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Inhibition of protein tyrosine phosphatase 1B by reactive oxygen species leads to maintenance of Ca^{2+} influx following store depletion in HEK 293 cells

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Abstract

Depletion of inositol 1,4,5 trisphosphate-sensitive Ca^{2+} stores generates a yet unknown signal, which leads to increase in Ca^{2+} influx in different cell types [J.W. Putney Jr., A model for receptor-regulated calcium entry, Cell Calcium 7 (1986) 1–12]. Here, we describe a mechanism that modulates this store-operated Ca^{2+} entry (SOC). Ca^{2+} influx leads to inhibition of protein tyrosine phosphatase 1B (PTP1B) activity in HEK 293 cells [L. Sternfeld, et al., Tyrosine phosphatase PTP1B interacts with TRPV6 in vivo and plays a role in TRPV6-mediated calcium influx in HEK293 cells, Cell Signal 17 (2005) 951–960]. Since Ca^{2+} does not directly inhibit PTP1B, we assumed an intermediate signal, which links the rise in cytosolic Ca^{2+} concentration and PTP1B inhibition. We now show that Ca^{2+} influx is followed by generation of reactive oxygen species (ROS) and that it is reduced in cells preincubated with catalase. Furthermore, Ca^{2+}-dependent inhibition of PTP1B can be abolished in the presence of catalase. H_2O_2 (100 μM) directly added to cells inhibits PTP1B and leads to increase in Ca^{2+} influx after store depletion. PP1, an inhibitor of the Src family tyrosine kinases, prevents H_2O_2-induced Ca^{2+} influx.

Our results show that ROS act as fine tuning modulators of Ca^{2+} entry. We assume that the Ca^{2+} influx channel or a protein involved in its regulation remains tyrosine phosphorylated as a consequence of PTP1B inhibition by ROS. This leads to maintained Ca^{2+} influx in the manner of a positive feedback loop.

Keywords: Store-operated Ca^{2+} influx; ROS; PTP1B; Src family kinases; Tyrosine phosphorylation; Mitochondria

1. Introduction

Depletion of inositol 1,4,5-trisphosphate (IP_3)-sensitive calcium stores leads to "capacitative" or "store-operated Ca^{2+} influx" (SOC) into pancreatic acinar cells [1] and in other cell types (for review, see Putney et al. [2]). The signal, by which SOC channels are opened, is however still unknown. Previously, we have shown that protein tyrosine phosphatase 1B (PTP1B) modulates store-operated Ca^{2+} influx in cells of the pancreatic acinar cell line AR42J and in HEK 293 cells [3]. In untransfected HEK 293 cells as well as in cells transfected with TRPV6 and/or co-transfected with PTP1B, the constitutive Ca^{2+} entry was not altered in the presence of the tyrosine phosphatase inhibitor DMHV. However, following depletion of intracellular Ca^{2+} stores, endogenous store-operated as well as TRPV6-mediated Ca^{2+} entry were increased in the presence of the tyrosine phosphatase inhibitor DMHV [3,4] and TRPV6 was tyrosine phosphorylated under these conditions [4]. We have demonstrated that Ca^{2+} influx following store depletion and the increase in cytosolic Ca^{2+} concentration led to inhibition of PTP1B activity in both, untransfected cells and cells transfected with TRPV6 [4]. Since PTP1B is not directly inhibited by Ca^{2+} [5] we assumed that Ca^{2+} influx activated a regulatory mechanism that finally led to inhibition

Abbreviations: ER, endoplasmic reticulum; PM, plasma membrane; SERCA, Ca^{2+} ATPase of endo(sarco)plasmic reticulum; SOCC, store-operated Ca^{2+} channel

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of PTP1B and consequently to increase in Ca^{2+} influx as long as tyrosine phosphorylation of the endogenous Ca^{2+} influx channel or of TRPV6, respectively, was maintained [4].

It was the aim of the present study to examine the regulatory mechanism, which is activated by Ca^{2+} influx and which results in transient inhibition of PTP1B. We have considered the Ca^{2+} signaling pathways, which play a role in the physiological stimulation of cells and which could be related to regulation of PTP1B activity [5–7]. Ca^{2+} is a messenger in different signaling pathways in response to cellular stimulation. Receptor-mediated stimulation of phospholipase C causes production of IP_3 and diacyl glycerol (DAG) in many cells. IP_3 releases Ca^{2+} from intracellular stores and DAG activates protein kinases C in Ca^{2+}-dependent or Ca^{2+}-independent ways, which have been suggested to inhibit PTP1B [8,9]. Furthermore, increase in cytosolic free Ca^{2+} concentrations also leads to stimulation of phospholipase A_2 [10–12], of NAD(P)H oxidases with generation of reactive oxygen species (ROS) and of nitric oxide synthase (NOS) with subsequent nitric oxide (NO^-) generation [13]. Inhibition of PTP1B by ROS due to reversible oxidation of cysteine 215 in the catalytic center of PTP1B has been described [14,15].

Our data provide evidence that Ca^{2+} influx following depletion of Ca^{2+} stores induces generation of ROS and that ROS inhibit PTP1B activity. Ca^{2+}-dependent inhibition of PTP1B activity is abolished and store-operated Ca^{2+} influx is reduced in cells preincubated with catalase. Furthermore, we show that the addition of an external source of ROS (H_2O_2) causes inhibition of PTP1B activity in HEK 293 and pancreatic AR42J cells and an increase in Ca^{2+} influx following store depletion. This increase is abolished in the presence of the Src family kinase inhibitor PP1.

Our data suggest that the cascade of events, which is engendered by depletion of intracellular Ca^{2+} stores by thapsigargin (tg) or acetylcholine (ACh) and followed by Ca^{2+} influx results in ROS generation and subsequent inhibition of PTP1B. We assume that this causes attenuated tyrosine dephosphorylation of target proteins involved in Ca^{2+} influx, which leads to maintenance of store-operated Ca^{2+} entry into the cell.

2. Materials and methods

2.1. Chemicals

Fura-2 AM, BAPTA-AM, 2′,7′-dichlorodihydrofluorescein diacetate (H_2-DCFDA) and thapsigargin were purchased from *Invitrogen* (*Molecular Probes*, Germany), carbonyl cyanide 3-chlorophenylhydrazone (CCCP), diphenyleneiodonium chloride (DPI), 1-oleoyl-2-acetylglycerol (OAG), GF-109203X, bis-(*N*,*N*-dimethyl-hydroxamido) hydrooxovanadate (DMHV), 12-(2-cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-50-oxo-5H-indolo(2,3-*a*)pyrrolo(3,4-*c*)-carbazole (GOE 6976), 1,2-dioctanoyl-*sn*-glycerol (DOG), apocynin and acetylcholine were from *Calbiochem* (Germany), 2-amino-ethyldiphenyl borate (2-APB), phorbol 12-myristate 13-acetate (PMA) and arachidonic acid (AA) were purchased from *AXXORA* (Germany), Dulbecco's modified Eagle's medium (DMEM), fetal calf serum and penicillin/streptomycin were obtained from *PAA Laboratories* (Germany). Bovine serum albumin (BSA) and all other chemicals (analytical grade) were from *Sigma* (Germany).

2.2. Buffers

Buffer A (in mM): NaCl 135, KCl 5, $MgCl_2$ 1, HEPES 20, glucose 10 (without Ca^{2+}), pH 7.4.
Buffer B (in mM): $CaCl_2$ 1.3, NaCl 135, KCl 5, $MgCl_2$ 1, HEPES 20, glucose 10, BSA 0.1%, pH 7.4.
Buffer C (in mM): NaCl 135, KCl 5, $MgCl_2$ 1, HEPES 20, glucose 10 (without Ca^{2+}), leupeptin 0.2, trypsin inhibitor 20 μg/ml, PMSF 1 μM, Triton X-100 0.2%, pH 7.4.

2.3. Cell culture

HEK 293 and AR42J cells (*ATCC*) were cultured on plastic *Petri* dishes in DMEM, supplemented with 10% fetal calf serum and penicillin/streptomycin, in a humidified atmosphere (8.5% CO_2) at 37 °C. The cells used for experiments were at a culture density of about 80%.

2.4. Determination of protein concentration

Protein concentrations of cell homogenates were determined according to Bradford [16] with bovine serum albumin as standard.

2.5. Measurement of intracellular calcium concentrations

The cells were detached from the *Petri* dish and loaded with fura-2 AM (7 μM) for 30 min at 37 °C. Thereafter cells were washed twice in *buffer B* and once in *buffer A* and transferred to an acryl stirring cuvette. Calcium measurements were performed at 37 °C in a cell suspension in the Ca^{2+} free *buffer A* at a protein concentration of 0.3 ± 0.1 mg/ml using a fluorescence spectrometer (SPEX, DM 3000) 340/380 nm excitation, 505 nm emission and slit-width 0.5 mm. Calcium was added as indicated and concentrations were calculated for each experiment according to Grynkiewicz et al. [17], using a dissociation constant (K_D) for Ca^{2+} of 224 nM.

2.6. Measurement of protein tyrosine phosphatase 1B activity

Attached cells were pretreated within the *Petri* dish according to the respective protocol as indicated in the figure legends. Reactions were stopped precisely at a particular time point by transferring *Petri* dishes to liquid nitrogen until complete freezing. Cells were then collected into ice-cold