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Sphingosine stimulates calcium mobilization in rat parotid acinar cells

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In fura-2-loaded parotid acinar cells, 50–200 μM sphingosine induced an increase in cytosolic Ca^{2+} ($[Ca^{2+}]_i$). When extracellular Ca^{2+} was chelated by EGTA, 50 μM sphingosine failed to increase $[Ca^{2+}]_i$, but 100 or 200 μM sphingosine induced a slight and transient increase in $[Ca^{2+}]_i$. The addition of $LaCl_3$ to the medium resulted in the same effect as chelation of extracellular Ca^{2+}. When cells were incubated in low Ca^{2+} medium containing sphingosine, and extracellular Ca^{2+} was subsequently added, a rapid increase in $[Ca^{2+}]_i$ depending on the concentration of sphingosine was shown. In low Ca^{2+} medium, a slight increase in $[Ca^{2+}]_i$ induced by high concentrations of sphingosine was not shown after the transient increase in $[Ca^{2+}]_i$ elicited by methacholine. Inhibitors of protein kinase C, H-7 and K252a, did not mimic the effect of sphingosine on $[Ca^{2+}]_i$. These results suggest that sphingosine stimulates Ca^{2+}-influx and further stimulates the release of Ca^{2+} from agonist-sensitive intracellular pools by a mechanism that is independent of protein kinase C.

Sphingosine; Ca^{2+} mobilization; Protein kinase C (rat parotid)

1. INTRODUCTION

Sphingosine, the basic unit of sphingolipids, has been suggested as one of the endogenous modulators of cell functions [1,2]. This molecule and related derivatives have been reported to inhibit protein kinase C activation in vitro [3]. Similarly, various events mediated by protein kinase C such as secretion and aggregation by agonists in platelets [4], oxidative burst in neutrophiles [5] and differentiation of HL-60 cells [6] are inhibited by sphingosine. On the other hand, in pituitary cells, thyrotropin-releasing hormone binding to membranes was inhibited by sphingosine independently of protein kinase C [7]. In A431 human epidermoid carcinoma cells and chinese hamster ovary cells, sphingosine increased the affinity and number of receptors for epidermal growth factor via a protein kinase C-independent mechanism [8].

Rat parotid acinar cells have been used extensively as an excellent model for the study of a signaling system related to Ca^{2+} mobilization [9,10]. In the present work, the effect of sphingosine on the changes in $[Ca^{2+}]_i$ in rat parotid acinar cells was investigated using the intracellular Ca^{2+} indicator, fura-2. The results indicate that sphingosine stimulates the increase in $[Ca^{2+}]_i$. The mechanism is mostly dependent upon the presence of extracellular Ca^{2+} and is independent of protein kinase C. The role of sphingosine on Ca^{2+} mobilization in parotid acinar cells is suggested.

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2. MATERIALS AND METHODS

2.1. *Materials*

Fura-2/AM was obtained from Dojindo Lab. (Japan). H-7 (1-(5-isoquinolinesulfonyl)-2-methylpiperadine) was from Seikagaku Kogyo (Japan). Collagenase (*Clostridium histolyticum*) was from Boehringer Mannheim (Germany). Other reagents were obtained from Sigma (USA).

2.2. *Preparation of parotid acinar cells*

Parotid glands were removed from male Sprague–Dawley rats (200–250 g) anaesthetized by sodium pentobarbital (50 mg/kg). Dispersed acinar cells were prepared by using trypsin and collagenase as described previously [11]. The cells were suspended at a concentration of 5–7 × 10^6 cells/ml in a Krebs–Ringer bicarbonate solution, containing NaCl (116 mM), KCl (5.4 mM), $MgSO_4$ (0.8 mM), $CaCl_2$ (1.8 mM), NaH_2PO_4 (0.96 mM), $NaHCO_3$ (25 mM), glucose (11.1 mM) and 1% bovine serum albumin (BSA), and incubated at 37°C in O_2/CO_2 (19 : 1). The cell preparations were over 90% viable, as determined by Trypan blue exclusion.

2.3. *Determination of $[Ca^{2+}]_i$*

$[Ca^{2+}]_i$ was determined with fura-2 essentially as described previously [12,13]. Briefly, the cells, suspended in a Krebs–Ringer bicarbonate solution containing 0.5% BSA, were incubated with fura-2/AM (2 μM) for 45 min at 37°C. The fura-2 loaded cells were washed (50 g for 5 min) twice, resuspended in a HEPES-buffered Krebs–Ringer solution containing NaCl (120 mM), KCl (5.4 mM), $MgSO_4$ (0.8 mM), $CaCl_2$ (1 mM), glucose (11.1 mM), HEPES (20 mM, pH 7.4) and 0.2% BSA, and kept at room temperature. To examine the effect of extracellular Ca^{2+}, fura-2-loaded cells were washed by centrifugation (50 × g for 5 min), and resuspended in a fresh Ca^{2+}-free HEPES-buffered Krebs–Ringer solution containing 1 mM EGTA in a quartz cuvette just before use. Sphingosine was dissolved in ethanol and added (the concentration of ethanol in the medium was 0.3%). The fluorescence of fura-2-loaded cells was measured with a CAF-100 spectrofluorimeter (Nihon Bunkou, Japan) with excitation at 340 nm and 380 nm and emission at 500 nm. $[Ca^{2+}]_i$ was calculated from the measurement of the ratio of fluorescence intensities [14]. Leakage of fura-2 was negligible by the addition of 50 μM $MnCl_2$ [15].

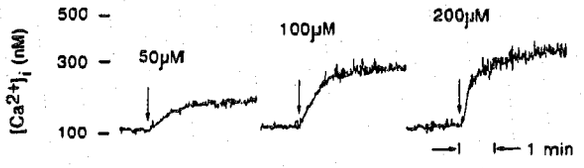

Fig. 1. The effect of sphingosine on $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+}. Fura-2-loaded parotid acinar cells were suspended with 50 μM, 100 μM and 200 μM sphingosine. The addition of sphingosine is indicated by arrows.

3. RESULTS AND DISCUSSION

Fig. 1 depicts a representative of the effect of 50 μM, 100 μM or 200 μM sphingosine on $[Ca^{2+}]_i$ in fura-2-loaded parotid acinar cells. At each concentration, sphingosine remarkably induced the elevation in $[Ca^{2+}]_i$, and the level reached a plateau during the initial 1-min stimulation. The concentration dependency of sphingosine on the changes in $[Ca^{2+}]_i$ is shown in Fig. 2.

Effect of extracellular Ca^{2+} on the increase in $[Ca^{2+}]_i$ induced by sphingosine was examined. As shown in Fig. 3, when cells were suspended in Ca^{2+}-free medium containing 1 mM EGTA (low Ca^{2+} medium), 50 μM sphingosine failed to increase $[Ca^{2+}]_i$. However, 100 μM and 200 μM sphingosine induced a very slight and transient increase in $[Ca^{2+}]_i$ even at a low extracellular Ca^{2+} concentration. These results suggest that the increase in $[Ca^{2+}]_i$ induced by sphingosine is mostly due to the influx of extracellular Ca^{2+}, and further induces the release of Ca^{2+} from intracellular pools.

The Ca^{2+} influx induced by sphingosine was further confirmed. As shown in Fig. 3, when cells were suspended with sphingosine in low Ca^{2+} medium for 3 min and extracellular Ca^{2+} was subsequently added, a

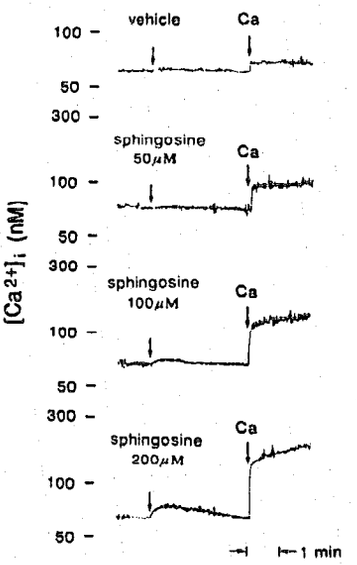

Fig. 3. The effect of sphingosine on $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. Fura-2-loaded parotid acinar cells were suspended with 50 μM, 100 μM and 200 μM sphingosine in Ca^{2+}-free medium containing 1 mM EGTA, and 3 min later, 3 mM Ca^{2+} was added to the medium.

rapid increase in $[Ca^{2+}]_i$ was induced. The increase in $[Ca^{2+}]_i$ depended on the concentration of suspended sphingosine (Fig. 4). In the other study on Ca^{2+} influx, the effect of $LaCl_3$, which blocks Ca^{2+} influx in parotid cells [9], was examined. When $LaCl_3$ was added to the medium, 50 μM sphingosine had no effect and 100 μM sphingosine resulted in a slight and transient increase in $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+} (Fig. 5), which showed the same results as shown by the depletion of extracellular Ca^{2+} as shown in Fig. 3. These results support the fact that sphingosine stimulates Ca^{2+} influx.

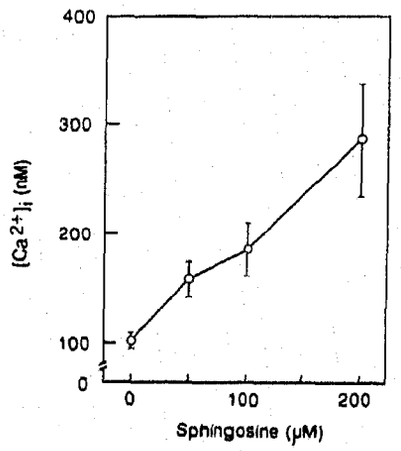

Fig. 2. Concentration-dependent effect of sphingosine on $[Ca^{2+}]_i$. The values of $[Ca^{2+}]_i$ in the cells incubated with sphingosine for 1 min are depicted. Results are means ± SE from 4–6 experiments.

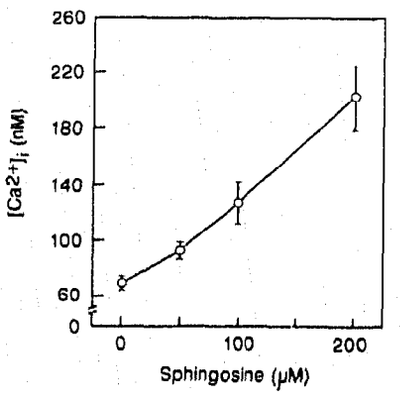

Fig. 4. The increase in $[Ca^{2+}]_i$ as a result of the addition of extracellular Ca^{2+}. After pre-incubation with sphingosine for 3 min in low Ca^{2+} medium, the addition of 3 mM extracellular Ca^{2+} increased $[Ca^{2+}]_i$. The values of $[Ca^{2+}]_i$ at 1 min after the addition of Ca^{2+} are depicted. Results are means ± SE from 4–6 experiments.

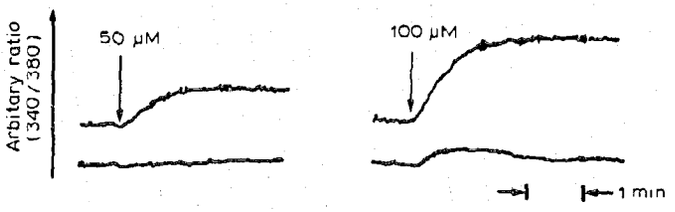

Fig. 5. The effect of sphingosine on $[Ca^{2+}]_i$ in the absence or the presence of $LaCl_3$. In the medium without (upper) or with (lower) 1 mM $LaCl_3$, cells were suspended with 50 μM or 100 μM sphingosine.

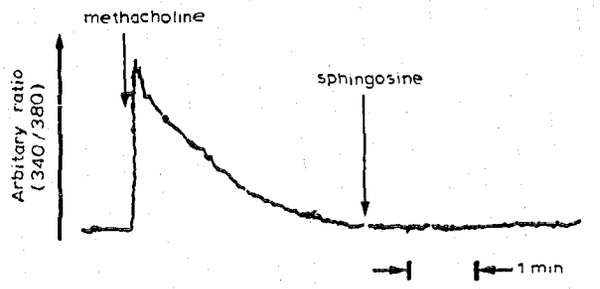

Fig. 6. No effect of sphingosine on $[Ca^{2+}]_i$ after stimulation with methacholine in the absence of extracellular Ca^{2+}. The cells were stimulated by 100 μM sphingosine after stimulation with 100 μM methacholine.

As for the slight increase in $[Ca^{2+}]_i$ induced by sphingosine, no increase in $[Ca^{2+}]_i$ was shown when 100 μM sphingosine was added to a low Ca^{2+} medium after a transient increase in $[Ca^{2+}]_i$ elicited by methacholine (Fig. 6). Hence, under these conditions, methacholine emptied the agonist-sensitive Ca^{2+} pools. This implies that sphingosine stimulates Ca^{2+} release from agonist-sensitive intracellular pools.

In many types of cells, the effect of sphingosine has been considered to attribute to the inhibition of protein kinase C activity [1–6]. If the effect of sphingosine on $[Ca^{2+}]_i$ in parotid cells was due to the inhibition of protein kinase C, other inhibitors of protein kinase C probably induce a similar effect on $[Ca^{2+}]_i$. Therefore, the effect of other protein kinase C inhibitors, H-7 and K252a, on $[Ca^{2+}]_i$ were examined. However, neither 50–100 μM H-7, nor K252a induced an increase of $[Ca^{2+}]_i$ in fura-2-loaded parotid acinar cells (data not shown).

In parotid acinar cells, activation of Ca^{2+}-mobilizing receptors such as muscarinic, cholinergic, α-adrenergic and substance P receptors results in the increase in $[Ca^{2+}]_i$ [9,10], and the increase persists in the absence of extracellular Ca^{2+} [12,13]. The agonists produce an increase in $[Ca^{2+}]_i$ even in low Ca^{2+} medium as induced by methacholine (Fig. 6). We demonstrate in this paper that sphingosine induced an increase in $[Ca^{2+}]_i$ and the effect was mostly dependent on extracellular Ca^{2+}. Therefore, it is unlikely that the increase in $[Ca^{2+}]_i$ by sphingosine is due to the activation of the Ca^{2+}-mobilizing receptors.

The increase in $[Ca^{2+}]_i$ induced by sphingosine consisted of extracellular Ca^{2+} influx and a slight release of Ca^{2+} from intracellular pools. The Ca^{2+} influx is considered to be a process of refilling the empty intracellular Ca^{2+} pools after the release stimulated by agonists [16], because the elevation of $[Ca^{2+}]_i$ was transient and smaller than that seen in the presence of extracellular Ca^{2+} when parotid cells were stimulated by Ca^{2+}-mobilizing agonists in the absence of extracellular Ca^{2+} [12,13]. As mechanisms, capacitive Ca^{2+} entry [16,17] and the role of inositol 1,3,4,5-tetrakisphosphate [18] have been demonstrated. Therefore, it may be possible that sphingosine relates to these mechanisms as a kind of modulator of the Ca^{2+} influx.

The release of Ca^{2+} from intracellular pools is stimulated by inositol 1,4,5-trisphosphate (IP_3) induced by receptor activation [9,10]. We previously demonstrated that sphingosine induced IP_3 formation [19]. However, the increase of IP_3 was much slower than that of $[Ca^{2+}]_i$. Furthermore, the IP_3 formation induced by sphingosine, even 200 μM sphingosine was completely blocked by the chelation of extracellular Ca^{2+}. Therefore, the release of Ca^{2+} from intracellular pools by sphingosine appears not to be caused by IP_3. Recently, Ghosh et al. have demonstrated that sphingosine-1-phosphate, the molecule converted from sphingosine within cells, stimulated Ca^{2+} release from intracellular pools [20]. This mechanism could be conceivable in parotid acinar cells, but it is necessary to carry out further investigations to elucidate this fact.

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REFERENCES

[1] Merrill Jr., A.H. and Stevens, V.L. (1989) Biochim. Biophys. Acta 1010, 131–139.
[2] Hannun, Y.A. and Bell, R.M. (1989) Science 243, 500–507.
[3] Hannun, Y.A., Loomis, C.R., Merrill Jr., A.H. and Bell, R.M. (1986) J. Biol. Chem. 61, 12604–12609.
[4] Hannun, Y.A., Greenburg, C.S. and Bell, R.M. (1987) J. Biol. Chem. 262, 13620–13626.
[5] Wilson, E., Olcott, M.C., Bell, R.M., Merrill Jr., A.H. and Lambeth, J.D. (1986) J. Biol. Chem. 261, 12616–12623.
[6] Merrill Jr., A.H., Sereni, A.M., Stevens, V.L., Hannun, Y.A., Bell, R.M. and Kinkade Jr., J.M. (1986) J. Biol. Chem. 261, 12610–12615.
[7] Winicov, I. and Gershengorn, M.C. (1988) J. Biol. Chem. 263, 12179–12182.
[8] Faucher, M., Girones, N., Hannun, Y.A., Bell, R.M. and Davis, R.J. (1988) J. Biol. Chem. 263, 5319–5327.
[9] Putney Jr., J.W. (1986) Annu. Rev. Physiol. 48, 75–88.
[10] Sugiya, H. and Furuyama, S. (1989) Biomed. Res. 10, 111–121.
[11] Sugiya, H., Tennes, K.A. and Putney Jr., J.W. (1987) Biochem. J. 244, 647–653.
[12] Merritt, J.E. and Rink, T.J. (1987) J. Biol. Chem. 262, 14912–14916.

[13] Merritt, J.E. and Rink, T.J. (1987) J. Biol. Chem. 262, 17361–17369.
[14] Grynkiewicz, G., Poenie, M. and Tsien, R.Y. (1985) J. Biol. Chem. 260, 3440–3450.
[15] Takemura, H., Thastrup, O. and Putney Jr., J.W. (1990) Cell Calcium 11, 11–17.
[16] Putney Jr., J.W. (1986) Cell Calcium 7, 1–12.
[17] Takemura, H. and Putney Jr., J.W. (1989) Biochem. J. 258, 409–412.
[18] Morris, A.P., Gallacher, D.V., Irvine, R.F. and Petersen, O.H. (1987) Nature 330, 653–655.
[19] Sugiya, H. and Furuyama, S. (1990) Cell Calcium 11, 469–475.
[20] Ghosh, T.K., Bian, J. and Gill, D.L. (1990) Science 248, 1653–1656.