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Na^+/Mg^{2+} transporter acts as a Mg^{2+} buffering mechanism in PC12 cells☆

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Abstract

Mg^{2+} buffering mechanisms in PC12 cells were demonstrated with particular focus on the role of the Na^+/Mg^{2+} transporter by using a newly developed Mg^{2+} indicator, KMG-20, and also a Na^+ indicator, Sodium Green. Carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone (FCCP), a protonophore, induced a transient increase in the intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$). The rate of decrease of $[Mg^{2+}]_i$ was slower in a Na^+-free extracellular medium, suggesting the coupling of Na^+ influx and Mg^{2+} efflux. Na^+ influxes were different for normal and imipramine- (a putative inhibitor of the Na^+/Mg^{2+} transporter) containing solutions. FCCP induced a rapid increase in $[Na^+]_i$ in the normal solution, while the increase was gradual in the imipramine-containing solution. The rate of decrease of $[Mg^{2+}]_i$ in the imipramine-containing solution was also slower than that in the normal solution. From these results, we show that the main buffering mechanism for excess Mg^{2+} depends on the Na^+/Mg^{2+} transporter in PC12 cells.

Keywords: KMG-20; FCCP; Sodium Green; Imipramine

Magnesium is an essential element for animals and plants. Approximately 300 enzymatic reactions are mediated by Mg^{2+} in cells, and it is considered that concentration change of intracellular Mg^{2+} ($[Mg^{2+}]_i$) regulates the metabolism of cells [1–6]. With this in mind, we used fluorometric techniques to investigate Mg^{2+} mobilization in PC12 cells. It has been known for some time that $[Mg^{2+}]_i$ can be increased by extracellular stimuli such as fructose, glutamate, or extracellular Mg^{2+} in hepatocytes, neurons, and endothelial cells [7–12]. Although Ca^{2+} has been reported to induce an increase in $[Mg^{2+}]_i$ as a result of release from organelles or via Mg^{2+} influx through non-specific cation channels [9,13], the full details of Mg^{2+} mobilization mechanisms are not well understood. Several chemical and physico-chemical Mg^{2+} buffering mechanisms have been described in terms of Mg^{2+} extrusion mechanisms. For example, Mg^{2+} efflux in chicken erythrocytes, examined using atomic absorption spectroscopy or radio-isotope techniques, has been explained in terms of a Na^+/Mg^{2+} transporter which was inhibited by amiloride or imipramine [14–16]. However these above reports mainly focused on Mg^{2+}, and did not directly observe the mobilization of a counter cation, Na^+. Another difficulty in mobilization studies is tracing the dynamics of Na^+ and Mg^{2+} in cells because most previous reports used extraction of these ions from cells or the radio-isotopic labeling of Mg^{2+}

☆ *Abbreviations:* FCCP, carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone; NGF, nerve growth factor.

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[17–19]. To overcome these previous difficulties in Mg^{2+} mobilization studies, we examined Mg^{2+} and Na^+ using ion-specific fluorescent indicators. For Mg^{2+} measurement, we developed a Mg^{2+} indicator, KMG-20 [20], which is the Mg^{2+}-specific fluorescent indicator with a much higher affinity for Mg^{2+} over Ca^{2+} ($K_dMg = 10\,mM$, $K_dCa = 33\,mM$) than commercially available Mg^{2+} indicators. We have previously confirmed that KMG-20 signaling is not disturbed by Ca^{2+} fluctuations in physiological Ca^{2+} concentrations [20]. We also used a Na^+ indicator, Sodium Green, to visualize the counter ion of Mg^{2+} transport. Here, we have analyzed the mechanism of the Mg^{2+} buffering system in PC12 cells after $[Mg^{2+}]_i$ was increased by exposure of the cells to carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone (FCCP), with particular emphasis on the Na^+/Mg^{2+} transporter. We found that the increase in Mg^{2+} in response to FCCP was transient, and we hypothesized that the main buffering system was via Mg^{2+} efflux through a transporter in the cell membrane. To examine this, we used a substituted extracellular solution or an inhibitor of the transporter, and confirmed that the Na^+/Mg^{2+} transporter in the plasma membrane works to eliminate the excess Mg^{2+} from PC12 cells.

Materials and methods

Chemical reagents. Cell membrane-permeable KMG-20 acetoxymethyl ester (KMG-20-AM) ({13-aza-3-oxa-4-oxotetracyclo [7.7.1.0⟨2, 7⟩.0⟨13, 17⟩] heptadeca-1(17),2(7),5,8-tetraen-5-ylcarbonyloxy} methyl acetate) was developed and synthesized [20]. Dulbecco's modified Eagle's medium (DMEM), horse serum (HS), and fetal bovine serum (FBS) were purchased from GIBCO (MD, USA). Sodium Green was from Molecular Probes (OR, USA). Poly-D-lysine (PDL), nerve growth factor (NGF), carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone (FCCP), and other reagents were from Sigma (MO, USA).

Cell culture. PC12 cells [21] were obtained from RIKEN Tsukuba Institute, and cells were cultured at 37 °C in DMEM containing heat-inactivated serums (10% HS and 5% FBS), 25 U/ml penicillin, and 25 μg/ml streptomycin, under a humidified atmosphere with 5% CO_2. For experimental use, cells (passage number 5–9) were cultured on glass coverslips coated with PDL and differentiated by culturing with 50 ng/ml NGF containing serum-free medium for 5–7 days.

Fluorescent measurements and analysis. KMG-20-AM was stored under 0 °C as a 10 mM stock solution in DMSO. Cells were incubated with 10 μM KMG-20-AM in the culture medium for 30 min at 37 °C and then washed twice with a recording (normal) solution containing (in mM): NaCl, 125; KCl, 5; $MgSO_4$, 1.2; $CaCl_2$, 2; KH_2PO_4, 1.2; glucose, 6; and Hepes, 25 (pH 7.4); and further incubated for 15 min for complete hydrolysis of the acetoxymethyl ester form of the KMG-20-AM loaded into the cells. Loading of Sodium Green-AM was carried out in the same way as that of the KMG-20-AM. Excitation wavelengths for KMG-20 and Sodium Green were at 440 and 488 nm, respectively. Fluorescence images were acquired with an inverted microscope (ECLIPSE TE300 Nikon) equipped with a 20× (S Fluor, Nikon) or a 40× (S Fluor, Nikon) objective, a 505 dichroic mirror, and a 535/55 barrier filter. A 150 W Xe lamp with a monochrometer unit was used for multiple excitations, and fluorescence was measured with a CCD camera (HiSCA, Hamamatsu Photonics). Data are reported as means ± SE and were compared using *t* tests.

Results and discussion

FCCP induces a transient increase in $[Mg^{2+}]_i$

In order to investigate the mobilization of Mg^{2+} in PC12 cells we chose to expose the cells to FCCP as the mechanism for inducing a change in $[Mg^{2+}]_i$. FCCP (5 μM) was applied to PC12 cells 30 s after commencing the measurement of the fluorescence signal (Fig. 1). FCCP resulted in an increase in the fluorescence signal, particularly in the cytosol where the KMG-20 is mainly localized (Fig. 1A). The FCCP-induced increase in $[Mg^{2+}]_i$ quickly decreased to a basal concentration within about 30 s of the onset of FCCP application (Fig. 1B). The estimated $[Mg^{2+}]_i$ at rest in these cells was about 0.7 mM, which is consistent with previous reports stating that resting $[Mg^{2+}]_i$ in mammalian cells is in the sub-millimolar range [2,22].

FCCP is a protonophore that collapses the proton gradient across the mitochondrial inner membrane and induces rapid ATP depletion [23]. After FCCP application, the remaining Mg–ATP^{2-} is decomposed to ADP and Mg^{2+}. We therefore expected that one source of intracellular Mg^{2+} would be the Mg–ATP^{2-} complex

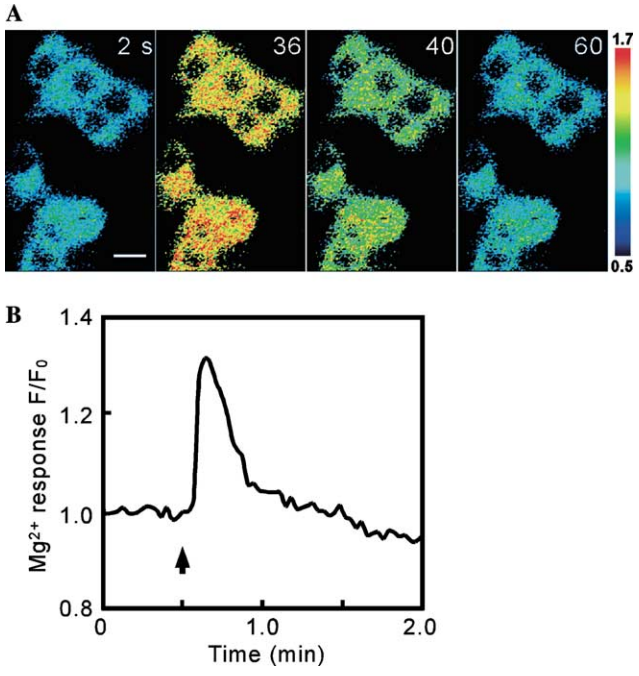

Fig. 1. FCCP induces a transient increase in PC12 cells. (A) Sequential fluorescent images of KMG-20 (10 μM)-loaded PC12 cells treated by FCCP (5 μM). Images were masked by a 3 × 3 median filter. Changes in fluorescence were mainly observed in the cytoplasm. $[Mg^{2+}]_i$ increased just after applying FCCP and decreased quickly. Times were indicated at upper right in each image (s). Bar = 10 μm. (B) This is a typical response for cells maintained in the normal solution. KMG-20 was excited at 440 nm and its emitted fluorescence acquired following passage through a 535 nm BP filter. Arrow indicates the timing of FCCP application.

existing in the cytosol. Although FCCP is generally known as an intracellular Ca^{2+} inducer [24], FCCP also increases $[Mg^{2+}]_i$ [23].

Extracellular Na^+ is necessary for the rapid elimination of intracellular Mg^{2+}

Given that FCCP induced a transient increase in $[Mg^{2+}]_i$ which was followed by a rapid decrease (Fig. 1), one would expect that if the rapid decrease was the result of the presence of an Na^+/Mg^{2+} transporter, then such an effect would not occur under Na^+-free conditions. To examine this hypothesis, the FCCP-induced increase in $[Mg^{2+}]_i$ was measured in a Na^+-free extracellular solution (Fig. 2A) which was achieved by substituting the sodium chloride in the extracellular solution for choline chloride. The increase in $[Mg^{2+}]_i$ in response to exposure to FCCP was similar in the normal (Rate of $F/F_0 = 1.80 \pm 0.58\ \text{min}^{-1}$, $n = 15$) and the Na^+-free solutions (Rate of $F/F_0 = 1.62 \pm 0.23\ \text{min}^{-1}$, $n = 14$). However, while $[Mg^{2+}]_i$ decreased rapidly after reaching a peak level in the normal solution, the rate of decrease of $[Mg^{2+}]_i$ was much slower in the Na^+-free solution. In this way, the rate of decrease in the Na^+-free solution was $0.14 \pm 0.04\ \text{min}^{-1}$ ($n = 15$) compared to that of $0.65 \pm 0.11\ \text{min}^{-1}$ ($n = 14$) in the normal solution (Fig. 2B). These results suggest that the Na^+/Mg^{2+} transporter could bring about a simultaneous influx of Na^+ and eliminate excess intracellular Mg^{2+}.

The Na^+/Mg^{2+} transporter eliminates excess Mg^{2+}

We directly illustrated an influx of Na^+ with the Na indicator, Sodium Green, in PC12 cells that was concomitant with the increase of $[Mg^{2+}]_i$. Sodium Green has high sensitivity to Na^+ and a substantially lower sensitivity to K^+ [25], and has been used successfully in rat hippocampal neurons and in guinea-pig cochlear hair cells [26,27].

In this experiment, we exposed PC12 cells to FCCP in the normal extracellular solution, and the measured change in fluorescence demonstrated that FCCP stimulation in this solution induced an increase in $[Na^+]_i$. The cells were then stimulated again with FCCP after the normal extracellular solution had been exchanged for one containing imipramine (200 μM, Fig. 3), which is a known inhibitor of the Na^+/Mg^{2+} transporter [28]. The second application of FCCP in the imipramine-containing solution induced a slower rise in $[Na^+]_i$.

It has recently been reported that FCCP induces Na^+ currents in bovine aortic endothelial cells [29]. The first rapid and the second gradual Na^+ increase can be accounted for by the induction of such Na^+ currents, however the difference in responses for the two protocols is probably caused by inhibition of the Na^+/Mg^{2+} exchange mechanism by imipramine.

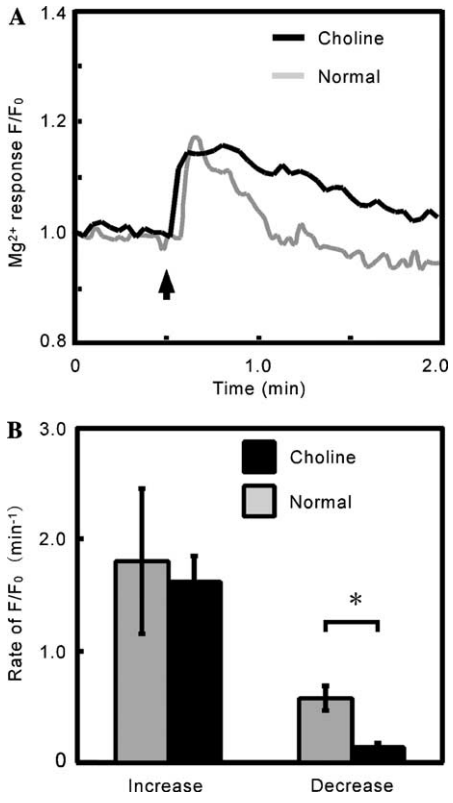

Fig. 2. $[Mg^{2+}]_i$ response under Na^+-free conditions. (A) KMG-20 (10 μM)-loaded PC12 cells were stimulated by FCCP (5 μM) in the Na^+-free solution (black line). All NaCl in extracellular solution was substituted by choline chloride. Gray line is the typical response in the normal solution. $[Mg^{2+}]_i$ increased, but its rate of decrease was slower compared with that seen when cells were maintained in the normal solution. This result indicates that extracellular Na^+ is required for Mg^{2+} extrusion. The difference can be attributed to the effect of the Na^+/Mg^{2+} transporter. Arrow indicates the timing of FCCP application. (B) Rates of increase and decrease of $[Mg^{2+}]_i$ were calculated. Although the rates of increase were similar, a significant difference exists in the rates of decrease of the fluorescence signal between the choline substituted solution and that of the normal solution ($p < 0.001$).

$[Mg^{2+}]_i$ in the PC12 cells was measured with KMG-20 in the presence of the imipramine-containing solution (Fig. 4A). The rate of increase of $[Mg^{2+}]_i$ induced by exposure of the cells to FCCP in the imipramine-containing solution was similar to that seen in the presence of the normal extracellular solution (Rate of $F/F_0 = 2.01 \pm 0.40\ \text{min}^{-1}$, $n = 9$). However, while $[Mg^{2+}]_i$ decreased quickly after its peak in the normal solution, its rate of decrease was slower in the imipramine-containing solution, and a plateau of high $[Mg^{2+}]_i$ was maintained. As such, the rate of decrease of $[Mg^{2+}]_i$ in the imipramine-containing solution was $0.05 \pm 0.02\ \text{min}^{-1}$ ($n = 9$, Fig. 4B) which was much lower than that measured in the normal extracellular solution.

These results indicate that the Na^+/Mg^{2+} transporter works to exchange Mg^{2+} for extracellular Na^+ after $[Mg^{2+}]_i$ is increased. Addition of imipramine or the

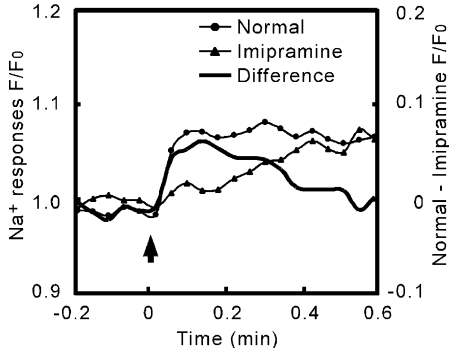

Fig. 3. Na^+ responses obtained with Na^+-sensitive fluorescent indicator. Sodium Green (10 μM)-loaded PC12 cells were stimulated by FCCP (5 μM). $[Na^+]_i$ increased rapidly in the normal solution (●), but only gradually in the imipramine-(200 μM) containing solution (▲). In the same figure, the difference between both responses has been superimposed (—). Because imipramine is an inhibitor of the Na^+/Mg^{2+} transporter, Na^+ influx by Na^+/Mg^{2+} exchange was suppressed and as such the difference between the two responses is dependent on the Na^+/Mg^{2+} transporter. Sodium Green was excited at 488 nm and its emitted fluorescence signal acquired following passage through a 535 nm BP filter. Arrow indicates the timing of imipramine application.

substitution of Na^+ with choline reduced the rate of decrease of $[Mg^{2+}]_i$, but did not completely suppress it (Figs. 2 and 4). For this reason, one could postulate that Mg^{2+} leaks from cells by non-specific ion channels and/or that Mg^{2+} is stored in intracellular organelles such as the ER or mitochondria; however analysis of these possibilities is outside of the scope of this study.

Absolute concentration of intracellular Mg^{2+} was not calculated in this study, because the concentration does not essentially concern with the rates of decrease of F/F_0. The resting $[Mg^{2+}]_i$ was calculated in our previous study, and the value is about 0.7 mM [20]. Concentration of total Mg^{2+} has been known in the range of 17–20 mM in several types of mammalian cells, and also approximately 4–5 mM of $[Mg^{2+}]_i$ was estimated to exist in the cytosol as a complex with ATP [1]. Because we used FCCP for a inhibitor of ATP synthesis at mitochondria, it could be thought that the $[Mg^{2+}]_i$ changes in Figs. 1, 2, and 4 were less than 5 mM.

The selection of Mg^{2+} indicators was very important for a study of this type because FCCP also induces an increase in intracellular Ca^{2+} [30]. Because conventional Mg^{2+} indicators have low affinity for Ca^{2+} (K_dCa (μM) of Magnesium Green, 7; Mag-fura-5, 31; and Mag-indo-1, 29) [31], we might misidentify Ca^{2+} signals as Mg^{2+} responses. While KMG-20 has a high specificity for Mg^{2+} compared with other indicators [20], it was therefore a favorable choice for the types of experiments carried out in this study.

Imaging with fluorescent indicators has many benefits for analyzing the roles of intracellular ions. However, few reports can be found in the literature concerning the Na^+/Mg^{2+} transporter as analyzed using imaging methods. Other methods such as atomic absorption

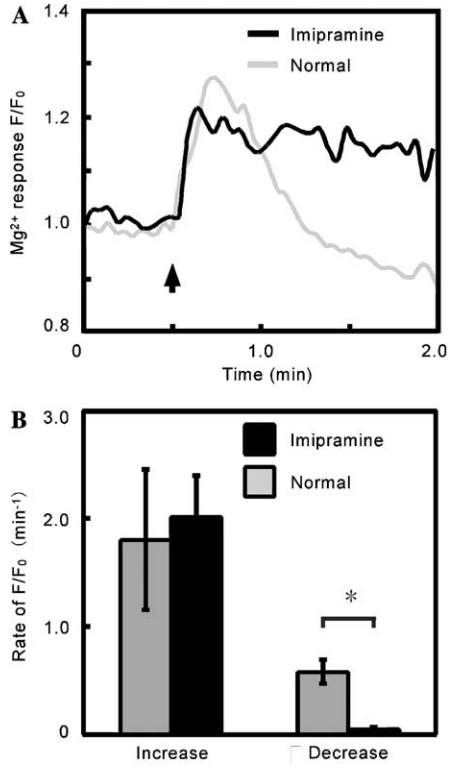

Fig. 4. $[Mg^{2+}]_i$ response in the presence of imipramine. (A) KMG-20 (10 μM)-loaded PC12 cells were stimulated by FCCP (5 μM) in the normal solution with 200 μM imipramine (imipramine-containing solution, black line). Gray line is the typical response in the normal solution. After a rapid increase in $[Mg^{2+}]_i$, the rate of decrease of the slope is low compared to that in the normal solution. The inhibition of the Na^+/Mg^{2+} transporter by imipramine resulted in $[Mg^{2+}]_i$ being sustained at a slightly higher level. Arrow indicates the timing of FCCP application. (B) Calculated rates of increase and decrease of $[Mg^{2+}]_i$. Although the rates of increase were similar, a significant difference between the imipramine-containing solution and the normal solution exists in the rates of decrease of the fluorescence signal ($p < 0.001$).

spectroscopy lose spatial resolution, as do radio-isotope techniques which are inferior to imaging techniques in terms of their temporal resolution. Mg^{2+} fluorescence imaging with KMG-20 is thus a reliable and promising method for analyzing intracellular Mg^{2+} mobilization.

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References

[1] A.M. Romani, A. Scarpa, Regulation of cellular magnesium, Front. Biosci. 5 (2000) D720–D734.
[2] H. Ebel, T. Günther, Magnesium metabolism: a review, J. Clin. Chem. Clin. Biochem. 18 (1980) 257–270.

[3] R.D. Grubbs, M.E. Maguire, Magnesium as a regulatory cation: criteria and evaluation, Magnesium 6 (1987) 113–127.
[4] B. Raju, E. Murphy, L.A. Levy, R.D. Hall, R.E. London, A fluorescent indicator for measuring cytosolic free magnesium, Am. J. Physiol. 256 (1989) C540–C548.
[5] L. Garfinkel, D. Garfinkel, Magnesium regulation of the glycolytic pathway and the enzymes involved, Magnesium 4 (1985) 60–72.
[6] R. DiPolo, G. Berberian, L. Beauge, In squid nerves intracellular Mg^{2+} promotes deactivation of the ATP-upregulated Na^+/Ca^{2+} exchanger, Am. J. Physiol. Cell Physiol. 279 (2000) C1631–C1639.
[7] V. Gaussin, P. Gailly, J.M. Gillis, L. Hue, Fructose-induced increase in intracellular free Mg^{2+} ion concentration in rat hepatocytes: relation with the enzymes of glycogen metabolism, Biochem. J. 326 (1997) 823–827.
[8] J.B. Brocard, S. Rajdev, I.J. Reynolds, Glutamate-induced increases in intracellular free Mg^{2+} in cultured cortical neurons, Neuron 11 (1993) 751–757.
[9] C. Cheng, I.J. Reynolds, Subcellular localization of glutamate-stimulated intracellular magnesium concentration changes in cultured rat forebrain neurons using confocal microscopy, Neuroscience 95 (2000) 973–979.
[10] A.K. Stout, Y. Li-Smerin, J.W. Johnson, I.J. Reynolds, Mechanisms of glutamate-stimulated Mg^{2+} influx and subsequent Mg^{2+} efflux in rat forebrain neurones in culture, J. Physiol. 492 (1996) 641–657.
[11] A. Zhang, B.T. Altura, B.M. Altura, Elevation of extracellular magnesium rapidly raises intracellular free Mg^{2+} in human aortic endothelial cells: is extracellular Mg^{2+} a regulatory cation? Front. Biosci. 2 (1997) a13–a17.
[12] H.J. Kennedy, Intracellular Mg^{2+} regulation in voltage-clamped Helix aspersa neurones measured with mag-fura-2 and Mg^{2+}-sensitive microelectrodes, Exp. Physiol. 83 (1998) 449–460.
[13] H. Gotoh, M. Kajikawa, H. Kato, K. Suto, Intracellular Mg^{2+} surge follows Ca^{2+} increase during depolarization in cultured neurons, Brain Res. 828 (1999) 163–168.
[14] T. Günther, J. Vormann, Mg^{2+} efflux is accomplished by an amiloride-sensitive Na^+/Mg^{2+} antiport, Biochem. Biophys. Res. Commun. 130 (1985) 540–545.
[15] T. Günther, J. Vormann, Characterization of Na^+/Mg^{2+} antiport by simultaneous $^{28}Mg^{2+}$ influx, Biochem. Biophys. Res. Commun. 148 (1987) 1069–1074.
[16] P.A. Tessman, A. Romani, Acute effect of EtOH on Mg^{2+} homeostasis in liver cells: evidence for the activation of an Na^+/Mg^{2+} exchanger, Am. J. Physiol. 275 (1998) G1106–G1116.
[17] C. Cefaratti, A. Romani, A. Scarpa, Characterization of two Mg^{2+} transporters in sealed plasma membrane vesicles from rat liver, Am. J. Physiol. 275 (1998) C995–C1008.
[18] C. Cefaratti, A. Romani, A. Scarpa, Differential localization and operation of distinct Mg^{2+} transporters in apical and basolateral sides of rat liver plasma membrane, J. Biol. Chem. 275 (2000) 3772–3780.
[19] A. Romani, C. Marfella, A. Scarpa, Regulation of magnesium uptake and release in the heart and in isolated ventricular myocytes, Circ. Res. 72 (1993) 1139–1148.
[20] Y. Suzuki, H. Komatsu, T. Ikeda, N. Saito, S. Araki, D. Citterio, D. Hisamoto, Y. Kitamura, T. Kubota, J. Nakagawa, K. Oka, K. Suzuki, Design and synthesis of Mg^{2+}-selective fluoroionophores based on a coumarin derivative and application for Mg^{2+} measurement in a living cell, Anal. Chem. 74 (2002) 1423–1428.
[21] L.A. Greene, A.S. Tischler, Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor, Proc. Natl. Acad. Sci. USA 73 (1976) 2424–2428.
[22] E. Kelepouris, R. Kasama, Z.S. Agus, Effects of intracellular magnesium on calcium, potassium and chloride channels, Miner. Electrol. Metab. 19 (1993) 277–281.
[23] A. Leyssens, A.V. Nowicky, L. Patterson, M. Crompton, M.R. Duchen, The relationship between mitochondrial state ATP hydrolysis $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ studied in isolated rat cardiomyocytes, J. Physiol. 496 (1996) 111–128.
[24] J.F. Buckman, I.J. Reynolds, Spontaneous changes in mitochondrial membrane potential in cultured neurons, J. Neurosci. 21 (2001) 5045–5065.
[25] H. Szmacinski, J.R. Lakowicz, Sodium Green as a potential probe for intracellular sodium imaging based on fluorescence lifetime, Anal. Biochem. 250 (1997) 131–138.
[26] J.E. Friedman, G.G. Haddad, Anoxia induces an increase in intracellular sodium in rat central neurons in vitro, Brain Res. 663 (1994) 329–334.
[27] G.D. Housley, N.P. Raybould, P.R. Thorne, Fluorescence imaging of Na^+ influx via P2X receptors in cochlear hair cells, Hearing Res. 119 (1998) 1–13.
[28] P.W. Flatman, L.M. Smith, Sodium-dependent magnesium uptake by ferret red cells, J. Physiol. 443 (1991) 217–230.
[29] K.S. Park, I. Jo, K. Pak, S.W. Bae, H. Rhim, S.H. Suh, J. Park, H. Zhu, I. So, K.W. Kim, FCCP depolarizes plasma membrane potential by activating proton and Na^+ currents in bovine aortic endothelial cells, Pflugers Arch. 443 (2002) 344–352.
[30] K. Medler, E.L. Gleason, Mitochondrial Ca^{2+} buffering regulates synaptic transmission between retinal amacrine cells, J. Neurophysiol. 87 (2002) 1426–1439.
[31] M. Zhao, S. Hollingworth, S.M. Baylor, Properties of tri- and tetracarboxylate Ca^{2+} indicators in frog skeletal muscle fibers, Biophys. J. 70 (1996) 896–916.