. For fluorescence excitation, we used a polychromatic light source (xenon-lamp based, Polychrome-III; Till Photonics, Martinsried, Germany), which was coupled into the microscope. We used a polychromatic light source (xenon-lamp based, Polychrome-III; Till Photonics, Martinsried, Germany), which was coupled into the microscope. Experiments were performed at 35 ± 1°C. All chemicals were obtained from Sigma (St. Louis, MO), except ruthenium red and 5(6)-carboxyeosin diacetate from Fluka (Buchs, Switzerland), Ru360 from Calbiochem (Nottingham, UK), and fura-2 (HEKA, Lambrecht, Germany).

**Calibration parameters.** In vivo calibration as described in Lee et al. (2000). The effective dissociation constant of fura-2 (K\(_{eq}\)) was calculated from the equation:

\[K_{eq} = [Ca^{2+}] \times (R_{max} - R_{min})/(R_{min} - R_{max}) ,\]

where [Ca\(^{2+}\)] was entered as 328 nm (assuming a dissociation constant (K\(_{d}\)) of BAPTA of 222 nm at pH 7.2). The estimated R\(_{max}\), R\(_{min}\), and K\(_{eff}\) (in μM) in somata measured using the upright microscope were typically 0.87, 5.72, and 1.32, respectively. Those in axon terminals measured using the inverted microscope were 0.27, 3.95, and 0.93, respectively. The K\(_{eq}\) of fura-2 was calculated as 201 nm from K\(_{eq}\) = K\(_{eff}\) \((\alpha + R_{max})/(\alpha + R_{min})\), where α is the isoelectric factor (Zhou and Neher, 1993).

For increasing time resolution and minimizing the photobleaching effect, we adopted the single-wavelength protocol (Helmchen et al., 1996; Lee et al., 2000). Images taken at 10 Hz with single wavelength excitation at 380 nm (F\(_{iso}\)) were preceded and followed by images with excitation at isosbestic wavelengths (360 nm). F\(_{iso}\) (isosbestic fluorescence) values were linearly interpolated between points just before and after the period of excursion at 380 nm. The ratio R = F\(_{max}\)/F\(_{iso}\) was converted to [Ca\(^{2+}\)] with the equation:

\[\frac{[Ca^{2+}]}{[Ca^{2+}]_{0}} = \frac{F_{max}F_{iso}}{F_{max}F_{iso}} \times (R - R_{min})/(R_{max} - R) \cdot \frac{1}{K_{eq}} .\]

**Ca\(^{2+}\) binding ratio.** Ca\(^{2+}\) binding ratios of endogenous buffers (K\(_{eq}\)) were estimated according to the single compartment model (Neher and Augustine, 1992; Helmchen et al., 1996; Lee et al., 2000). The differential Ca\(^{2+}\) binding ratio of a Ca\(^{2+}\) buffer, X, is defined as:

\[\kappa_X = \frac{\partial \ln X_{Ca}}{\partial \ln [Ca^{2+}]_{iso}} = \frac{[X]_i K_{d,X}}{[Ca^{2+}]_{iso} + K_{d,X}} .\]

The Ca\(^{2+}\) clearance rate exerted by a linear Ca\(^{2+}\) clearance mechanism from a single compartment can be described as:

\[\frac{d\Delta [Ca^{2+}]}{dt} = -\alpha \cdot \frac{\Delta [Ca^{2+}]}{S} .\]

Where \(\Delta [Ca^{2+}]_1\) and \(\Delta [Ca^{2+}]_0\) are magnitudes of total and free Ca\(^{2+}\) excursions from the basal level, respectively, and α is a Ca\(^{2+}\) clearance rate constant. From the definition of Ca\(^{2+}\) binding ratio:

\[\frac{\Delta [Ca^{2+}]}{dt} = \frac{\Delta [Ca^{2+}]}{S} = \frac{\Delta [Ca^{2+}]}{S} 	imes (1 + K_{sX} + K_{pX}) ,\]

where K\(_{sX}\) and K\(_{pX}\) are Ca\(^{2+}\) binding ratios of the Ca\(^{2+}\) indicator dye (fura-2) and endogenous Ca\(^{2+}\) buffers, respectively. And thus, Equation 5 can be rewritten in terms of \(\Delta [Ca^{2+}]_0\), as:

\[\frac{\Delta [Ca^{2+}]}{dt} = -\alpha \cdot [Ca^{2+}]_{iso}(1 + K_{sX} + K_{pX}) .\]

Decay phase of a Ca\(^{2+}\) transient (\(\Delta [Ca^{2+}]_{0}(t)\)) following a pulse of Ca\(^{2+}\) influx can be described by the following equations:

\[\Delta [Ca^{2+}]_{0}(t) = A \cdot \exp(-t/\tau) \]

\[\tau = (1 + K_{sX} + K_{pX})/\gamma .\]

Since

\[\Delta [Ca^{2+}]_{0}(0) = Q/(2FV_s) \]

\[\Delta [Ca^{2+}]_{0}(0) = A \cdot (1 + K_{sX} + K_{pX}) .\]

The value for Q can be measured from time integration of Ca\(^{2+}\) current evoked by short depolarization pulse. Estimates of the endogenous Ca\(^{2+}\)
binding ratio, $k_s$ can be obtained from the negative x-axis intercepts of plots of $\tau$, $A$, or $(A/Q)^{-1}$ versus $k_p$.

**Ca$^{2+}$ clearance rate constant.** In our previous paper (Lee et al., 2002), to describe the Ca$^{2+}$ clearance power of a cell when Ca$^{2+}$ transients do not show a single exponential decay, we used the instantaneous Ca$^{2+}$ decay rate constant ($\lambda_{i-o}$) at the peak of a Ca$^{2+}$ transient, which is defined as $-d[Ca^{2+}]/dt|_{t=0}/[Ca^{2+}]_t$. When the Ca$^{2+}$ transient is fitted to the biexponential function, $A_1 \cdot \exp(-\lambda_{1} \cdot t) + A_2 \cdot \exp(-\lambda_{2} \cdot t)$, $\lambda_{i-o}$ value can be calculated by the following equation:

$$\lambda_{i-o} = (A_1 \cdot \lambda_1 + A_2 \cdot \lambda_2)/(A_1 + A_2).$$

(13)

Because the genuine Ca$^{2+}$ clearance power of the cell is represented by $d[Ca^{2+}]/dt$ rather than by $d[Ca^{2+}]/dt|_{t=0}/[Ca^{2+}]_t$, we corrected $\lambda_{i-o}$ for endogenous and exogenous Ca$^{2+}$ buffers by using Equation 9 and obtained the instantaneous Ca$^{2+}$ clearance rate constant at the peak of the Ca$^{2+}$ transient, $\gamma_{i-o}$ as follows:

$$\gamma_{i-o} = \lambda_{i-o} \cdot (1 + k_e + k_f).$$

(14)

Please note that the symbol, $\lambda_{i-o}$, corresponds to $\gamma_{i-o}$ in our previous paper (Lee et al., 2002). In the present paper, however, we introduced a new symbol, $\lambda$, to distinguish the decay rate constant from the clearance rate constant, $\gamma$.

**Calculation of relative contribution curve of a clearance mechanism, X, as a function of $[Ca^{2+}]_t$.** We quantified contribution of a clearance mechanism, $X$, to the entire Ca$^{2+}$ clearance at given $[Ca^{2+}]_t$ level as follows: (1) We calculated $d[Ca^{2+}]/dt$ from two Ca$^{2+}$ transients before and after the application of an inhibitor of $X$, and plotted as a function of $[Ca^{2+}]_t$; (2) Each $d[Ca^{2+}]/dt$ plot was fitted with a fourth-order polynomial function; (3) The difference between the polynomial fit ($f_{control}$) to the $d[Ca^{2+}]/dt$ curve under control condition and that ($f_{inhibitor}$) in the presence of the inhibitor was regarded as the contribution made by $X$; (4) The relative contribution made by $X$ ($R_X$) to the entire Ca$^{2+}$ removal mechanism was calculated according to the equation:

$$R_X = (f_{control} - f_{inhibitor})/f_{control},$$

and plotted as a function of $[Ca^{2+}]_t$ (see Fig. 4, open gray circles for exemplary plots).

**Data analysis.** Data were analyzed with IgorPro (version 4.1; WaveMetrics, Lake Oswego, OR). Statistical data are expressed as mean ± SEM, and $n$ indicates the number of cells studied. The significance of the differences in $\gamma_{i-o}$ or in $R_X$ were evaluated using Student’s t test with a significance level of 0.01. Because all the plot and analyses were performed as a function of $[Ca^{2+}]_t$, hereafter $[Ca^{2+}]_t$ and $[Ca^{2+}]_1$ will be used interchangeably for convenience.

In situ hybridization and immunohistochemistry. The procedures for synthesis of riboprobes and in situ hybridization have been described in detail in our recent paper (Lee et al., 2002). For immunohistochemistry, male Sprague Dawley rats (11–12 weeks old; 325 ± 25 gm) were perfused transcardially with 40 ml of 0.1 M PBS, followed by 400 ml 4% paraformaldehyde in PBS. The brains and pituitaries were removed and post-fixed by immersion in the same fixative for 16 hr at 4°C. Pituitary or tissue blocks containing hypothalami were cryoprotected in 25% sucrose in PBS at 4°C overnight, then frozen by immersion into prechilled isopentane in liquid nitrogen. For hypothalami, serial 16-μm-thick coronal sections were cut with cryostat and adhered to gelatin-coated slides. For neurohypophyses, serial transverse 6-μm-thick sections were collected onto gelatin-coated slides. For neurohypophyses, serial transverse 6-μm-thick sections were collected onto gelatin-coated slides. For neurohypophyses, serial transverse 6-μm-thick sections were collected onto gelatin-coated slides. For neurohypophyses, serial transverse 6-μm-thick sections were collected onto gelatin-coated slides. For neurohypophyses, serial transverse 6-μm-thick sections were collected onto gelatin-coated slides. For neurohypophyses, serial transverse 6-μm-thick sections were collected onto gelatin-coated slides. For neurohypophyses, serial transverse 6-μm-thick sections were collected onto gelatin-coated slides. For neurohypophyses, serial transverse 6-μm-thick sections were collected onto gelatin-coated slides. For neurohypophyses, serial transverse 6-μm-thick sections were collected onto gelatin-coated slides.

Results

We investigated the Ca$^{2+}$ clearance mechanisms in the somata and axon terminals of supraoptic MNCs by analyzing the decay phases of Ca$^{2+}$ transients evoked in response to depolarizing pulses. Because the decay rate of free Ca$^{2+}$ $-d[Ca^{2+}]/dt$ is affected not only by Ca$^{2+}$ clearance mechanisms but also by the concentrations of endogenous and exogenous Ca$^{2+}$ buffers, we first estimated the endogenous Ca$^{2+}$ binding ratios ($k_s$) in two loci. Using these values, we converted $-d[Ca^{2+}]/dt$ into the Ca$^{2+}$ clearance rate ($-d[Ca^{2+}]/dt$), which represents the genuine power of Ca$^{2+}$ clearance mechanisms in the cell.

**Endogenous Ca$^{2+}$ binding ratios in axon terminals and somata of MNCs.** Endogenous Ca$^{2+}$ binding ratios ($k_s$) in the axon terminals and somata of MNCs were estimated according to the single compartment model (Neher and Augustine, 1992). To evoke Ca$^{2+}$ transients, we used depolarizing pulses of relatively short duration (from −60 to 0 mV; 20 msec for somata, 30 msec for axon terminals). Under these conditions, the initial amplitudes of Ca$^{2+}$ transients were lower than 200 nm, and Ca$^{2+}$ transients were well fitted by monoeponential functions.

Somata of MNCs were loaded with an internal solution containing 250 μM fura-2. Time course of fura-2 loading within the soma of the MNC is shown in Figure 1Aa (open circles). After the break-in, Ca$^{2+}$ transients were evoked every 1 min. Ca$^{2+}$ transients measured at various fura-2 were depicted near the fura-2 loading trace. As cytosolic fura-2 concentration increased, amplitudes and decay rates of Ca$^{2+}$ transients decreased. To estimate $k_s$ in the somata of MNCs, time constants (Fig. 1Ab, open circles, left ordinate) and inverse of amplitudes (Fig. 1Ab, filled gray circles, right ordinate) were plotted against the incremental Ca$^{2+}$ binding ratio of fura-2 ($k'_s$). The $k'_s$ values obtained using Equations 9 and 11 were 79 ± 2.6 (n = 8) and 63 ± 5 (n = 8), respectively, showing no significant difference. As the single compartment model predicts, A$\tau$ showed no significant dependence on $k'_s$ (Fig. 1Ab, inset).

When axon terminals were loaded with 250 μM fura-2, there were negligible effects of the exogenous buffer on Ca$^{2+}$ transients, indicating that 250 μM fura-2 is not sufficient to compete with endogenous buffers. We therefore increased the fura-2 concentration to 500 μM. The time course of fura-2 loading into the axon terminal is shown in Figure 1Ba. Shortly after the break-in, Ca$^{2+}$ currents were evoked by depolarizing pulses. The method to estimate $k_s$ in the soma was similar to that used in axon terminals, except that the initial amplitude of each Ca$^{2+}$ transient (A) was normalized to the total charge of corresponding Ca$^{2+}$ influx (Q) to compensate for the error caused by rundown of Ca$^{2+}$ current (Fig. 1Ba, inset). For this purpose, we used a Ca$^{2+}$-based pipette solution. As shown in Figure 1Bh, and $(A/Q)^{-1}$ were plotted against $k'_s$ and $k_p$ was calculated according to Equations 9 and 12. Mean values for $k_s$ estimates from analyses of $\tau$ and $(A/Q)^{-1}$ were 179 ± 35 (n = 3) and 187 ± 19 (n = 3), respec-
tively. These values are not substantially different from the value reported by Stuenkel (1994) (174 ± 17.6) in the same preparation. These results indicate that the endogenous Ca2+ binding ratio is significantly higher in the axon terminals than in the soma of MNCs (p < 0.01 by Student’s t test).

**Ca2+ clearance in soma and axon terminals of MNCs**

To investigate Ca2+ clearance mechanisms in the axon terminals and soma of MNCs, Ca2+ transients were evoked by depolarizing pulses, the durations of which were adjusted such that the peak [Ca2+] fell within the range 1.0 ± 0.2 μM (typically, 200 msec in axon terminals and 300 msec in soma). To avoid inhibition of K+-dependent Na+/Ca2+ exchange activity, we used a K+-rich pipette solution containing 50 μM fura-2 (Lee et al., 2002). The Ca2+ transients were recorded from the soma (Fig. 2Aa, black line) and the axon terminal (Fig. 2Ab, black line) under control condition. From decay phases of these Ca2+ transients, we calculated values for $-d[Ca^{2+}]_i/dt$ and plotted them as a function of $\Delta[Ca^{2+}]_i$ (Fig. 2B, insets). These results indicated that $-d[Ca^{2+}]_i/dt$ is faster in the axon terminal than in the soma. To take into account the difference in $\kappa_s$ in the two loci, $-d[Ca^{2+}]_i/dt$ was converted into $-d(Ca^{2+})_i/dt$ according to Equation 6 (Fig. 2C; rectangles, soma; circles, axon terminal). Estimates for $\gamma_{r=0}$, which represents the Ca2+ clearance power of a cell at the peak of Ca2+ transient, were obtained from the corresponding $\lambda_{r=0}$ according to Equation 14. The mean values for $\gamma_{r=0}$ in somata and axon terminals were 81.3 ± 5.0 sec$^{-1}$ (n = 9) and 250.3 ± 23.5 sec$^{-1}$ (n = 7), respectively (Fig. 2D), indicating that Ca2+ clearance power is about threefold larger in axon terminals than in soma.

![Figure 1](image1.png)

**Figure 1.** Representative examples for estimation of endogenous Ca2+ binding ratios ($\kappa_s$) in the soma of a MNC (A) and in the neurohypophysial axon terminal (B). Aa. Time course of fura-2 (250 μM) loading during whole-cell patch recording (absissa: elapsed time after breakin). Fura-2 concentration was calculated from isosbestic fluorescence averaged from ROI drawn on the soma. Exponential fit to the loading curve was superimposed ($r = 175.8$ sec). After break-in, Ca2+ transients were evoked every 1 min by depolarizing pulses from −60 to 0 mV (20 msec duration). Four exemplar Ca2+ transients are shown along the fura-2 loading curve. Calibration: 2 sec, 20 nM. Monoexponential functions were fitted to the decay phases of four Ca2+ transients (r = 1273.7, 1615.9, 2138.2, and 2973.3 msec, from left to right). Ab. Plots of time constants (open circles, left ordinate) and inverse of initial amplitudes (1/[$Ca^{2+}$]) (gray filled circles, right ordinate) versus incremental Ca2+ binding ratio of fura-2 ($\kappa_B$). $\kappa_B$ were estimated from x-axis intercepts of regression lines. The plot of $A_r$ versus $\kappa_s$ (inset) shows no significance dependence on $\kappa_s$. B0. Time course of fura-2 (500 μM) loading in the NHP axon terminal. Ca2+ transients were evoked by depolarizing pulses (30 msec duration) during whole-cell patch recording. Four exemplar Ca2+ transients (calibration: 2 sec, 20 nM) are shown, and their decay phases were well fitted by exponential functions whose $r$ values are 4225.2, 6426.9, 8970.7, and 1089.6 msec, Bb. Plots of time constants (open circles, left ordinate) and inverse of normalized amplitudes (gray filled circles, right ordinate) versus $\kappa_B$. Inset, Plot of $A_r$ versus $\kappa_B$.

![Figure 2](image2.png)

**Figure 2.** Effects of external Na+ reduction (replaced with Li+) on Ca2+ transients of soma and axon terminals. A. Micrograph of the MNC soma in supraoptic nucleus (Aa) and that of axon terminals dissociated from neurohypophysis (Ab). Ba. Two Ca2+ transients from the soma were superimposed in control condition (black line) and after the 10 min superfusion with low Na+ aCSF (24 μM, gray line). K+ pipette solution containing 50 μM fura-2 was used for the internal solution. Ca2+ transients were evoked by depolarizing pulses (from −60 to 0 mV; 300 msec duration). Bb. Two Ca2+ transients recorded in the axon terminal (black line: control; gray line: low Na+ condition). The same internal solution was used as Ba. Ca2+ transients were evoked by 200 msec depolarization.Insets, Time derivatives of decay phases of Ca2+ transients ($-d(Ca^{2+})_i/dt$) were plotted as a function of $\Delta(Ca^{2+})_i$, and fitted with fourth-order polynomial equations (open circles and black lines: control condition, filled circles and gray lines: low Na+ condition). C. Time derivatives of total Ca2+ ($d(Ca^{2+})_i/dt$) decay as a function of $\Delta(Ca^{2+})_i$, which were obtained from Ca2+ transients illustrated in B. Four plots obtained from the soma (rectangles) and the axon terminal (circles) were superimposed. Values for $d(Ca^{2+})_i/dt$ were calculated according to Equation 6, assuming that $\kappa_s$ values for soma and axon terminal are 79 and 187, respectively, and that $\kappa_B$ of fura-2 is 201 μM. Plots of $d(Ca^{2+})_i/dt$ were fitted with fourth-order polynomial equations. D. Mean values for instantaneous Ca2+ clearance rate constants ($\gamma_{r=0}$) calculated using Equation 14 before (indicated by “Na”) and after extracellular Na+ reduction (indicated by “Li”). Asterisk indicates statistical significance (p < 0.01).
superimposed (Fig. 2Aa), indicating that removal of external Na\(^+\) had no effect on Ca\(^{2+}\) transients. In eight other cells, no change in Ca\(^{2+}\) transients was observed at least 10 min after superfusion with low Na\(^+\)–ACSF. The mean value for $\gamma_{i=0}$ after reduction of extracellular Na\(^+\) (81.3 ± 5.0 sec\(^{-1}\); n = 9) was not significantly different from that under control conditions ($p = 0.27$, by Student’s t test for paired samples) (Fig. 2C). In contrast, the decay phases of Ca\(^{2+}\) transients in NHP axon terminals were significantly slowed by reduction of extracellular Na\(^+\) to 20 mM (Fig. 2Bb), consistent with our previous report (Lee et al., 2002). The mean values for $\gamma_{i=0}$ before and after reduction of extracellular Na\(^+\) were 250.3 ± 23.5 sec\(^{-1}\) and 87.5 ± 5.1 sec\(^{-1}\), respectively ($n = 7$; $p < 0.01$) (Fig. 2D).

In Figure 2G, plots of $-\frac{d[Ca^{2+}]_i}{dt}$ as a function of $[Ca^{2+}]_i$, obtained from the soma (rectangles) and the axon terminal (circles) under conditions of normal (open symbols) and low [Na\(^+\)] (closed symbols) were superimposed. The $-\frac{d[Ca^{2+}]_i}{dt}$ plot of the axon terminal under low Na\(^+\) condition overlapped almost completely with that of the soma under normal Na\(^+\) conditions, indicating that the Ca\(^{2+}\) clearance power of the axon terminal in the absence of Na\(^+\)/Ca\(^{2+}\) exchange is equivalent to that of the soma. This finding was confirmed by results for $\gamma_{i=0}$ under each condition (Fig. 2D). Above results suggest that greater Ca\(^{2+}\) clearance power in axon terminals than in somata can be attributed to Na\(^+\)/Ca\(^{2+}\) exchanger and that Ca\(^{2+}\) clearance in the soma is mediated by mechanisms other than Na\(^+\)/Ca\(^{2+}\) exchange.

### NCKX2 is highly localized to axon terminals

To test whether the polarization of Na\(^+\)/Ca\(^{2+}\) exchange activity in axon terminals is caused by the specific localization of Na\(^+\)/Ca\(^{2+}\) exchange proteins, we performed immunohistochemistry in SON and in NHP. Because we showed in our previous report (Lee et al., 2002) using in situ hybridization technique that NCKX2 is a major isoform of NCKX present in SON (Fig. 3A), the expression of NCKX2 was examined.

The location of SON was confirmed by the adjacent optic chiasm. NHP was distinguished from the adjacent intermediate lobe of the hypophysis by the lower density of nuclei that were counterstained with cresyl violet. NCKX2-positive signals were visualized with dianaminobenzidine tetrahydrochloride as brown color. Although there were few NCKX2-positive signals in SON (Fig. 3B), intense staining for NCKX2 was observed in NHP, in a form resembling Herring bodies (Fig. 3C, arrows).

**Figure 3.** Expression of NCKX2 or its transcripts in the supraoptic nucleus (SON) and the neurohypophysis. In SON, positive signals for NCKX2 transcripts were revealed by in situ hybridization (A). NCKX2 mRNA was detected using digoxigenin-labeled NCKX2 antisense riboprobes. Somata of MNCs were strongly positive to NCKX2. However, the micrograph obtained from immunohistochemistry indicates that NCKX2-immunoreactive signals are very weak in SON (B). In neurohypophysis, intense NCKX2-immunoreactive signals were localized in numerous Herring bodies (C, arrows). Note the difference in staining density between B and C, IL, Intermediate lobe; OC, optic chiasm; SON, supraoptic nucleus. Scale bars, 50 μm.

**Figure 4.** Effects of specific inhibitors of SERCA pumps, mitochondria, and PMCA pumps on Ca\(^{2+}\) transients of MNCs somata. Aa, Two Ca\(^{2+}\) transients evoked in control condition (black line) and in the presence of 1 μM thapsigargin (gray line) were superimposed. Ab, Plots of total Ca\(^{2+}\) decay rate ($-\frac{d[Ca^{2+}]_i}{dt}$) as a function of $[Ca^{2+}]_i$, which were obtained from decay phases of two Ca\(^{2+}\) transients in Aa according to Equation 6. Fourth-order polynomial fits were superimposed on the plots (continuous lines). The difference between two polynomial fits to $-\frac{d[Ca^{2+}]_i}{dt}$ curves was plotted as a dotted line (marked by “SERCA pumps”). Relative contribution of SERCA to Ca\(^{2+}\) clearance ($R_{SERCA}$) was calculated according to the Equation 15 and plotted as a function of $\Delta (Ca^{2+})$, (gray open circles, right ordinate). Ac, Bar graph representing mean values for $\gamma_{i=0}$ normalized to the control condition. TG, Thapsigargin; CPA, cyclopiazonic acid (10 μM). Ba, Two Ca\(^{2+}\) transients in the control condition (black line) and in the presence of 10 μM Ru360 (gray line) were superimposed. Bb, $-\frac{d[Ca^{2+}]_i}{dt}$ and $R_{Mito}$ were plotted by the similar procedure as in Ab. Bc, Normalized mean values for $\gamma_{i=0}$ averaged from eight cells in control condition, in the presence of CPA, and in the presence of CPA and CE were summarized.
Other $Ca^{2+}$ clearance mechanisms in somata of MNCs

Because Na"/Ca"$^{2+}$ exchange contributed little to $Ca^{2+}$ clearance in the somata of MNCs, we investigated the contribution of other putative $Ca^{2+}$ clearance mechanisms: SERCA, mitochondrial $Ca^{2+}$ uniporter, and PMCA.

To study the contribution of SERCA pumps to $Ca^{2+}$ clearance, we examined the effects of thapsigargin (TG; 1 $\mu$M) and cyclopiazonic acid (CPA; 10 $\mu$M), specific inhibitors of the SERCA pump, to the decay phase of $Ca^{2+}$ transients. Because the effects induced by these inhibitors depend on the incubation time (Sagara et al., 1992), we superfused the slice with SERCA pump inhibitors ~10 min before estimating their effects. Figure 4Aa illustrates representative $Ca^{2+}$ transients before and after the addition of 1 $\mu$M TG to the extracellular solution. The $Ca^{2+}$ decay rate was significantly slowed down by TG.

The contribution of mitochondrial $Ca^{2+}$ uptake was measured using Ru360 and ruthenium red, blockers of mitochondrial $Ca^{2+}$ uniporters. The superfusion of 10 $\mu$M Ru360 for 30 min caused a slowing of $Ca^{2+}$ decay (Fig. 4Bb). Extracellular perfusion of 20 $\mu$M ruthenium red and intracellular application of 10 $\mu$M ruthenium red showed similar effects as Ru360.

The role of PMCA pumps in $Ca^{2+}$ clearance was tested using 5(6)-carboxymerocyanine diacetate (CE). It was reported that CE can inhibit SERCA pumps as well as PMCA pumps (Fierro et al., 1998). To assess the role of PMCA pumps by CE, SERCA pumps were inhibited by 10 $\mu$M CPA or 1 $\mu$M thapsigargin before the addition of CE. Comparison of two $Ca^{2+}$ transients in the presence of CPA (control condition) and 15 min after additional application of CE (40 $\mu$M) revealed that CE slowed the $Ca^{2+}$ decay phase (Fig. 4Ca). Because CPA or thapsigargin was present in the extracellular perfusion solutions throughout the experiment, it is likely that CE-induced decrease in $Ca^{2+}$ decay rate is caused by PMCA inhibition.

We calculated $-d[Ca^{2+}]_i/dt$ and $\gamma_{i=0}$ from the $Ca^{2+}$ transients before and after the application of each blocker, as described in Figure 2. The relative contribution of each mechanism to $Ca^{2+}$ clearance over the whole range of $[Ca^{2+}]_i$ was calculated from $-d[Ca^{2+}]_i/dt$ using Equation 15, and is shown as open circles superimposed on each $-d[Ca^{2+}]_i/dt$ plot (right ordinates; see Materials and Methods for details). We found that $R_{SERCA}$ increased as $[Ca^{2+}]_i$ increased, reaching its maximal value (46.4%) (Fig. 4Ab). $R_{SERCA}$ calculated according to the equation ($\gamma_{i=0,con} - \gamma_{i=0,TG}$)/$\gamma_{i=0,con}$ was 47.6%. A similar value was obtained when $R_{SERCA}$ was calculated from $\gamma_{i=0}$ in the presence of CPA ($\gamma_{i=0,CPA}$). TG decreased $\gamma_{i=0}$ by 48.2 ± 5.4% ($n = 6$) and CPA decreased by 52.3 ± 2.6% ($n = 5$), with no significant difference observed between these two agents ($p = 0.54$) (Fig. 4Ac). $R_{Mitochondria}$ obtained from the Ru360 results showed an increase as $[Ca^{2+}]_i$ increased until it reached a maximum value (28.6%) (Fig. 4Bb). The decreases in $\gamma_{i=0}$ induced by Ru360 and by extracellular and intracellular ruthenium red were 28.2 ± 16.2% ($n = 6$), 26.4 ± 7.5% ($n = 3$), and 25.5 ± 1.4% ($n = 3$), respectively (Fig. 4Bc). The relative contribution of PMCA ($R_{PMCA}$) was characteristic in that $R_{PMCA}$ steeply increased and reached a maximal value at a lower $[Ca^{2+}]_i$ range than the other $Ca^{2+}$ clearance mechanisms (Fig. 4Cb). After reaching the maximal value, $R_{PMCA}$ decreased as $[Ca^{2+}]_i$ increased. In the presence of CPA where $\gamma_{i=0}$ was 47.6% of the control value, CE-induced decrease in $\gamma_{i=0}$ was 41.0 ± 3.96% ($n = 8$), indicating that the decrease in $\gamma_{i=0}$ that can be ascribed to CE-induced PMCA inhibition is 19.5%.

Figure 5A summarizes the relative contribution of each mechanism to somatic $Ca^{2+}$ clearance at a given $[Ca^{2+}]_i$ range. Because the experiments of PMCA inhibition were performed in the presence of SERCA pump inhibitors, $R_{PMCA}$ was corrected for normal conditions. After reaching the maximal value at relatively low $[Ca^{2+}]_i$, range, $R_{PMCA}$ steeply decreased. In contrast, $R_{SERCA}$ and $R_{Mitochondria}$ increased as $[Ca^{2+}]_i$ increased, reaching their maximum contributions. This can be interpreted as a transition of the major $Ca^{2+}$ clearance mechanism from a low-capacity, high-affinity mechanism to high-capacity, low-affinity mechanisms as $[Ca^{2+}]_i$ increases. In Figure 5B, the relative contributions of each $Ca^{2+}$ clearance mechanism calculated using $\gamma_{i=0}$ value were summarized. Mean values of the relative contribution of SERCA pumps, mitochondrial uptake, and PMCA pumps were 47.8 ± 7.2% ($n = 9$), 27.4 ± 6.9% ($n = 6$), and 21.1 ± 4.5% ($n = 8$), respectively. By taking the sum of these three mechanisms, we could account for $>90\%$ of $Ca^{2+}$ clearance. This result is consistent with the finding that contribution of Na"/Ca"$^{2+}$ exchange to $Ca^{2+}$ clearance in the somata of MNCs is negligible (Fig. 2).

Discussion

Although recent studies increased our understanding of differences in $Ca^{2+}$ regulation mechanisms among neuronal cell types, little is known about the differences in $Ca^{2+}$ regulation mechanisms in different parts of a single neuron. We have previously shown that, in axon terminals of rat neurohypophyses, the K"-dependent Na"/Ca"$^{2+}$ exchanger, NCKX, plays a dominant role, accounting for $~60\%$ of the total $Ca^{2+}$ clearance (Lee et al., 2002). In the present study, we investigated the differences in $Ca^{2+}$ regulation mechanisms between axon terminals and somata of MNCs and found that the two compartments of MNCs clear $Ca^{2+}$ loads in different ways: (1) the endogenous $Ca^{2+}$...
binding ratio \((\kappa_0)\) and \(\text{Ca}^{2+}\) clearance rate constant \((\gamma_{i=0})\) in axon terminals were significantly higher than in somata of MNCs; (2) the distribution of NCXX is polarized to axon terminals; (3) sequestration of \(\text{Ca}^{2+}\) via SERCA pumps is a major \(\text{Ca}^{2+}\) clearance mechanism in the somata of MNCs. These results indicate that the two compartments of MNCs clear \(\text{Ca}^{2+}\) loads in different ways, and that the higher \(\text{Ca}^{2+}\) clearance power in axon terminals is caused by the polarized distribution of NCXX.

### Subcellular localization of \(\text{Na}^+/\text{Ca}^{2+}\) exchangers in neurons

Two different families of \(\text{Na}^+/\text{Ca}^{2+}\) exchangers, NCX and NCXX, are expressed in various regions of the brain. Three different genes for NCX isoforms and four different genes for NCXX isoforms have been cloned (Nicoll et al., 1990, 1996; Li et al., 1994; Tsoi et al., 1998; Kraev et al., 2001; Li et al., 2002). Distributions of each gene in various regions of the brain have been studied by in situ hybridization. Apart from NCXX1, which has the most restricted tissue distribution, being found only in retinal photoreceptor (Prinsen et al., 2000), transcripts for the other NCX isoforms and for all NCX isoforms are widely distributed throughout the brain (Tsoi et al., 1998; Kraev et al., 2001; Lee et al., 2002; Li et al., 2002).

However, information on the subcellular localization of \(\text{Na}^+/\text{Ca}^{2+}\) exchangers is very limited. In cultured hippocampal neurons (Reuter and Porzig, 1995; Canitano et al., 2002) and the ciliary ganglia of the chick (Juhaszova et al., 2000), immunocytochemical studies have shown the preferential localization of NCX to presynaptic nerve terminals. Immunogold electron microscopy in rat brain section, however, revealed that NCX1 is localized to both the presynaptic and the postsynaptic sites of excitatory synapses (Canitano et al., 2002). Functional studies can also be useful in demonstrating the expression and the role of \(\text{Na}^+/\text{Ca}^{2+}\) exchangers. Slowing of \(\text{Ca}^{2+}\) decay by removal of \(\text{Na}^+\) has been observed in the somata of some neurons (Mironov et al., 1993; Fierro et al., 1998). Changes in \([\text{Ca}^{2+}]\), in presynaptic nerve terminals can be indirectly monitored by measuring miniature IPSCs (mIPSCs). In acutely isolated neurons containing functional GABAergic presynaptic boutons, a transient increase in mIPSCs was induced by removal of external \(\text{Na}^+\) in the presence of ouabain, suggesting the presence of NCX in these nerve terminals (Doi et al., 2002). Flux studies using synaptosomal preparations have shown that the efflux of radiolabeled \(\text{Ca}^{2+}\) could be attenuated by removal of external \(\text{Na}^+\) and accelerated by reintroduction of external \(\text{Na}^+\) (Sanchez-Armass and Blaustein, 1987).

Although these results are thought to represent the contribution of NCX, the possible contribution of NCXX cannot be excluded because these studies were not designed to distinguish between their activities. In the present study, however, we performed functional tests together with immunocytochemical assays, and we found that NCXX does not function in the somata but is localized specifically to axon terminals of supraoptic MNC neurons. To our knowledge, our studies are the first to demonstrate the subcellular localization of a specific \(\text{Ca}^{2+}\) regulation mechanism in different parts of a single neuron. We cannot yet state whether the specific localization of NCXX to axon terminals is specific to supraoptic MNC neurons or generally found in other regions. It also needs to be investigated in future studies whether the localization and role of NCXX are distinguished from those of NCX or overlap with each other.

### Ca\(^{2+}\) clearance mechanisms in the somata of MNCs

Neuronal cells clear \(\text{Ca}^{2+}\) loads by extrusion across the plasma membrane or by sequestration into intracellular organelles. In the present study, we characterized the relative contribution of \(\text{Ca}^{2+}\) clearance mechanisms in the somata of MNCs using pharmacological and ion substitution methods, showing that sequestration of \(\text{Ca}^{2+}\) into the ER via SERCA pumps is the dominant form of \(\text{Ca}^{2+}\) removal. ER is capable of rapidly sequestering \(\text{Ca}^{2+}\) and functions as a major \(\text{Ca}^{2+}\) clearance mechanism in dendrites and the somata of some neurons (Markram et al., 1995; Fierro et al., 1998). The presence of SERCA pumps in somata of MNCs was previously suggested by the finding that SERCA pump inhibitors prevented the generation of depolarizing afterpotentials and phasic firing activity (Li and Hatton, 1997).

Recently, the role of mitochondria in cytosolic \(\text{Ca}^{2+}\) handling has been investigated in various cell types. In adrenal chromaffin cells, mitochondrial \(\text{Ca}^{2+}\) uptake has been shown to play a major role in \(\text{Ca}^{2+}\) clearance (Herrington et al., 1996; Park et al., 1996). In addition to directly taking up cytosolic \(\text{Ca}^{2+}\) via Ca\(^{2+}\) uniporters, mitochondria contribute to \(\text{Ca}^{2+}\) clearance indirectly by providing fuel to ATP-dependent Ca\(^{2+}\) pumps. In synaptic terminals of retinal bipolar neurons, mitochondria have been shown to contribute to \(\text{Ca}^{2+}\) clearance primarily as an energy source for other clearance mechanisms, and mitochondrial \(\text{Ca}^{2+}\) uptake was observed only when PMCA pump, which is the major form of \(\text{Ca}^{2+}\) clearance in this tissue, was inhibited (Zeiske et al., 2000). In somata of neurons, however, contributions of mitochondria to \(\text{Ca}^{2+}\) clearance has not been investigated.

For investigating mitochondrial functions, the protonophore carbonyl cyanide m-chlorophenylhydrazone (CCCP) has been frequently used as a mitochondrial inhibitor. Because protonophores dissipate transmembrane potential of intracellular organelles including mitochondria, many cytotoxic materials including \(\text{Ca}^{2+}\) can be released from them. These nonspecific effects make protonophores not useful for assaying the role of mitochondria in \(\text{Ca}^{2+}\) clearance. We found that bath application of CCCP significantly increased resting \([\text{Ca}^{2+}]\) in the somata of MNCs (data not shown). The increase of resting \([\text{Ca}^{2+}]\) by CCCP was observed in the absence of extracellular \(\text{Ca}^{2+}\), suggesting that it was released from intracellular stores. Thus, we investigated the mitochondrial contribution to \(\text{Ca}^{2+}\) clearance using specific inhibitors of mitochondrial \(\text{Ca}^{2+}\) uniporters. Application of ruthenium red and Ru360 had no effect on resting \(\text{Ca}^{2+}\) concentration, although they slightly decreased peak \(\text{Ca}^{2+}\) amplitude. The \(\text{Ca}^{2+}\) decay rate measured in the presence of ruthenium red did not differ from that measured in the presence of Ru360 (\(p = 0.92\); Student’s t test). These findings suggest that the direct contribution of mitochondria to \(\text{Ca}^{2+}\) clearance, by taking up cytosolic \(\text{Ca}^{2+}\) via \(\text{Ca}^{2+}\) uniporters, accounts for ~25% of the total clearance in the somata of MNCs.

### Physiological implications

Because secretion of vasopressin and oxytocin is accompanied by AP bursts in MNCs (15–20 Hz; Cazalis et al., 1985), somata and axon terminals frequently undergo large \(\text{Ca}^{2+}\) loads in physiological conditions. When we mimicked the burst spikes by applying a train of 3 msec depolarization pulses at a frequency of 15 Hz for 5 sec, the increase in \([\text{Ca}^{2+}]\) was similar to that evoked by a depolarizing pulse from −60 to 0 mV for 300 msec, as used in the present study. Thus, SERCA pumps and NCXX, which are low-affinity, high-capacity \(\text{Ca}^{2+}\) clearance mechanisms in somata and axon terminals, respectively, might play a dominant role in...
Ca\(^{2+}\) clearance at the physiological range of Ca\(^{2+}\) loads associated with peptide hormone secretion. The results of the present study indicate that the axon terminals and somata of MNCs clear Ca\(^{2+}\) loads in different ways. Extrusion of Ca\(^{2+}\) across the plasma membrane via K\(^{-}\)-dependent Na\(^+/\)Ca\(^{2+}\) exchange accounts for \(\geq60\%\) of Ca\(^{2+}\) clearance in axon terminals, whereas sequestration of Ca\(^{2+}\) into the ER via SERCA pumps is the dominant Ca\(^{2+}\) clearance mechanism in the somata of MNCs. Including mitochondrial Ca\(^{2+}\) uptake, \(\geq70\%\) of Ca\(^{2+}\) clearance is mediated by sequestration of Ca\(^{2+}\) into the intracellular organelles in the somata of MNCs. Why axon terminals and somata of MNCs adopt different Ca\(^{2+}\) clearance mechanisms? The differences in \(\kappa_s\) and surface-to-volume ratio between two compartments may provide a possible explanation. Because the Ca\(^{2+}\) decay rate constant is inversely proportional to \(\kappa_s\), axon terminals require higher Ca\(^{2+}\) clearance power to attain a similar Ca\(^{2+}\) decay rate as somata. This requirement may be met by localized expression of NCKX2 in NHP axon terminals. The diameters of axon terminals of MNCs range from 2 to 15 \(\mu\)m, whereas those of somata range from 30 to 50 \(\mu\)m. Because axon terminals have higher surface-to-volume ratio, extrusion of Ca\(^{2+}\) across the plasma membrane can be the most effective mechanism for Ca\(^{2+}\) clearance. In contrast to axon terminals, extrusion across the plasma membrane may not be very effective in clearing Ca\(^{2+}\) from the somata of MNCs because of low surface-to-volume ratio. Thus, using intracellular stores seems to be a good strategy for overcoming low surface-to-volume ratio.

References