Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 336 (2005) 157–162
BBRC
www.elsevier.com/locate/ybbrc

Hyperosmotic stress stimulates inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate formation independently of bis-diphosphoinositol tetrakisphosphate modulation ☆

Xavier Pesesse [a,*], Alexandre Leyman [a], Tomas Luyten [b], Ludwig Missiaen [b], Christophe Erneux [a]

[a] *Interdisciplinary Research Institute (IRIBHM), Université Libre de Bruxelles, Campus Erasme, 808 Route de Lennik, 1070 Bruxelles, Belgium*
[b] *Laboratorium voor Fysiologie, K.U. Leuven, Campus Gasthuisberg O/N, 49 Herestraat, 3000 Leuven, Belgium*

Received 1 August 2005
Available online 18 August 2005

Abstract

Hyperosmotic stress induces water diffusion out of the cell, resulting in cell shrinkage, and leading to DNA damage, cell cycle arrest, and cytoskeletal reorganization. A previous report showed that low concentrations of sorbitol (200 mM) could increase up to 25-fold the concentration of $InsP_8$ in animal cells. Here, we investigate the effect of sorbitol (200 mM) on the inositol 1,4,5-trisphosphate ($InsP_3$) and inositol 1,3,4,5-tetrakisphosphate ($InsP_4$) pathway. A 3- to 4-fold increase in $InsP_3$ and $InsP_4$ levels after sorbitol challenge was observed. It was prevented by the phospholipase C inhibitor U-73122 but was insensitive to the MAP kinase inhibitor U0126. We also observed an increase in the free intracellular [Ca^{2+}] and the occurrence of Ca^{2+} oscillations in response to sorbitol. A hyperosmotic stress could therefore affect the levels of both hyperphosphorylated inositol phosphates and $InsP_3$/$InsP_4$-signalling molecules.

Keywords: Inositol 1,4,5-trisphosphate; 1,3,4,5-Tetrakisphosphate; Diphosphoinositol polyphosphates; Hyperosmotic stress

It is well known that stress such as UV, osmotic shock, heat shock, and cytokines are external stimuli that are able to activate different cell signalling mechanisms (for review [1]). Unbalance in osmolality across the plasma membrane leads to major changes in cell size and ions, metabolites or water transport. Osmotic shock can induce cell proliferation too by increasing interleukin expression [2]. Cells react by activating their intracellular protein kinase cascade or by rapid synthesis of compatible osmolytes like *myo*-inositol, sorbitol or taurine [3].

In yeast, the osmoregulatory signalling pathway has been well studied involving scaffolding proteins and protein kinase phosphorylation steps. In mammalian, one of the most important pathways involved in the transduction of signals from the cell membrane to the nucleus is the mitogen/stress-activated protein kinase (MAPK, p38, and JNK) pathway [4]. Activation of this pathway has been shown to enhance cell resistance to a variety of stress signals [5].

In mammalian cells, little is known about the effects of osmotic stress on other signalling pathways. The rapid hydrolysis of phosphatidylinositol 4,5-bisphosphate to $InsP_3$ and 1,2-diacylglycerol plays an essential role in cellular physiology [6]. Particularly, $InsP_3$ mediates

☆ *Abbreviations:* $InsP_3$, inositol 1,4,5-trisphosphate; $InsP_4$, inositol 1,3,4,5-tetrakisphosphate; $InsP_6$, inositol hexakisphosphate; $InsP_7$, diphosphoinositol pentakisphosphate; $InsP_8$, bis-diphosphoinositol tetrakisphosphate; PLC, phospholipase C.

* Corresponding author. Fax: +32 2 5554655.
E-mail address: xpesesse@ulb.ac.be (X. Pesesse).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.08.049

the release of Ca^{2+} from internal Ca^{2+} stores. $InsP_3$ is also a precursor in the formation of a wide family of other derived molecules such as $InsP_4$ which has been shown to play a crucial role in T lymphocytes [7]. The metabolism of $InsP_3$ is also producing higher phosphorylated inositol phosphates such as diphosphoinositol pentakisphosphate ($InsP_7$) and bis-diphosphoinositol tetrakisphosphate ($InsP_8$) found in all organisms or cells from fungi to humans [8]. They have the property to contain high energetic phosphate bonds and it has recently been shown that $InsP_7$ could serve as phosphate donor to nuclear proteins of *Saccharomyces cerevisiae* [9]. Other functions of diphosphoinositol polyphosphates have been proposed including telomere length regulation and messenger RNA transport control [10,11]. Recently, we have investigated the effect of a sorbitol challenge on the metabolism of inositol polyphosphates. We have shown that a low concentration (0.2 M) of sorbitol, which does not provoke cell death within 24 h, induces an important increase in the levels of intracellular $InsP_8$ (up to 25-fold) associated with a decrease of its precursor $InsP_7$, suggesting the activation of an unknown $InsP_7$ kinase. Moreover, this $InsP_8$ production was sustained upon long-term sorbitol challenge and regulated mainly by the MAP (ERK1/2 and p38) kinase protein pathway [12].

Here, we report on the stimulatory effect of hyperosmotic stress on $InsP_3$ and $InsP_4$ levels together with their consequences for the Ca^{2+} response after sorbitol challenging. The kinetics of production of these two inositol phosphates (i.e., $InsP_3$ and $InsP_4$) were quite slow and lasted for at least 2 h as compared to the classical short-term kinetics of IP_3 production (within minutes) in response to ATP.

Materials and methods

Materials. [^{3}H]*myo*-Inositol was from Amersham. [^{3}H]$InsP_3$, [^{3}H]$InsP_4$, and [^{3}H]$InsP_6$ used as standards to calibrate the HPLC SAX column were from Perkin-Elmer. Sorbitol was from Sigma. DDT_1-MF_2 cells were provided by Dr. H. De Smedt (K.U. Leuven, Leuven, Belgium). $InsP_6$ was from Sigma.

Cell labelling and inositol phosphate analysis. DDT_1-MF_2 cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% CO_2. Culture medium was supplemented with 1 μM sodium pyruvate, 100 U/ml penicillin G, and 100 μg/ml streptomycin. Cells were harvested by brief trypsinization and 6×10^5 cells were seeded in 6 cm tissue culture plates and labelled with 50 μCi/ml [^{3}H] *myo*-inositol for 3 days. After completion of the labelling protocol, the cells were pre-incubated with different inhibitors or DMSO as control and stimulated by ATP or challenged by sorbitol. Cells were quenched with ice-cold 0.6 M perchloric acid containing 0.2 mg/ml $InsP_6$ and neutralized with 1 M K_2CO_3. The perchlorate precipitates were pelleted by centrifugation (10,000*g*, 5 min). Inositol phosphates were separated by HPLC (4.6×250 mm Partisphere SAX) at a flow rate of 1 ml/min using a protocol adapted to determine the complete profile of inositol phosphates: it was reached by mixing Buffer A (1 mM Na_2EDTA) and Buffer B (Buffer A containing 1.3 M $(NH_4)_2HPO_4$, pH 3.85, with H_3PO_4): 0–10 min, 0% B; 10–30 min, 0–45% B; 30–100 min, 45–100% B; 100–110 min, 100% B). To assess the relative levels of the $InsP_3$ or $InsP_4$ isomers, the protocol described above was modified by using a gradient of buffer A (1 mM Na_2EDTA) and Buffer B (Buffer A plus 2 M $NH_4H_2PO_4$, pH 3.35, with H_3PO_4): 0–1 min, 0% B; 1–6 min, 0–50% B; 6–56 min, 50–100% B; 56–61 min, 100% B). Fractions of 1 ml were collected and mixed with scintillation liquid for radioactivity measurements. When possible, the radioactivity was also directly determined with an online detector from Raytest.

Ca^{2+} measurements. Single-cell [Ca^{2+}] measurements were performed using a laser-scanning MRC-1024 UV system (Bio-Rad, Hertfordshire, UK) attached to an inverted Nikon Diaphot 300 epifluorescence microscope with a CF Fluor $40 \times (NA = 1.3)$ oil immersion objective. Briefly, after removing the culture medium and washing the cells, they were incubated for 30 min with 5 μM Indo-1-AM dissolved in a modified Krebs solution containing 135 mM NaCl, 5.9 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 11.6 mM Hepes, and 11.5 mM glucose (pH 7.3). The cells were then further incubated for 1–1.5 h in the absence of Indo-1. During the experiment, the cells were continuously superfused from a pipette placed on top of the cell. The scan speed was 1 images per 1.5 s and the temperature 25 °C. The signals were not averaged.

Results

Hyperosmotic stress increases $InsP_3$ and $InsP_4$ levels in intact cells

We recently reported that DDT_1-MF_2 cells challenged with 0.2 M sorbitol for 30 min show a dramatic increase in the levels of $InsP_8$ (up to 25-fold) [12]. In response to sorbitol, the mechanism underlying that increase was due in part to MAP kinase activation. We therefore questioned whether low phosphorylated inositol phosphates, i.e., $InsP_3$ and $InsP_4$ were also modified by a hyperosmotic stress. In those experiments, $InsP_3$ and $InsP_4$ levels were increased by at least 3-fold when cells were challenged for 30 min with 0.2 M sorbitol (Figs. 1A and B). No changes in the levels of $InsP_5$ or $InsP_6$ were observed (not shown). As expected, levels of $InsP_8$ were increased about 10-fold in this particular experiment.

We compared the effect of ATP and sorbitol on the kinetics of $InsP_3$/$InsP_4$ formation. DDT_1-MF_2 cells were stimulated for different time periods by 100 μM ATP (Fig. 2A). A transient increase in $InsP_3$ level (up to 30-fold) was observed with a maximum at 15 s. It returned to basal level after 3 min. $InsP_4$ level was increased up to 3-fold at 5 or 10 s and stayed elevated up to 3 min (Fig. 2A). In the case of sorbitol, the increase of $InsP_3$ started at 5 min, was maximal at 30 min, and declined after 2 h. $InsP_4$ level was increased with a maximum at 60 min and decreased at 45% of its control value after 2 h. Finally, the Ins(1,3,4)P_3 level was maintained at a 10-fold higher level than basal until at least 30 min (Fig. 2B). The data suggest that Ins(1,3,4)P_3 formation mainly results from the dephosphorylation of $InsP_4$ by the type-I $InsP_3$/$InsP_4$ 5-phosphatase [13].

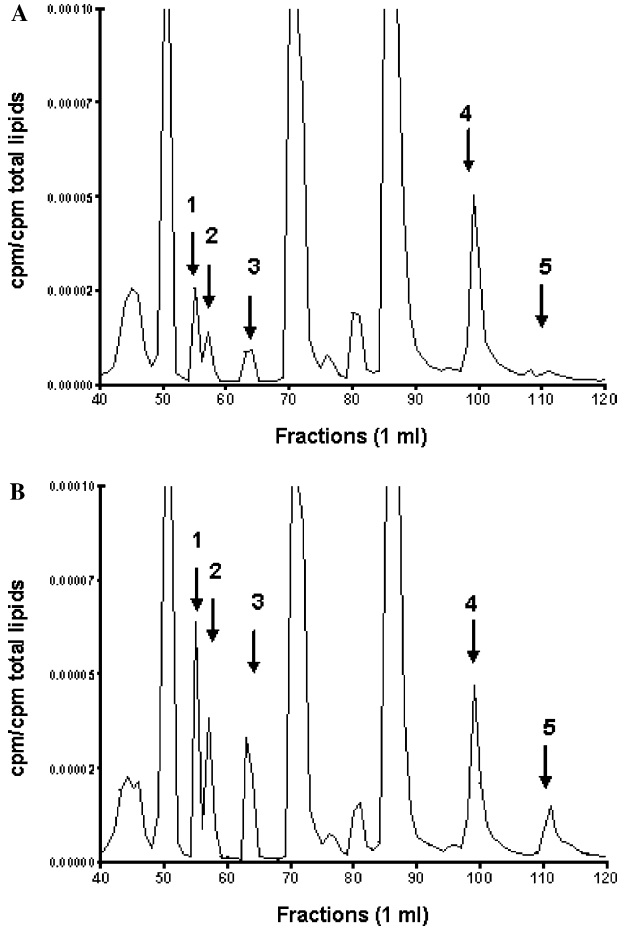

Fig. 1. Inositol polyphosphate levels in DDT_1-MF_2 cells challenged by sorbitol. [^{3}H]Inositol labelled cells were incubated in the presence of 0.2 M sorbitol (B) or water (A) for 30 min. Tritiated inositol phosphates were extracted as described in Materials and methods and analysed by anion exchange HPLC. The radioactivity (in ordinate) was normalized with respect to the total radioactivity present in the lipid fraction. Data presented are representative of two different experiments. 1, Ins(1,3,4)P_3; 2, InsP_3; 3, InsP_4 pool; 4, InsP_7; 5, InsP_8.

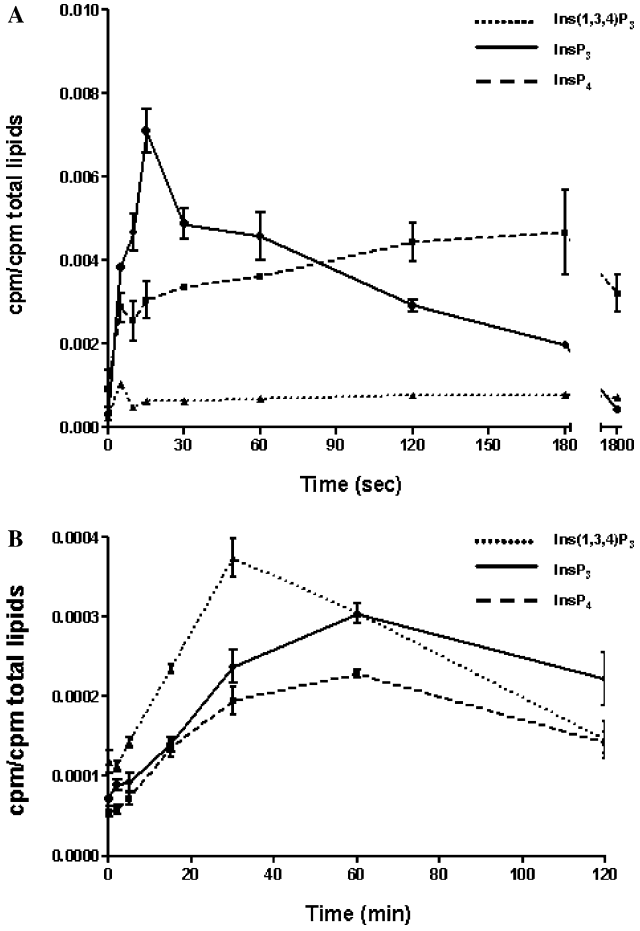

Fig. 2. Time-course of inositol polyphosphate production in DDT_1-MF_2 cells in response to different stimuli. Inositol phosphate levels were stimulated by 100 μM ATP (A) or 0.2 M sorbitol (B). They were determined as referred to in Materials and methods and in Fig. 1. Data are means of triplicate ± SEM.

PLC and Ser/Thr protein phosphatases but not MAP kinases are involved in this process

We have shown that the stimulatory effect of sorbitol on InsP_3 level was inhibited by pre-treatment with 50 μM of the PLC inhibitor U-73122 (Fig. 3), suggesting that the effect of sorbitol on InsP_3 results from an effect (direct or indirect) on PLC. In many types of cells, MAP kinases ERK1/2 have important roles in differentiation, survival, and proliferation [4]. To investigate whether members of the ERK family are involved in the osmotic stress effect on InsP_3/InsP_4, we used U0126, a MEK inhibitor which specifically attenuates the activation of ERKs. The net increase in InsP_8 formation was prevented in the presence of U0126 as reported before (Table 1 and [12]). In contrast in the presence of sorbitol, InsP_3 or InsP_4 levels were not affected by U0126, suggesting that the MAP kinase pathway was not involved. Therefore, the fact that InsP_3 upregulation was not affected by U0126 (in contrast to InsP_8 levels) argues against the hypothesis that InsP_8 could serve as an intermediate in the effect of sorbitol on PLC activity.

A low concentration of sorbitol leads to Ca^{2+} oscillations

Ca^{2+} is an intracellular second messenger involved in the control of a wide range of effects such as proliferation, differentiation, transcription or muscle contraction [14]. It has been shown before that osmotic cell swelling induced by hypotonic stress is associated with a raise in free intracellular [Ca^{2+}] [15]. Since our data on hyperosmotic stress indicated an activation of PLC, we questioned whether this would also produce a Ca^{2+} response.

DDT_1-MF_2 cells from a Coverglass chamber have been challenged with 0.2 M sorbitol in 1.5 mM Ca^{2+}-containing Krebs medium. By single cell measurements, we observed a Ca^{2+} response assessed by fluorescence

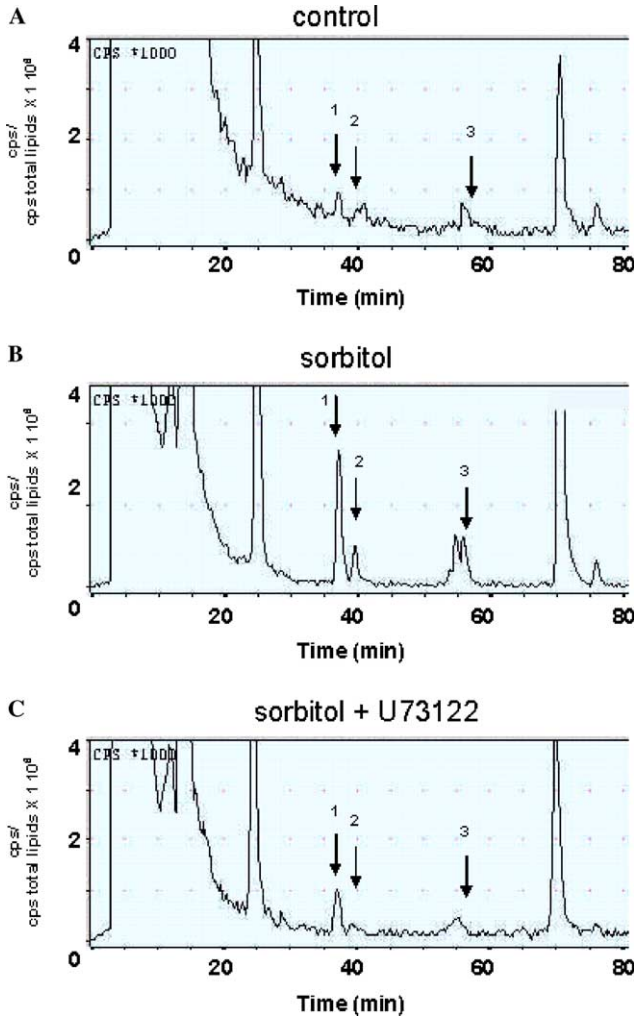

Fig. 3. Effect of a PLC inhibitor on inositol polyphosphate production. DDT_1-MF_2 cells were pre-incubated (B) or not (A,C) with 50 μM U73122 for 1 h and challenged for 30 min (B,C) or not (A) with 0.2 M sorbitol. Soluble inositol phosphates were isolated and analysed by HPLC. Data presented are from one representative experiment out of two. 1, Ins(1,3,4)P_3; 2, $InsP_3$; 3, $InsP_4$.

Table 1
Effect of U0126 on inositol phosphate levels after osmotic stress

	DMSO		U0126	
	No sorbitol	30 min	No sorbitol	30 min
$InsP_8$	0.97 ± 0.09	12.14 ± 0.29	0.79 ± 0.03	6.93 ± 0.90
$InsP_3$	9.42 ± 0.42	31.37 ± 0.85	8.79 ± 0.03	29.01 ± 0.26
$InsP_4$	4.74 ± 0.07	25.48 ± 0.05	5.90 ± 0.64	25.19 ± 1.04

$InsP_3$, $InsP_4$, and $InsP_8$ levels were determined in DDT_1-MF_2 cells challenged (30 min) or not (no sorbitol) with 0.2 M sorbitol during 30 min. Results are expressed as a ratio of specific cpm normalized with respect to the total lipid value (cpm/lipid cpm × 10^{-4}). The data are means of duplicate ± SD.

changes of Indo 1 at 405 and 460 nm (Figs. 4A and B) and the occurrence of Ca^{2+} oscillations (Fig. 4C). At higher concentrations of sorbitol, we could clearly observe an increase in Ca^{2+} response that returned to basal

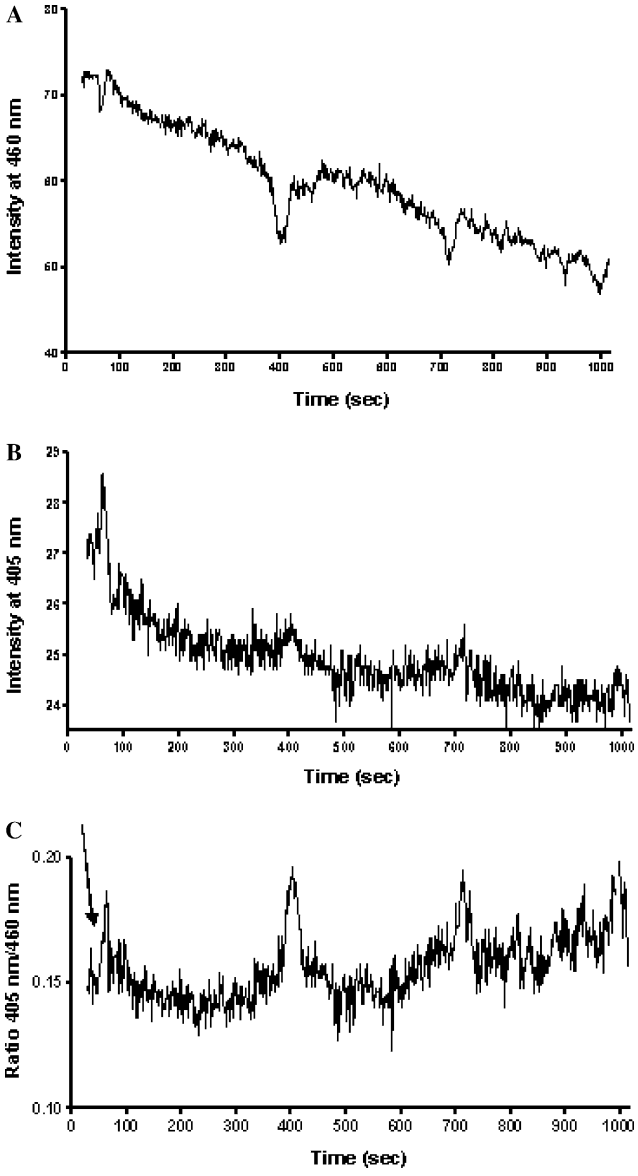

Fig. 4. Fluorescence and [Ca^{2+}] changes in response to sorbitol in DDT_1-MF_2 cells. Time-course of the emitted fluorescence at a wavelength of 460 nm (A) or 405 nm (B) after addition of 0.2 M sorbitol (arrow). (C) Ratio between the emission at 405 and at 460 nm, which corresponds to the free [Ca^{2+}].

level after 5–10 min (data not shown). The Ca^{2+} peaks induced by 0.2 M sorbitol were of smaller intensity as compared to those induced by ATP (100 μM). The upregulation of $InsP_3$ was also more pronounced with ATP (100 μM) as compared to sorbitol (0.2 M).

Discussion

Cellular stress has been largely studied in plants and mammalian cells the last 10 years [16]. However, the effect of stress on phosphoinositide signalling is not yet fully understood and subject to controversy. For

example, it has been shown that mechanical stress could mobilize Ca^{2+} by an $InsP_3$-insensitive way in vascular smooth muscle cells [17] or by an $InsP_3$-sensitive pathway in THP-1 cells [18]. In addition, a wide range of stresses including heat shock, hypotonic stress or oxidative stress can raise the level of intracellular Ca^{2+} [16,19]. It is, however, not clear whether this always involves an increase in PLC activity.

Recently, we showed that the amount of $InsP_8$ could be increased up to 25-fold following a 30 min hyperosmotic stress. The regulation of this effect was mediated by the MAP kinase pathway which is, to our knowledge, an unusual way to control soluble inositol phosphates. The concentration of sorbitol used in those experiments did not affect the viability of the DDT_1-MF_2 cells, even after 24 h of treatment [12]. We proposed that $InsP_8$ was a sensor of hyperosmotic stress that could mediate other signalling pathways. A molecular dissection making a link between highly phosphorylated inositol phosphates and $InsP_3$ has been obtained in mice lacking the IPMK (also know as IPK2). Cells derived from those knockout mice show the absence of higher phosphorylated inositol phosphates as compared to normal cells [20].

Despite the fact that it has been reported that PLC could be activated by short-term osmotic stress in other cells [21,22], it is surprising that long-term effects of hyperosmotic challenge can lead to an increase in the formation of $InsP_3$ and $InsP_4$ in a sustained manner. We demonstrated here that 30 min incubation with 0.2 M sorbitol increases by 3- and 4-fold the amount of $InsP_3$ and $InsP_4$ in DDT_1-MF_2 cells. We have shown that this regulation is PLC dependent since it was prevented by the presence of U73122, a specific PLC inhibitor. However, in contrast to $InsP_8$, it was not dependent on the MAP kinase pathway. It is well known that most agonists which increase PLC activity do not modify the levels of $InsP_8$. Our data suggest that sorbitol-induced increase in the formation of $InsP_3$ and $InsP_8$ follows two different and independent mechanisms.

The effect of sorbitol differs from the classical ATP-induced PLC activation mechanism. In our experiments, ATP (100 μM) increases the $InsP_3$ level up to 35-fold within 15 s and the concentration drops down after 3 min to nearly basal level. On the contrary, sorbitol increases the amount of $InsP_3$ by 3- to 4-fold in a sustained manner up to at least 2 h. This slower kinetics of $InsP_3$ production are not unique in the inositol phosphate field: for example, the levels of Ins(1,4,5,6)P_4 or Ins(3,4,5,6)P_4 are known to be increased after a long time stimulation [23]. This prolonged production of $InsP_3$ could reflect two different manners of PLC activation (or the involvement of different isoforms) leading to different Ca^{2+} responses as suggested in neonatal rat cardiomyocytes [24]. Moreover, it has been shown that an intact cytoskeleton was required for agonist-induced Ca^{2+} signalling in NIH3T3 cells [25]. In another model in bone marrow-derived macrophage precursors, M-CSF in a model of cell differentiation induces the translocation of PLC-γ2 to the cell membrane [26]. Therefore, in the case of sorbitol challenging, modulation of cytoskeleton may also affect signal transduction, PLC activation, and the generation Ca^{2+} oscillations.

Acknowledgments

We thank Drs. Colette Moreau, Humbert De Smedt, Fabrice Vandeput, Jing Zhang, Katrien Backers, and Nathalie Paternotte for many helpful discussions. This work was supported by grants from the Fonds de la Recherche Scientifique Médicale, Action de Recherche Concertée of the Communauté Française de Belgique. This work was executed in the framework of research network IAPV-O5 (Belgium Science Policy). X.P. is Chargé de Recherche FNRS and A.L. is supported by a FRIA fellowship.

References

[1] P. Dent, A. Yacoub, J. Contessa, R. Caron, G. Amorino, K. Valerie, M.P. Hagan, S. Grant, R. Schmidt-Ullrich, Stress and radiation-induced activation of multiple intracellular signaling pathways, Radiat. Res. 159 (2003) 283–300.
[2] W.H. Loomis, S. Namiki, R.S. Ostrom, P.A. Insel, W.G. Junger, Hypertonic stress increases T cell interleukin-2 expression through a mechanism that involves ATP release, P2 receptor, and p38 MAPK activation, J. Biol. Chem. 278 (2003) 4590–4596.
[3] F. Lang, G.L. Busch, H. Volkl, The diversity of volume regulatory mechanisms, Cell Physiol. Biochem. 8 (1998) 1–45.
[4] E. Zwick, P.O. Hackel, N. Prenzel, A. Ullrich, The EGF receptor as central transducer of heterologous signalling systems, Trends Pharmacol. Sci. 20 (1999) 408–412.
[5] J. Yaglom, C. O'Callaghan-Sunol, V. Gabai, M.Y. Sherma, Inactivation of dual-specificity phosphatases is involved in the regulation of extracellular signal-regulated kinases by heat shock and hsp72, Mol. Cell. Biol. 23 (2003) 3813–3824.
[6] M.J. Berridge, R.F. Irvine, Inositol phosphates and cell signalling, Nature 341 (1989) 197–205.
[7] V. Pouillon, R. Hascakova-Bartova, B. Pajak, F. Adam, F. Bex, V. Dewaste, C. Van Lint, O. Leo, C. Erneux, S. Schurmans, Inositol 1,3,4,5-tetrakisphosphate is essential for T lymphocyte development, Nat. Immunol. 11 (2003) 1136–1143.
[8] S.B. Shears, The versatility of inositol phosphates as cellular signals, Biochim. Biophys. Acta 1436 (1998) 49–67.
[9] A. Saiardi, R. Bhandari, A.C. Resnick, A.M. Snowman, S.H. Snyder, Phosphorylation of proteins by inositol pyrophosphates, Science 306 (2004) 2101–2105.
[10] A. Saiardi, A.C. Resnick, A.M. Snowman, B. Wendland, S.H. Snyder, Inositol pyrophosphates regulate cell death and telomere length through phosphoinositide 3-kinase-related protein kinases, Proc. Natl. Acad. Sci. USA 102 (2005) 1911–1914.
[11] J.D. York, A.R. Odom, R. Murphy, E.B. Ives, S.R. Wente, A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export, Science 285 (1999) 96–100.
[12] X. Pesesse, K. Choi, T. Zhang, S.B. Shears, Signaling by higher inositol polyphosphates. Synthesis of bisdiphosphoinositol

tetrakisphosphate ("$InsP_8$") is selectively activated by hyperosmotic stress, J. Biol. Chem. 279 (2004) 43378–43381.
[13] F. De Smedt, A. Boom, X. Pesesse, S.N. Schiffmann, C. Erneux, Post-translational modification of human brain type I inositol-1,4,5-trisphosphate 5-phosphatase by farnesylation, J. Biol. Chem. 271 (1996) 10419–10424.
[14] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling, Nat. Rev. Mol. Cell Biol. 1 (2000) 11–21.
[15] L. Missiaen, H. De Smedt, J.B. Parys, I. Sienaert, S. Vanlingen, G. Droogmans, B. Nilius, R. Casteels, Hypotonically induced calcium release from intracellular calcium stores, J. Biol. Chem. 271 (1996) 4601–4604.
[16] L. Xiong, K.S. Schumaker, J.K. Zhu, Cell signaling during cold, drought, and salt stress, Plant Cell 14 (Suppl.) (2002) S165–S183.
[17] M.J. Mohanty, X. Li, Stretch-induced Ca^{2+} release via an IP_3-insensitive Ca^{2+} channel, Am. J. Physiol. Cell Physiol. 283 (2002) C456–C462.
[18] N. Ashida, H. Takechi, T. Kita, H. Arai, Vortex-mediated mechanical stress induces integrin-dependent cell adhesion mediated by inositol 1,4,5-trisphosphate-sensitive Ca^{2+} release in THP-1 cells, J. Biol. Chem. 278 (2003) 9327–9331.
[19] E. Bakondi, M. Gonczi, E. Szabo, P. Bai, P. Pacher, P. Gergely, L. Kovacs, J. Hunyadi, C. Szabo, L. Csernoch, L. Virag, Role of intracellular calcium mobilization and cell-density-dependent signaling in oxidative-stress-induced cytotoxicity in HaCaT keratinocytes, J. Invest. Dermatol. 121 (2003) 88–95.
[20] J.P. Frederick, D. Mattiske, J.A. Wofford, L.C. Megosh, L.Y. Drake, S.T. Chiou, B.L. Hogan, J.D. York, An essential role for an inositol polyphosphate multikinase, Ipk2, in mouse embryogenesis and second messenger production, Proc. Natl. Acad. Sci. USA 102 (2005) 8454–8459.
[21] E. Ueno, T. Haruta, T. Uno, I. Usui, M. Iwata, A. Takano, J. Kawahara, T. Sasaoka, O. Ishibashi, M. Kobayashi, Potential role of Gab1 and phospholipase C-γ in osmotic shock-induced glucose uptake in 3T3-L1 adipocytes, Horm. Metab. Res. 33 (2001) 402–406.
[22] D.M. Cohen, S.R. Gullans, W.W. Chin, Urea signaling in cultured murine inner medullary collecting duct (mIMCD3) cells involves protein kinase C, inositol 1,4,5-trisphosphate (IP_3), and a putative receptor tyrosine kinase, J. Clin. Invest. 97 (1996) 1884–1889.
[23] J. Mishra, U.S. Bhalla, Simulations of inositol phosphate metabolism and its interaction with $InsP_3$-mediated calcium release, Biophys. J. 83 (2002) 1298–1316.
[24] E.A. Woodcock, C.J. Mitchell, T.J. Biden, Phospholipase C delta(1) does not mediate Ca^{2+} responses in neonatal rat cardiomyocytes, FEBS Lett. 546 (2003) 325–328.
[25] C.M. Pedrosa Ribero, J. Reece, J.W. Putney Jr., Role of the cytoskeleton in calcium signaling in NIH 3T3 cells, J. Biol. Chem. 272 (1997) 26555–26561.
[26] M.T. Dearden-Badet, G. Mouchiroud, Re-distribution of phospholipase C gamma 2 in macrophage precursors is mediated by the actin cytoskeleton under the control of the Src kinases. Cell Signal. (2005), in press.