Acta Neurobiol. Exp. 1997, 57: 353

Sphingosine modulates Ca^{2+} signals *via* phospholipase C dependent pathway in glioma C6 cells

Rafał Czajkowski, Paweł Sabała and **Jolanta Barańska**

Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Email: baranska@nencki.gov.pl

INTRODUCTION AND METHODS. Sphingosine, as a natural constituent of cells and potent protein kinase C inhibitor, attracts considerable experimental attention (1,2). Apart from PKC-dependent activities, sphingosine has been reported to act on intracellular Ca^{2+} stores, causing inositol(1,4,5)triphosphate formation and Ca^{2+} mobilization, however the molecular mechanism of this action is not well understood (3). Sphingosine modulates calcium signals evoked by thapsigargin, a selective inhibitor of Ca^{2+}-ATPase, pumping Ca^{2+} into the endoplasmatic reticulum (ER) (4). The aim of this study was to examine the mechanism of sphingosine action on intracellular calcium stores in glioma C6 cells. In this type of cells, the major features of the membrane transport mechanisms seem to be similar to normal glial cells (5). Cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% v/v calf serum, penicilin (50 IU/ml), streptomycin (50 µg/ml) and 2 mM L-glutamine under humified atmosphere of 5% CO_2. Intracellular Ca^{2+} level was measured using fluorescent dye fura-2 and digital video imaging system, as described in (4). Cells were preincubated for 30 min before the experiment with 10 mM neomycin, phospholipase C (PLC) and phospholipase D (PLD) inhibitor, or 5 min before the experiment with 150 mM ethanol. Control cells remained untreated. One min before the experiment cells were washed with medium containing 0,5 mM EGTA to remove extracellular calcium. Each trace shown in Figures represents a mean value from one representative experiment and each experiment was conducted at least three times.

RESULTS AND DISCUSSION. Sphingosine at the concentration of 100 µM is depleting ER calcium stores, causing a rise in intracellular calcium ranging from 50 to 100 nM (Fig. 1B). It also decreases Ca^{2+} response caused by 100 nM thapsigargin (Fig. 1A,B). Preincubation with 10 mM neomycin, a high affinity ligand for PIP_2, which inhibits PLC and PLD activitity in brain, partially eliminates the effect of sphingosine on thapsigargin-elicited Ca^{2+} signal (Fig. 1C). This effect is not observed after preincubation with 150 mM ethanol (Fig. 1D), which acts as a substrate for PLD, blocking its normal activity by production of phosphatidylethanol instead phosphatidic acid (1). These results suggest that sphingosine may act on intracellular Ca^{2+} stores and stimulate Ca^{2+} mobilization *via* process mediated rather by PLC than by PLD activation.

This work was supported by Committee for Scientific Research, Poland, grant 6PO4A01011.

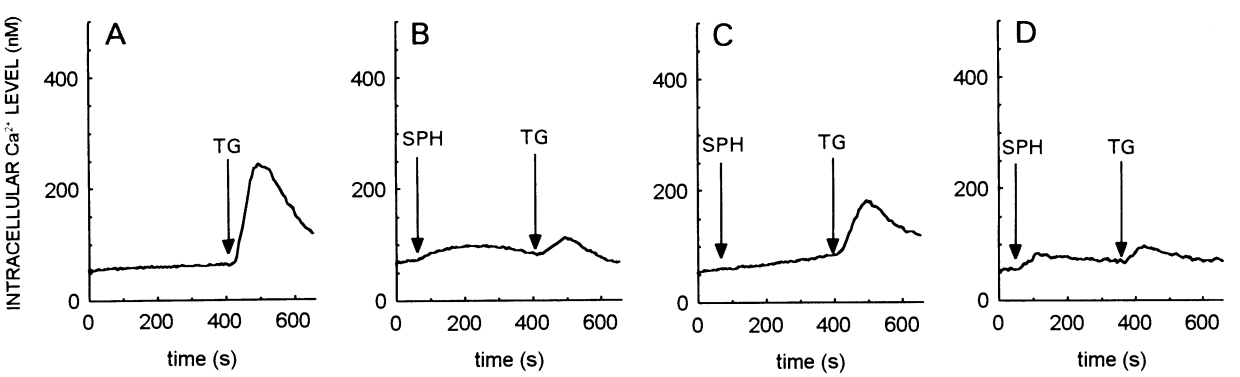

Fig. 1. Efect of 100 µM sphingosine (SPH) on on intracellular Ca^{2+} level and on Ca^{2+} signals elicited by 100 nM thapsigargin (TG) in glioma C6 cells in the absence of extracellular Ca^{2+} without (B) and with preincubation with neomycin (C) or ethanol (D).

1. Divecha N., Irvine R.F. (1995) Cell 80: 269-278.
2. Spiegel S., Merrill A.H. Jr (1996) FASEB J. 10: 1388-1397.
3. Chao C.P., Laulerderkind S.J., Ballou L.R. (1994) J. Biol. Chem. 269: 5849-5856.
4. Sabała P., Wiktorek M., Czarny M., Chaban V., Barańska J. (1996) Acta Neurobiol. Exp. 56: 507-513.
5. Brismar T. (1995) Glia 15: 231-243.

Accepted 25 October 1997