Deviant Nicotinic Acid Adenine Dinucleotide Phosphate (NAADP)-mediated Ca\(^{2+}\) Signaling upon Lysosome Proliferation

George D. Dickinson\(^1\), Grant C. Churchill\(^2\), Eugen Brailoiu\(^3\), and Sandip Patel\(^4\)

Received for publication, February 10, 2010
Published, JBC Papers in Press, March 15, 2010, DOI 10.1074/jbc.C110.112573

From the \(^1\)Department of Cell and Developmental Biology, University College London, London WC1E 6BT, United Kingdom, the \(^2\)Department of Pharmacology, University of Oxford, Oxford OX1 3QT, United Kingdom, and the \(^3\)Department of Pharmacology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

Accumulating evidence suggests that the endolysosomal system is a novel intracellular Ca\(^{2+}\) pool mobilized by the second messenger, nicotinic acid adenine dinucleotide phosphate (NAADP). Although lysosomes in neurons are known to proliferate in numerous neurodegenerative diseases and during the normal course of aging, little is known concerning the effect of lysosomal proliferation on Ca\(^{2+}\) homeostasis. Here, we induce proliferation of lysosomes in primary cultures of rat hippocampal neurons and PC12 cells through chronic treatment with the cathepsin inhibitor, Z-Phe-Ala-diazomethylketone. We demonstrate that lysosome proliferation increases the size of the lysosomal Ca\(^{2+}\) pool and enhances Ca\(^{2+}\) signals in response to direct cellular delivery of NAADP and glutamate, an identified NAADP-producing agonist. Our data suggest that deregulated lysosomal Ca\(^{2+}\) signaling through NAADP may contribute to neuronal dysfunction and highlight the usefulness of lysosomal hydrolase inhibition in probing NAADP action.

Changes in the concentration of cytosolic Ca\(^{2+}\) ions form the basis of a ubiquitous signal transduction pathway important for a vast number of cellular processes (1). Cytosolic Ca\(^{2+}\) levels are regulated by a tightly controlled system of Ca\(^{2+}\) channels, pumps, exchangers, and buffers (1). In the central nervous system, Ca\(^{2+}\) is essential for vital processes such as neurotransmitter release and synaptic plasticity (2). Loss of Ca\(^{2+}\) homeostasis has devastating consequences as exemplified by cell death in response to Ca\(^{2+}\) overload during glutamate toxicity (2).

Over the last 15 years, an entirely new Ca\(^{2+}\)-signaling pathway has been described that is controlled by the Ca\(^{2+}\)-mobilizing messenger, nicotinic acid adenine dinucleotide phosphate (NAADP)\(^2\) (3–6). Like the better characterized messengers, inositol 1,4,5-triphosphate and cyclic ADP-ribose, NAADP is produced upon cell stimulation and generates spatio-temporally complex Ca\(^{2+}\) signals deriving from intracellular Ca\(^{2+}\) stores (3, 4). NAADP is active in the nervous system, as evidenced by the presence of NAADP binding sites (7), an enzymatic route for NAADP metabolism (8), and the demonstration of changes in cytosolic Ca\(^{2+}\) in response to NAADP in neuronal preparations (9–14). Indeed, NAADP has been shown to regulate neurotransmission (10, 15), neurite outgrowth (11), neuronal differentiation (13), and depolarization (14). Moreover, glutamate has recently been identified as an NAADP-dependent agonist (16) linking, for the first time, the actions of NAADP to a major excitatory neurotransmitter in the brain.

In stark contrast to inositol 1,4,5-triphosphate and cyclic ADP-ribose, in most systems studied, NAADP appears to target novel Ca\(^{2+}\)-permeable channels located not on the endoplasmic reticulum (ER; an established Ca\(^{2+}\) store) but instead on acidic Ca\(^{2+}\) stores (3–5). These channels have recently been identified as the two-pore channels that localize to the endolysosomal system (17–19). This location is consistent with the many studies that have shown blockade of NAADP-mediated Ca\(^{2+}\) signals by the lysosomotropic agent, glycyrrhetinic acid, and naphthylamine β-naphthylamide (GPN) (12, 13, 16, 18, 20–24). Acidic stores of Ca\(^{2+}\) are thus a novel determinant of Ca\(^{2+}\) signals highlighting the fundamentally different mode of action of NAADP when compared with its messenger counterparts.

Lysosomes are dynamic, heterogeneous organelles with an internal pH of 4.5–5 and are best known as the terminal degradative compartments of the endocytic and autophagic pathways (25, 26). Depending on cell type, they typically occupy between 0.5 and 5% of the volume of a cell and, in neurons, are ~0.5 μm in diameter (25, 26). The critical role of lysosomes in the brain is highlighted by the numerous neurodegenerative diseases attributable to lysosome dysfunction, including Niemann-Pick disease type C, frontotemporal dementia, neuronal ceroid lipofuscinoses, Down syndrome, Creutzfeldt-Jakob, and Alzheimer, Parkinson, and Huntington disease (27). All of these diseases are characterized by impaired lysosomal function and clearance of waste products, an accumulation of lysosomes and associated autophagic vacuoles, and extensive neurodegeneration of the cortex and hippocampus. Similar lysosomal proliferation has been reported in a variety of aged cells, including neurons (28), and in cells undergoing replicative senescence (29). That lysosomes are now recognized as a readily mobilized pool of Ca\(^{2+}\) raises the intriguing possibility that their proliferation may contribute to disturbed Ca\(^{2+}\) homeostasis and thus cellular dysfunction. Indeed, recent studies have shown altered lysosomal Ca\(^{2+}\) stores in a lysosomal storage disorder (30).

\(^{*}\) This work was supported by grants from the Alzheimer’s Research Trust, Research into Ageing, and the Biotechnology and Biological Sciences Research Council (Grant BB/G013721/1 to S. P. and Grant BB/D012694/1 to G. C. C.).

\(^{†}\) The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.

\(^{1}\) To whom correspondence should be addressed. E-mail: patel.s@ucl.ac.uk.

\(^{2}\) The abbreviations used are: NAADP, nicotinic acid adenine dinucleotide phosphate; NAADP-AM, acetoxymethyl ester of NAADP; ZPAD, Z-Phe-Ala-diazomethylketone; DMSO, dimethyl sulfoxide; ER, endoplasmic reticulum; GPN, glycyrrhetinic acid, β-naphthylamide; HBS, HEPES-buffered saline; CCD, cooled coupled device; RT-PCR, reverse transcription-PCR.

© 2010 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.
In the present study, we examined the effect of proliferating lysosomes on NAADP-mediated Ca\(^{2+}\) signaling in primary cultured rat hippocampal neurons and the rat pheochromocytoma PC12 cell line. We demonstrate, for the first time, that lysosome proliferation, induced by treating cultured cells with the cathepsin B and L inhibitor Z-Phe-Ala-diazomethylketone (ZPAD), results in exaggerated NAADP-mediated Ca\(^{2+}\) signals in response to both the cell-permeant NAADP analogue NAADP-AM and the NAADP-linked neurotransmitter, glutamate.

**MATERIALS AND METHODS**

**Cell Culture**—Hippocampal neurons were isolated from 3-day-old rat pups (Sprague-Dawley, University College London breeding colony) and maintained as mixed cultures of neurons and glia, as described previously (16) with some modifications. Dissected hippocampi from two pups were finely chopped in ice-cold Ca\(^{2+}\)/Mg\(^{2+}\)-free Hank's balanced salt solution and incubated with 0.25% (w/v) Trypsin-EDTA for 7 min at 37 °C. The suspension was then washed with Hank's balanced salt solution and resuspended in advanced DMEM/F12 medium supplemented with 10% (v/v) fetal bovine serum, 2 mMM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were dissociated by passage through an 18-gauge needle and plated on glass coverslips (VWR International, 25 mm, thickness number 1; coated with 20 µg/ml poly-L-lysine) by overnight incubation at 37 °C in a humidified atmosphere of 95% air, 5% CO\(_2\). The cells were then maintained in Neurobasal\textsuperscript{TM} medium supplemented with 2% (v/v) B-27 and 2 mM L-glutamine for 6–7 days prior to experimentation. All reagents were used from Invitrogen. Neurons were identified by their phase-bright rounded appearance and processes, and their identity was confirmed during Ca\(^{2+}\) imaging by monitoring whether they responded to depolarization (see below).

PC12 cells (ATCC\textsuperscript{®} number CRL-1721\textsuperscript{TM}) were maintained in RPMI 1640 medium (Sigma) supplemented with 10% (v/v) heat-inactivated horse serum, 5% (v/v) fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin (all from Invitrogen). Prior to experimentation, cells were plated onto glass coverslips at a density of 200,000 cells/ml.

**Proliferation of Lysosomes**—Lysosome proliferation was induced using the method described by Bednarski et al. (31). 10–200 µM ZPAD (Bachem; dissolved in DMSO) was added to the culture medium of plated cells for 5–10 days and replenished with each regular medium change. DMSO was added to matched controls to a final concentration of 0.05% (v/v).

**LyoTracker Imaging**—Cells were incubated with 1 µM LysoTracker Red (Invitrogen) in HEPES-buffered saline (HBS) composed of 156 mM NaCl, 3 mM KCl, 2 mM MgSO\(_4\), 1.25 mM KH\(_2\)PO\(_4\), 2 mM CaCl\(_2\), 10 mM glucose, and 10 mM HEPES (pH 7.4) at 37 °C for 30 min in the dark. Cells were washed with HBS, and fluorescence images captured by either epifluorescence or confocal microscopy.

Epifluorescence microscopy was carried out using an Olympus IX71 inverted microscope fitted with either a 10× 0.40 UPLanApo or a 100× 1.35 NA UPLanApo oil immersion objective. Fluorescence images (emission > 590 nm) were captured with a cooled coupled device (CCD) camera following excitation from a monochromatic light source (TILL Photonics). Confocal microscopy was performed using an Axiovert 200M inverted microscope (Carl Zeiss, Inc.) equipped with a confocal scanner (LSM 510, Carl Zeiss, Inc.), a 63× 1.4 NA Plan Apochromat oil immersion objective, and a long pass 560 emission filter. In both cases, the excitation wavelength was 543 nm.

**Transmission Electron Microscopy**—Cells were fixed with 2% glutaraldehyde, 1.5% formaldehyde in 0.1 M sodium cacodylate buffer, pH 7.3, and then treated with 1% osmium, 1.5% K\(_2\)Fe(CN)\(_6\) in 0.1 M cacodylate buffer, pH 7.3, dehydrated in a graded ethanol-water series, cleared in propylene oxide, and infiltrated with agar resin. Ultrathin sections were cut using a diamond knife on a Reichert Ultracut E microtome and collected on 300 mesh grids, stained with uranyl acetate and lead citrate. Samples were viewed with a Joel 1010 transition electron microscope, and the images were recorded using a Gatan Orius CCD camera. Imagej software (32) was used to count and measure the area of lysosomes and mitochondria.

**Quantitative RT-PCR**—Total RNA was extracted using the RNeasy mini kit (Qiagen) following the manufacturer’s instructions, including the on-column digestion of DNA using the RNase-free DNase set (Qiagen). Quantitative RT-PCR was performed as described previously (17). Briefly, the Improm-II reverse transcription system (Promega) was used to prepare cDNA with oligo(dT) primers and gene-specific oligonucleotide primers corresponding to the nucleotide sequences of rat LAMP-1 (forward, 5’-GTAGCAACCTTCAAGAGG-3’; reverse, 5’-GGTCACTTGGCCACCT3’), LAMP-2 (forward, 5’-ACCTGAGCTGTGTCACCT3’; reverse, 5’-GGA GTGAGTGTCTGATAG-3’), and glyceraldehyde-3-phosphate dehydrogenase (forward, 5’-GTAAGCTTCCACCCACGGCAAG-3’; reverse, 5’-TTCTCCATGTTGAGGTACACGC-3’). RNA samples were denatured for 2 min at 94 °C and then subjected to 40 cycles of denaturation (15 s at 94 °C), annealing (30 s at 60 °C), and extension (30 s at 72 °C) with the SYBR Green JumpStart Taq ReadyMix (Sigma-Aldrich). Expression levels were normalized to glyceraldehyde-3-phosphate dehydrogenase following simultaneous amplification.

**Measurement of Cytosolic Ca\(^{2+}\) Concentration**—Cells were loaded with the fluorescent ratiometric Ca\(^{2+}\) indicator fura-2 by incubation with 2.5 µM fura-2 AM and 0.005% (v/v) pluronic acid (Invitrogen) in HBS for 30 min at room temperature in the dark. The cells were washed with HBS and mounted in a viewing chamber on the stage of the Olympus IX71 microscope described above fitted with a 20× 0.75 NA UPlanApo/340 objective. Fura-2 fluorescence (emission > 440 nm) images were captured every 3 s using a CCD camera (TILL Photonics) following alternate excitation at 340 and 380 nm delivered by a monochromator (TILL Photonics). Recordings were made for 60 s to allow the determination of the basal fluorescence ratio prior to stimulation. Cells were depolarized by the addition of K\(^{+}\)-enriched HBS (containing 40 mM K\(^{+}\) as an equimolar substitution of Na\(^{+}\)). Experiments were also performed in nominally Ca\(^{2+}\)-free HBS, which contained 1 mM EGTA in place of Ca\(^{2+}\) where indicated.

During each experiment, 30–60 cells were analyzed by calculating the mean 340/380 nm ratio at each time point within
user-defined regions of interest using TILLvisION software. The magnitude of each response upon stimulation ($\Delta R$) was determined by subtracting the mean of the basal ratios for 30–60 s prior to stimulation from the peak fluorescence ratio. Cells were considered responsive to a given agonist if the change in $\Delta R$ was 20% or greater than the basal ratio.

**RESULTS AND DISCUSSION**

Previous studies have demonstrated that lysosomal proliferation can be induced both in vitro (31, 33, 34) and in vivo (35, 36) by pharmacological inhibition of select lysosomal hydrolases. Here, we treated primary cultured rat hippocampal neurons and PC12 cells with ZPAD for 10 or 5 days, respectively, and examined its effect on lysosome morphology. First, we compared the distribution of the weak fluorescent base, LysoTracker Red, in live cells. Epifluorescence and confocal imaging indicated that ZPAD treatment had a dramatic effect on LysoTracker staining in both cell types. As shown in the epifluorescence images in Fig. 1A, 50 μM ZPAD induced dense perinuclear labeling in neurons consistent with a marked up-regulation of acidic compartments. From wide field epifluorescence images of cultures of PC12 cells, LysoTracker Red staining was increased by ZPAD in a concentration-dependent manner (Fig. 1B, top panels). At the highest concentration tested (200 μM), there was an ~10-fold increase in fluorescence (supplemental Fig. S1A). Higher magnification images of individual cells indicated that ZPAD increased the number of labeled structures (Fig. 1B, bottom panels). This was confirmed in both cell types by confocal microscopy (Fig. 1C). Second, we examined ZPAD-treated cells by electron microscopy. The effect of 50 μM ZPAD on the morphology of a typical neuron and PC12 cell is shown in Fig. 1, D and E. Higher magnification images are shown in supplemental Fig. S2. As evident, ZPAD increased the number and size of electron-dense lysosomes (Fig. 1, D and E). Pooled data from several cells quantifying lysosomal number and area are shown in Fig. 1F. Analysis of frequency distributions of lysosome size indicated that ZPAD treatment induced a concentration-dependent rightward shift (supplemental Fig. S1, B and C). In contrast, ZPAD had little effect on the number, size (Fig. 1, D–F), or morphology (supplemental Fig. S2) of mitochondria. These results, showing selective proliferation of lysosomes, are consistent with previous studies conducted on cultured hippocampal slices treated with ZPAD for 6 days (31). Finally, we examined the effects of ZPAD on transcript levels of the lysosomal proteins LAMP-1 and LAMP-2. As shown in Fig. 1G, 50 μM ZPAD induced a 2–3-fold increase in expression of the lysosomal proteins LAMP-1 and LAMP-2.
REPORT: Altered Lysosomal Ca²⁺ Homeostasis

FIGURE 2. Lysosome proliferation selectively potentiates lysosomal Ca²⁺ signals. A–D, cytosolic Ca²⁺ signals from cultures treated with either DMSO (left) or 50 μM ZPAD (right). A, responses from neurons sequentially stimulated with thapsigargin (Tg, 1 μM) and GPN (200 μM) in the absence of extracellular Ca²⁺. B and C, responses from neurons (B) and PC12 cells (C) stimulated with 250 nM and 1 μM NAADP-AM, respectively. Experiments were performed in the presence of extracellular Ca²⁺. D, responses from neurons stimulated with 10 μM glutamate (Glu) in the absence of extracellular Ca²⁺. E, responses from neurons stimulated with 40 mM K⁺ in the presence of extracellular Ca²⁺. All traces are population averages from 3–4 separate cultures. The total number of cells analyzed (n) is indicated. F, summary (mean ± S.E.) showing the magnitude of the responses (ΔR) under the various conditions.

markers. Taken together, the above analysis suggests that lysosome proliferation can be readily induced in culture by inhibition of lysosomal hydrolases.

Having successfully induced lysosome proliferation, we examined the effect of lysosome proliferation on the size of the lysosomal Ca²⁺ stores. To achieve this, cytosolic Ca²⁺ levels were measured in fura-2-loaded cells in response to GPN. GPN is a cell-permeable cathepsin C peptide substrate that, when cleaved, causes osmotic lysis of lysosomes (37). Cells were stimulated with GPN in the absence of extracellular Ca²⁺ (to prevent Ca²⁺ influx) and following treatment with thapsigargin (to deplete endoplasmic reticulum Ca²⁺ stores). Although Ca²⁺ signals in response to thapsigargin were not affected by ZPAD, those to GPN were markedly increased (Fig. 2A). Peak responses to GPN were elevated ~4-fold (Fig. 2F). These data show that ZPAD increases the size of the lysosomal Ca²⁺ store but has no effect on ER Ca²⁺ stores.

Because NAADP has been proposed to mobilize acidic Ca²⁺ stores, we next examined the effect of lysosomal proliferation on NAADP-mediated Ca²⁺ signals. As a charged molecule, NAADP, like other second messengers, is incapable of passing through the plasma membrane. The recent development of a cell-permeant acetoxyethyl ester of NAADP (NAADP-AM) (38) has provided an excellent pharmacological tool for directly investigating the effect of NAADP on Ca²⁺ signals, circumventing the more complicated and/or invasive techniques of liposome delivery and microinjection (14, 30, 39). The addition of NAADP-AM evoked robust Ca²⁺ signals in both cell types (Fig. 2, B and C), whereas the addition of NAADP had no effect (data not shown). In neurons, a typical “bell-shaped” concentration-effect relationship for NAADP-AM was observed (supplemental Fig. S3) as reported previously in cortical neurons (38).

We compared NAADP-AM-mediated cytosolic Ca²⁺ changes in cells treated with 50 μM ZPAD or DMSO. As shown in Fig. 2, B and C, ZPAD treatment potentiated Ca²⁺ signals in both neurons and PC12 cells. The mean peak Ca²⁺ response (ΔR) of neurons and PC12 cells to NAADP-AM following ZPAD treatment was 1.5–2-fold higher (Fig. 2F). Taken together, the above data indicate that the proliferation of lysosomes by ZPAD results in an increase in the mobilizable NAADP-sensitive intracellular Ca²⁺ pool consistent with the increases in total Ca²⁺ content (Fig. 2A). These data also provide independent evidence that NAADP-sensitive Ca²⁺ channels are expressed on the acidic stores of these cell types (13, 16).

Our knowledge concerning the physiological cues that engage the NAADP pathway is currently limited, particularly in the nervous system. We have recently shown that NAADP-sensitive Ca²⁺ stores in neurons are recruited through NAADP production by the neurotransmitter glutamate (16). We therefore investigated the impact of lysosome proliferation on Ca²⁺ signaling evoked by this neurotransmitter. As shown in Fig. 2D, ZPAD treatment substantially potentiated Ca²⁺ signals in response to glutamate. Ca²⁺ signals in response to 10 μM ATP and 100 μM carbachol, however, were not significantly affected (data not shown). Finally, we compared Ca²⁺ signals in response to chemical depolarization (with high K⁺) in the presence of extracellular Ca²⁺ to stimulate Ca²⁺ entry through voltage-sensitive Ca²⁺ channels. Ca²⁺ signals were similar in control and ZPAD-treated cultures, further attesting to the specificity of ZPAD in modulating cytosolic Ca²⁺ levels (Fig. 2E). These data, summarized in Fig. 2F, indicate that lysosome proliferation selectively potentiates Ca²⁺ signals in response to an identified NAADP-coupled neurotransmitter.

To conclude, we show that pharmacological inhibition of lysosome hydrolases induces marked proliferation of the lysosomal system and that this is associated with an increase in the total size of the acidic Ca²⁺ store and exaggerated Ca²⁺ signals.
in response to direct intracellular delivery of NAADP and to the NAADP-forming neurotransmitter, glutamate. ZPAD-treated cells are likely to provide an extremely tractable system for defining changes in Ca\(^{2+}\) signaling and other processes associated with neurodegenerative diseases and aging in which lysosomal proliferation features. Indeed, deregulated Ca\(^{2+}\) signaling through Ca\(^{2+}\) stores has already been associated with neuronal dysfunction, although this is ascribed to changes in the ER (40, 41). Potential changes in lysosomal Ca\(^{2+}\) signaling, however, are not mutually exclusive because NAADP is thought to provide a “trigger” release of Ca\(^{2+}\), which is then amplified by Ca\(^{2+}\)-sensitive ER Ca\(^{2+}\) channels (3–6). Thus, altered activity of NAADP-sensitive channels is likely to have profound effects on downstream Ca\(^{2+}\) signals and Ca\(^{2+}\)-dependent function. Exaggerated NAADP-mediated signaling as a potential contributor to homeostasis loss therefore represents a novel mechanism underlying deregulated Ca\(^{2+}\) signals. Additionally, although the use of the lysosomotropic agent, GPN, which decreases lysosomal number, has been widely used to study neuronal dysfunction, although this is ascribed to changes in lysosomal Ca\(^{2+}\) stores has already been associated with neuronal dysfunction, although this is ascribed to changes in the ER (40, 41). Potential changes in lysosomal Ca\(^{2+}\) signaling, however, are not mutually exclusive because NAADP is thought to provide a “trigger” release of Ca\(^{2+}\), which is then amplified by Ca\(^{2+}\)-sensitive ER Ca\(^{2+}\) channels (3–6). Thus, altered activity of NAADP-sensitive channels is likely to have profound effects on downstream Ca\(^{2+}\) signals and Ca\(^{2+}\)-dependent function. Exaggerated NAADP-mediated signaling as a potential contributor to homeostasis loss therefore represents a novel mechanism underlying deregulated Ca\(^{2+}\) signals. Additionally, although the use of the lysosomotropic agent, GPN, which decreases lysosomal number, has been widely used to probe NAADP action (12, 13, 16, 18, 20–24), we suggest that ZPAD, which has the opposite effect on lysosomes, could prove equally useful.

Acknowledgments—We thank Mark Turmaine and Mary Rahman for assistance with the electron microscopy and quantitative PCR and Dev Churamani, Robert Hooper, and Chi Li for useful discussions.

REFERENCES

1. Berridge, M. J., Lipp, P., and Bootman, M. D. (2000) Nat. Rev. Mol. Cell Biol. 1, 11–21
2. Berridge, M. J. (1998) Neuron 21, 13–26
3. Patel, S. (2004) Biol. Cell 96, 19–28
4. Lee, H. C. (2005) J. Biol. Chem. 280, 33693–33696
5. Yamasaki, M., Churchill, G. C., and Galione, A. (2005) FEBS J. 272, 4598–4606
6. Guse, A. H., and Lee, H. C. (2008) Sci. Signal 1, re10
7. Patel, S., Churchill, G. C., Sharp, T., and Galione, A. (2000) J. Biol. Chem. 275, 36495–36497
8. Berridge, G., Cramer, R., Galione, A., and Patel, S. (2002) Biochem. J. 365, 295–301
9. Bak, J., White, P., Timár, G., Missiaen, L., Genazzani, A. A., and Galione, A. (1999) Curr. Biol. 9, 751–754
10. Chameau, P., Van de Vrede, Y., Fossier, P., and Baux, G. (2001) Pflugers Arch. 443, 289–296
11. Brailoiu, E., Hoard, J. L., Filipeano, C. M., Brailoiu, G. C., Dun, S. L., Patel, S., and Dun, N. J. (2005) J. Biol. Chem. 280, 5646–5650
12. Heidemann, A. C., Schipke, C. G., and Kettenmann, H. (2005) J. Biol. Chem. 280, 35630–35640
13. Brailoiu, E., Churamani, D., Pandey, V., Brailoiu, G. C., Tuluc, F., Patel, S., and Dun, N. J. (2006) J. Biol. Chem. 281, 15923–15928
14. Brailoiu, G. C., Brailoiu, E., Parkesh, R., Galione, A., Churchill, G. C., Patel, S., and Dun, N. J. (2009) Biochem. J. 419, 91–97
15. Brailoiu, E., Patel, S., and Dun, N. J. (2003) Biochem. J. 373, 313–318
16. Pandey, V., Chuang, C. C., Lewis, A. M., Aley, P. K., Brailoiu, E., Dun, N. J., Churchill, G. C., and Patel, S. (2009) Biochem. J. 422, 503–512
17. Brailoiu, E., Churamani, D., Cai, X., Schlau, M. G., Brailoiu, G. C., Gao, X., Hooper, R., Boulware, M. J., Dun, N. J., Marchant, J. S., and Patel, S. (2009) J. Cell Biol. 186, 201–209
18. Calcraft, P. J., Ruas, M., Pan, Z., Cheng, X., Arredouani, A., Hao, X., Tang, J., Rietdorf, K., Teboul, L., Chuang, K. T., Lin, P., Xiao, R., Wang, C., Zhu, Y., Lin, Y., Wyatt, C. N., Parrington, J., Ma, J., Evans, A. M., Galione, A., and Zhu, M. X. (2009) Nature 459, 596–600
19. Brailoiu, E., Hooper, R., Cai, X., Brailoiu, G. C., Kebeler, M. V., Dun, N. J., Marchant, J. S., and Patel, S. (2010) J. Biol. Chem. 285, 2897–2901
20. Churchill, G. C., Okada, Y., Thomas, J. M., Genazzani, A. A., Patel, S., and Galione, A. (2002) Cell 111, 703–708
21. Yamasaki, M., Masgrau, R., Morgan, A. J., Churchill, G. C., Patel, S., Ashcroft, S. J., and Galione, A. (2004) J. Biol. Chem. 279, 7234–7240
22. Duman, J. G., Chen, L., Palmer, A. E., and Hille, B. (2006) Traffic 7, 859–872
23. Menteney, A., Burdakov, A., Charpentier, G., Petersen, O. H., and Cancela, J. M. (2006) Curr. Biol. 16, 1931–1937
24. Soares, S., Thompson, M., White, T., Isbell, A., Yamasaki, M., Prakash, Y., Lund, F. E., Galione, A., and Chini, E. N. (2007) Am. J. Physiol. Cell Physiol. 292, C227–C239
25. Holtzman, E. (1989) Lysosomes, Plenum Press, New York
26. Saftig, P. (2005) Lysosomes, Landes Bioscience/Springer-Verlag, Georgetown, TX
27. Nixon, R. A., Yang, D. S., and Lee, J. H. (2008) Autophagy 4, 590–599
28. Lynch, G., and Bi, X. (2003) Neurochem. Res. 28, 1725–1734
29. Hwang, E. S., Yoon, G., and Kang, H. T. (2009) Cell Mol. Life Sci. 66, 2509–2524
30. Lloyd-Evans, E., Morgan, A. J., He, X., Smith, D. A., Elliott-Smith, E., Silence, J. D., Churchill, G. C., Schuchman, E. H., Galione, A., and Platt, F. M. (2008) Nat. Med. 14, 1247–1255
31. Bednarski, E., Ribak, C. E., and Lynch, G. (1997) J. Neurosci. 17, 4006–4021
32. Abramoff, M. D., Magelhaes, P. J., and Ram, S. J. (2004) Biophotonics Int. 11, 36–42
33. Bi, X., Zhou, J., and Lynch, G. (1999) Exp. Neurol. 158, 312–327
34. Yong, A. P., Bednarski, E., Gall, C. M., Lynch, G., and Ribak, C. E. (1999) Exp. Neurol. 157, 150–160
35. Ivy, G. O., Schottler, F., Wenzel, J., Baudry, M., and Lynch, G. (1984) Science 226, 985–987
36. Ivy, G. O. (1992) Ann. N.Y. Acad. Sci. 674, 89–102
37. Berg, T. O., Stromhaug, P. E., Berg, T., and Seglen, P. O. (1994) Eur. J. Biochem. 221, 595–602
38. Parkesh, R., Lewis, A. M., Aley, P. K., Arredouani, A., Rossi, S., Taveiras, R., Vasudevan, S. R., Rosen, D., Galione, A., Dowden, J., and Churchill, G. C. (2008) Cell Calcium 43, 531–538
39. Macgregor, A., Yamasaki, M., Rakovic, S., Sanders, L., Parkesh, R., Churchill, G. C., Galione, A., and Terrar, D. A. (2007) J. Biol. Chem. 282, 15302–15311
40. Bezprozvanny, I., and Mattson, M. P. (2008) Trends Neurosci. 31, 454–463
41. Green, K. N., and LaFerla, F. M. (2008) Neuron 59, 190–194