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Maitotoxin-induced nerve growth factor production accompanied by the activation of a voltage-insensitive Ca^{2+} channel in C6-BU-1 glioma cells

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1 The aim of the present study was to determine the effects of maitotoxin on nerve growth factor production and the Ca^{2+} influx in clonal rat glioma cells (C6-BU-1).

2 Maitotoxin (1–10 ng ml^{-1}) induced a profound increase in $^{45}Ca^{2+}$ influx in an extracellular Ca^{2+}-dependent manner. However, high KCl had no effect at all. These effects were supported by the results from the analysis of intracellular Ca^{2+} concentration using fura 2.

3 The maitotoxin-induced $^{45}Ca^{2+}$ influx was inhibited by inorganic Ca^{2+} antagonists, such as Mg^{2+}, Mn^{2+} and Co^{2+}. The inhibitory effect of Co^{2+} was antagonized by increasing the extracellular Ca^{2+} concentrations.

4 Maitotoxin (3 ng ml^{-1}) as well as A-23187 (1 μM) and dibutyryl cyclic AMP (0.5 mM) caused an acceleration of nerve growth factor (NGF) production in C6-BU-1 cells, as determined by NGF enzyme immunoassay.

5 Reverse transcription polymerase chain reaction (RT–PCR) analysis showed that maitotoxin (10 ng ml^{-1}) enhanced the expression of NGF mRNA, which was abolished by the removal of extracellular Ca^{2+}. A-23187 also accelerated its expression.

6 These results suggest that maitotoxin activates a voltage-insensitive Ca^{2+} channel and accelerates NGF production mediated through a Ca^{2+} signalling pathway in C6-BU-1 glioma cells.

Keywords: Maitotoxin; rat glioma cell (C6-BU-1); calcium channel; calcium influx; nerve growth factor (NGF); NGF mRNA

Introduction

It is now generally accepted that Ca^{2+} plays an important role in various signalling processes in cellular activity (Campbell, 1983). Calcium channels are one of the key factors in the control of the intracellular Ca^{2+} level, and thus are important in cellular signalling processes such as maintenance of protein synthesis (Wong *et al*., 1993).

Numerous toxins have been shown to be very useful pharmacological tools for studying on ion channels and drug receptors (Ohizumi, 1997). For instance, tetrodotoxin has contributed to the understanding of the Na^+ channel. Maitotoxin, the most potent marine toxin obtained from toxic dinoflagellate and poisonous fishes inhabiting tropical and subtropical seas, has been reported to produce many responses in a wide variety of mammalian cells, including the stimulation of hormone and neurotransmitter secretion, contraction of cardiac and smooth muscles, and the stimulation of inositol phosphate production (Gusovsky & Daly, 1990). All these diverse actions are dependent on the stimulation of Ca^{2+} influx into the cells.

Neurons cannot proliferate and regenerate as they are terminally differentiated cells. Therefore, neurotrophic factors are essential to maintain and organize neurons functionally. Glial cells support neuron by releasing neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) (Rudge *et al*., 1995) and glia-derived neurotrophic factor (GDNF) (Lin *et al*., 1993). NGF has been extensively investigated showing pleiotrophic effects such as the induction of neuronal differentiation, promoting the survival and the prevention of apoptosis in both central and peripheral nervous systems (Levi-Montalcini, 1987).

It has been reported that C6 cells, derived from rat glial tumours induced by N-nitrosomethylurea (Benda *et al*., 1968), synthesize/secrete NGF upon the stimulation by β-adrenergic agonists, forskolin or cyclic AMP analogues (Schwartz, 1988; Mocchetti *et al*., 1989; Fukumoto *et al*., 1994; Colangelo *et al*., 1996). These studies indicated that cyclic AMP increased NGF synthesis and secretion by initiating its gene expression. Furthermore, the expression of the NGF mRNA is dramatically enhanced when primary astrocytes are exposed to phorbol ester (Neveu *et al*., 1992). This effect is in part mimicked by diacylglycerol, and prevented by an inhibitor of protein kinase C (PKC). These data provide evidence that PKC exerts as a key enzyme in the up-regulation of NGF synthesis. Nuclear factor κB (NFκB) is known to play an important role in regulation of gene expression involved in immune and inflammatory responses in brain (Kaltschmidt *et al*., 1994; O'Neill & Kaltschmidt, 1997). It has recently been shown that an activation of NFκB by lipopolysaccharide, ceramide or sphingomyelinase induces NGF synthesis (Galve-Roperh *et al*., 1997; Heese *et al*., 1998). It is assumed that an induction of NGF synthesis by interleukin-1β, tumour necrosis factor-α or activated vitamin D_3 depends on the accumulation of ceramide (Okazaki *et al*., 1994; Hannun *et al*., 1996). In addition, certain growth factors, cytokines, interleukins, proteases and serum have been shown to express the NGF mRNA dramatically in primary astrocytes (Carman-krzan &

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Wise, 1993; Jehan *et al*., 1995). Thus, it is thought that the expression of NGF mRNA is under the regulation of multiple signalling pathways.

In spite of many reports concerning NGF synthesis, the role of Ca^{2+} in the production still remains unknown. It is supposed that those pathways to induce NGF synthesis are not always independent but correlated by cross-talk (Jehan *et al*., 1995; Colangelo *et al*., 1996). Therefore, it is important to investigate the role of Ca^{2+}, one of the representative second messengers, in the regulation of NGF gene expression. In this report, we examined the effects of maitotoxin on Ca^{2+} channel activation and NGF release in C6-BU-1 glioma cells.

Methods

Materials

Maitotoxin and A-23187 were obtained from Wako (Osaka, Japan). Dulbecco's modified Eagle's medium (DMEM) and horse serum were purchased from ICN Biochemicals, Inc. (Costa Mesa, CA, U.S.A.). Foetal calf serum was purchased from Cell Culture Laboratory (Cleveland, OH, U.S.A.). The following materials were obtained from the companies as indicated. $^{45}CaCl_2$, Amersham (Buckinghamshire, U.K.); tetracaine, dibutyryl cyclic AMP (dbcAMP), Sigma (St. Louis, MO, U.S.A.); tetrodotoxin, Sankyo (Tokyo, Japan); fura 2 acetoxy methylester, 3-(4,5-dimethylthiazol-2-yl-) diphenyltetrazolium bromide (MTT), Dojindo (Kumamoto, Japan); NGF enzyme-linked immunosorbent assay (ELISA) kit, Boehringer Mannheim (Mannheim, Germany); RNA extraction kit, Pharmacia biotech (Piscataway, NY, U.S.A.); Reverse transcription polymerase chain reaction (RT–PCR) kit, Toyobo Co, Ltd (Osaka, Japan). All other chemicals were reagent grade.

Cell culture

Clonal rat glioma cells (C6-BU-1) were kindly supplied to us by Dr Amano (The Mitsubishi-Kagaku Institute of Life Sciences). The cells were maintained in DMEM containing 5% foetal calf serum and 5% heat-inactivated horse serum. Two days before experiments, these cells were subcultured on 35 mm plastic dishes (Becton Dickinson, Lincoln Park, NJ, U.S.A.) at a density of 2×10^5 cells $dish^{-1}$ for C6-BU-1 cells.

Measurement of $^{45}Ca^{2+}$ influx by maitotoxin

For assay of $^{45}Ca^{2+}$ influx, cells cultured on 35 mm plastic dishes were washed once with a wash solution containing (mM): NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgSO_4$ 0.8, glucose 5.5 and HEPES 50, pH 7.3 at 37°C. After aspiration, $^{45}Ca^{2+}$ influx was inititated by adding 0.6 ml of assay solution containing (mM): sucrose 260, KCl 5.4, $^{45}CaCl_2$ 1.8, (0.3–0.9 Ci per dish), glucose 5.5 and HEPES 50, pH 7.3 with or without supplements of 10 ng ml^{-1} of maitotoxin or 46 mM KCl. After the desired incubation period at 37°C, the assay solution was removed to stop the $^{45}Ca^{2+}$ influx and the cells were washed with the ice-cold wash solution quickly four times within 15 s. After lysis of the cells in 1 ml of 1% Triton X-100, the radioactivity was determined by scintillation counting. The protein content was measured in parallel plates. The background $^{45}Ca^{2+}$ influx in the absence of maitotoxin and high KCl has been subtracted from each set of values.

Measurement of intracellular free Ca^{2+} concentration with fura 2

Intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by the fura 2 assay as described previously (Ohkubo *et al*., 1996).

NGF enzyme immunoassay

NGF secretion from C6-BU-1 cells was examined by ELISA. Samples for NGF ELISA were prepared as follows. C6-BU-1 cells were seeded into 24-well multiplates and allowed to grow to confluence. The day before incubation, the medium was replaced with serum-free DMEM. After washing with serum-free DMEM, drugs in DMEM/1% BSA without the serum were added to the wells. The cells were cultivated for 24 h, and 500 μl of the conditioned medium was collected. The NGF content in the medium was measured as extracellular NGF by the sandwich ELISA, according to the instructions of the NGF ELISA kit.

RT–PCR analysis

NGF mRNA expression was examined by using a RT–PCR technique as described previously (Honma *et al*., 1998). The sense primer (5′-CTT CAG CAT TCC CTT GAC AC-3′, 316–335 of rat NGF cDNA) and the antisense primer (5′-AGC CTT CCT GCT GAG CAC ACA-3′, 889–909) were conserved regions of the cDNA from rat NGF (Amand *et al*., 1996). The NGF cDNA fragment was amplified 35 cycles (94°C for 60 s, 57°C for 60 s, 72°C for 10 s). Glyceraldehyde 3-phosphate dehydrogenase (G3PDH) transcripts were used as a positive control. PCR products, which had been migrated by electrophoresis on 2% agarose gels and stained by ethidium bromide, were analysed by an image scanner (Foto/Eclipse, Fotodyne Inc., WI, U.S.A.).

MTT assay

C6-BU-1 cells were seeded on 96-well plates (200 μl $well^{-1}$) at a density of 5×10^5 cells ml^{-1}. At the end of the experiment, MTT (0.1 mg) was added to each well and the plates were incubated for 4 h at 37°C. After centrifugation at $350 \times g$ for 5 min, the medium was replaced with dimethyl sulphoxide. The absorbance of reduced MTT at 595 nm was measured with a plate reader (Taglialatela *et al*., 1997).

Measurement of protein content

Protein content was determined by dye-binding method with BSA as the standard (Bradford, 1976).

Statistical analysis

Data were expressed as mean values or means $\pm$ s.e.mean, and the significant difference was analysed with unpaired Student's *t*-test.

Results

Effect of maitotoxin on Ca^{2+} influx

Figure 1a shows the time courses of $^{45}Ca^{2+}$ influx into C6-BU-1 cells after the addition of maitotoxin (10 ng ml^{-1}) or high KCl (51.4 mM). The $^{45}Ca^{2+}$ influx into C6-BU-1 cells was

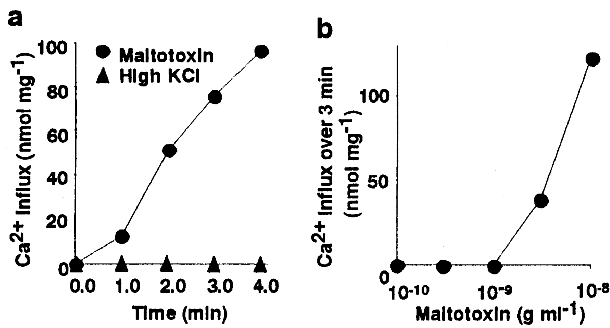

Figure 1 Stimulation of Ca^{2+} influx into C6-BU-1 cells by maitotoxin. (a) Time courses of $^{45}Ca^{2+}$ influx by the treatment with maitotoxin (10 ng ml^{-1}), and high KCl (51.4 mM). (b) Concentration-response curve for the effect of maitotoxin on the $^{45}Ca^{2+}$ influx over 4 min. $^{45}Ca^{2+}$ influx was initiated by adding 0.6 ml of (mM): sucrose 260, KCl 5.4, $^{45}CaCl_2$ 1.8, glucose 5.5 and HEPES 50, pH 7.3 supplemented with maitotoxin or KCl 46 mM. After desired incubation period at 37°C, the assay solution was removed to stop the influx, and then the cells were washed quickly four times. The cells were dissolved in 1% Triton X-100 and then the radioactivity was counted. The background $^{45}Ca^{2+}$ influx (0.25 nmol mg^{-1} min^{-1}) in the absence of maitotoxin and high KCl has been subtracted.

profoundly increased by matitotoxin after a brief period, but was not affected by high KCl. Maitotoxin induced a concentration-dependent increase in the $^{45}Ca^{2+}$ influx into C6-BU-1 cells at concentrations above 1 ng ml^{-1} (Figure 1b).

Substitution of sucrose with isotonic NaCl (130 mM) reduced the $^{45}Ca^{2+}$ influx induced by maitotoxin to about one-twenty-fifth; however, the significant $^{45}Ca^{2+}$ influx was still observed after treatment with maitotoxin (Figure 2a and b).

It is well known that the function of Ca^{2+} channels is blocked by inorganic Ca^{2+} channel blockers such as Mn^{2+}, Mg^{2+} and Co^{2+} or by local anaesthetics, and that inhibitory effects of these Ca^{2+} channel blockers are competitively antagonized by external Ca^{2+} (Fleckenstein, 1971). Thus, the effects of various inorganic Ca^{2+} channel antagonists on maitotoxin-induced $^{45}Ca^{2+}$ influx were studied both in Na^+-containing and Na^+-free solutions (Figure 2a and b). The $^{45}Ca^{2+}$ influx induced by maitotoxin was markedly suppressed by divalent cations such as Mg^{2+}, Mn^{2+} and Co^{2+} (5 mM) and tetracaine (1 mM), and was slightly suppressed by tetrodotoxin (1 μM) and Na^+ (5 mM).

Figure 2c shows the inhibitory effect of Co^{2+} on the maitotoxin-stimulated $^{45}Ca^{2+}$ influx into C6-BU-1 cells under

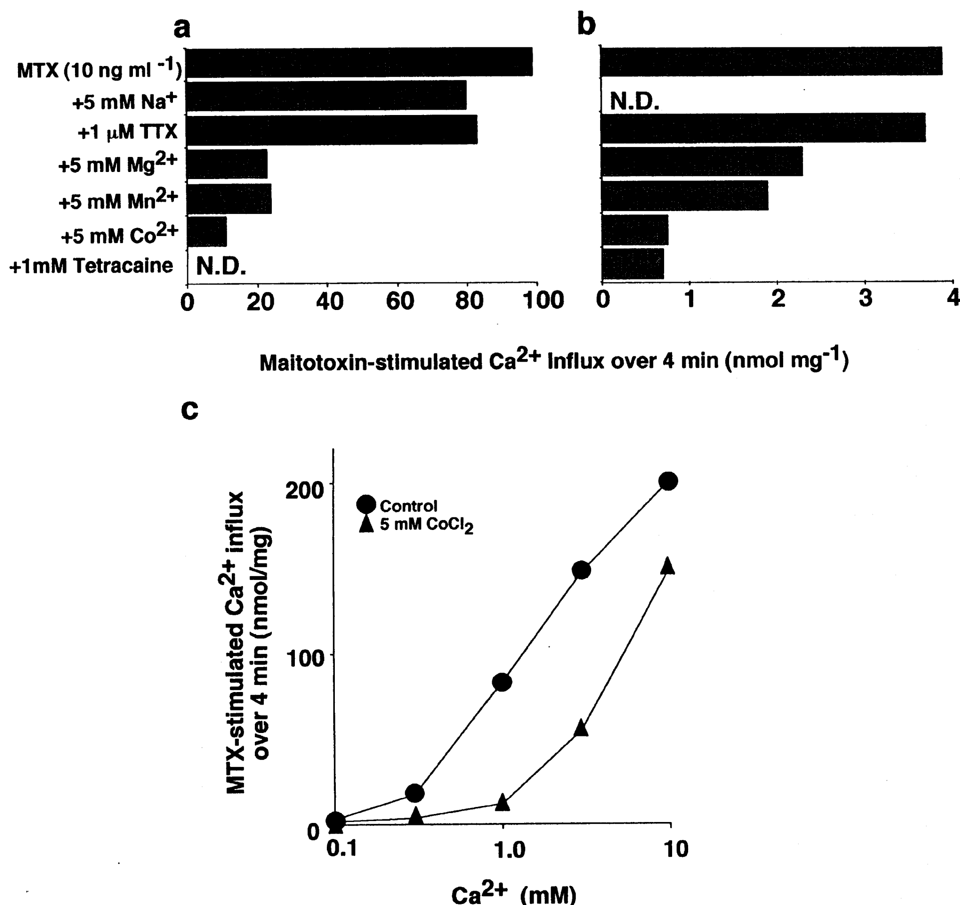

Figure 2 Characteristics of maitotoxin-induced $^{45}Ca^{2+}$ influx. (a and b) Effects of several agents on the $^{45}Ca^{2+}$ influx into C6-BU-1 cells induced by maitotoxin (MTX, 10 ng ml^{-1}) in Na^+-free (a) and Na^+-containing solutions. (b) $^{45}Ca^{2+}$ influx over 4 min was measured as described before, and the background influx (0.73 and 0.53 nmol mg^{-1} min^{-1} for a and b, respectively) has been subtracted from each set of values. The composition of Na^+-containing solution was the same as that of assay solution described before except all the sucrose was substituted with 130 mM NaCl. (c) Effect of maitotoxin (MTX, 10 ng ml^{-1}) on the log concentration response curve for Ca^{2+} in the presence or absence of Co^{2+}. $^{45}Ca^{2+}$ influx into C6-BU-1 cells over 4 min in the Na^+-free assay solution was measured as described before and the background influx (0.22, 0.34, 0.58, 1.02 and 2.15 nmol mg^{-1} min^{-1} at 0.1, 0.3, 1, 3 and 10 mM of $CaCl_2$, respectively) has been subtracted from each set of values. Results are mean of two determinations. N.D., not determined.

various concentrations of external Ca^{2+}. The average rate of the maitotoxin-stimulated $^{45}Ca^{2+}$ influx over 4 min was increased upon increasing the concentration of external Ca^{2+} from 0.1 to 10 mM. In 1 mM Ca^{2+}, Co^{2+} (5 mM) caused the inhibition of the $^{45}Ca^{2+}$ influx by 89%, which was overcome by increasing the concentration of external Ca^{2+} to 10 mM.

Intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by using fura 2. Maitotoxin (10 ng ml^{-1}) caused $[Ca^{2+}]_i$ elevation (Figure 3a). The $[Ca^{2+}]_i$ elevation was weakly suppressed by pretreatment with Mg^{2+} (5 mM) (Figure 3b), and was completely inhibited by the removal of extracellular Ca^{2+} with EGTA (5 mM) (Figure 3c). High KCl (50 mM) caused neither change in basal $[Ca^{2+}]_i$ nor in maitotoxin-induced $[Ca^{2+}]_i$ elevation in C6-BU-1 cells (Figure 3e), indicating that the maitotoxin-induced $[Ca^{2+}]_i$ elevation was attributed to Ca^{2+} influx through a voltage-insensitive Ca^{2+} channel. Since the addition of high KCl resulted in the hypertonic condition in this experiment, we also carried out the experiment using 50 mM NaCl instead of KCl, neglecting the hypertonic effect (Figure 3f).

Effect of maitotoxin on NGF synthesis and secretion from C6-BU-1 cells

Since maitotoxin sometimes causes cell toxicity due to excess Ca^{2+} influx, cell viability was examined by the MTT-method (Taglialatela *et al.*, 1997) in order to determine an appropriate incubation time and inappropriate maitotoxin concentration for NGF measurement. The result showed that almost all the cells remained alive under the following conditions: 3 ng ml^{-1} of maitotoxin for 24 h, and 10 ng ml^{-1} of maitotoxin for 3 h, while 50% of cells turned out to be dead with 10 ng ml^{-1} of maitotoxin for 24 h (data not shown). To examine the effect of maitotoxin on NGF synthesis and secretion, NGF content in the culture medium was measured by using ELISA. NGF contents in the conditioned medium were increased upon the treatment with maitotoxin (3 ng ml^{-1}) and A-23187 (1 μM) as well as dbcAMP (0.5 mM) for 24 h (Figure 4). In addition, the NGF mRNA expression in C6-BU-1 cells was examined by using RT–PCR methods. Maitotoxin (10 ng ml^{-1}) increased the expression of NGF mRNA, dependent on the extracellular Ca^{2+} concentration. EGTA abolished maitotoxin-induced the enhancement (Figure 5a). A-23187-induced NGF mRNA expression was also reversed by EGTA treatment as in the case of maitotoxin (Figure 5b). The results clearly demonstrated that NGF mRNA expression in C6-BU-1 cells was increased by maitotoxin in an extracellular Ca^{2+}-dependent manner.

Discussion

In the present experiments, maitotoxin induced a profound increase in Ca^{2+} influx into non-excitable glioma cells whereas high KCl treatment did not affect Ca^{2+} level at all. The effects of maitotoxin on C6-BU-1 cells were markedly suppressed by various Ca^{2+} channel blockers. The inhibitory effect of Co^{2+} was antagonized by external Ca^{2+} and became less obvious in the higher Ca^{2+} concentration range. These observations support the results that voltage-insensitive Ca^{2+} channels exist

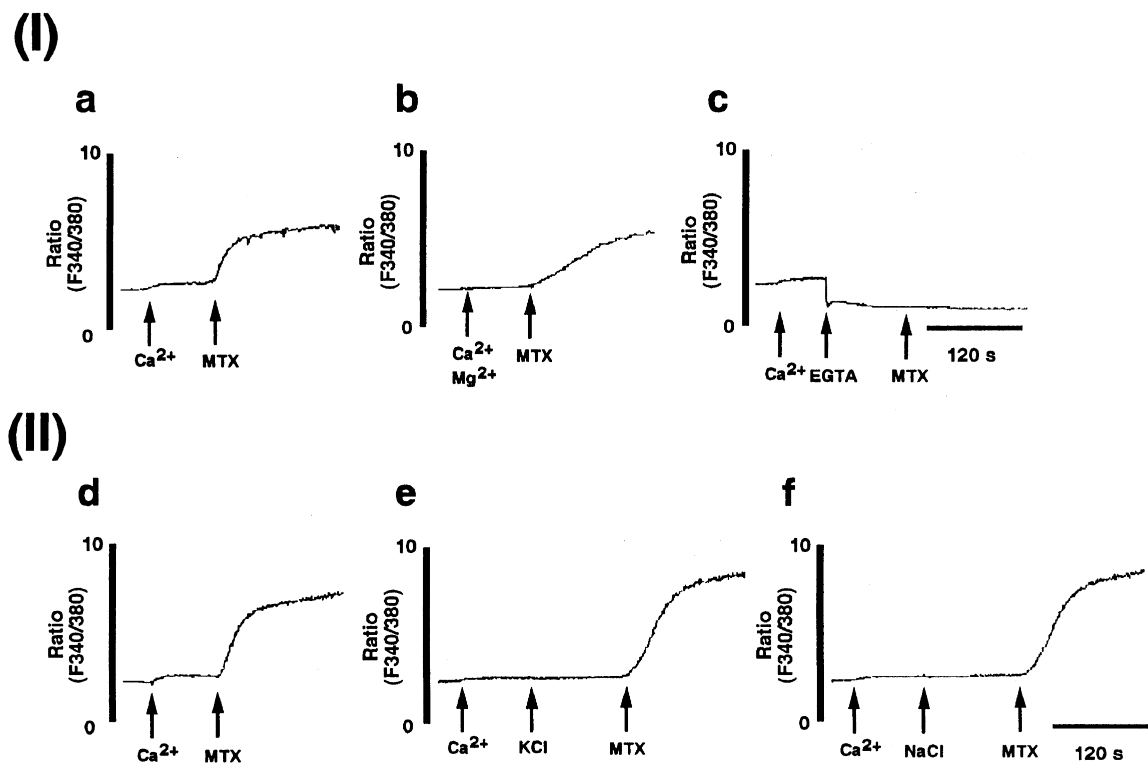

Figure 3 Maitotoxin-induced extracellular Ca^{2+}-dependent voltage-insensitive Ca^{2+} influx. (I) Extracellular Ca^{2+} dependence of maitotoxin-induced Ca^{2+} influx in C6-BU-1 cells. $[Ca^{2+}]_i$ was measured as described in Methods. The cells loaded with fura 2 acetoxy methylester were suspended in the Tyrode solution. (a) Maitotoxin (MTX, 10 ng ml^{-1}) was added 90 s after addition of 1 mM Ca^{2+} (control for b and c). (b) Maitotoxin (MTX, 10 ng ml^{-1}) was added 90 s after addition of 5 mM Mg^{2+} and 1 mM Ca^{2+}. (c) Maitotoxin (MTX, 10 ng ml^{-1}) was added after addition of 5 mM EGTA in the presence of 1 mM Ca^{2+}. (II) Maitotoxin-induced Ca^{2+} influx through voltage-insensitive Ca^{2+} channel. (d) Maitotoxin (MTX, 10 ng ml^{-1}) was added 90 s after addition of 1 mM Ca^{2+} (control for e and f). (e) Maitotoxin (MTX, 10 ng ml^{-1}) was added 120 s after addition of high KCl (50 mM) in the presence of 1 mM Ca^{2+}. (f) Maitotoxin (MTX, 10 ng ml^{-1}) was added 120 s after addition of high NaCl (50 mM) in the presence of 1 mM Ca^{2+}.

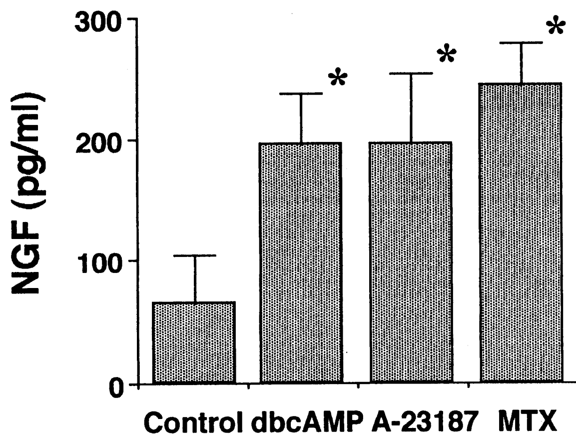

Figure 4 NGF secretion from C6-BU-1 cells in response to dbcAMP, A-23187 and maitotoxin. After incubation with dbcAMP (0.5 mM), A-23187 (1 μM) and maitotoxin (MTX, 3 ng ml^{-1}) for 24 h, the medium from C6-BU-1 cell cultures were collected, and NGF content was measured using an ELISA. Values are the means±s.e.mean of three determinations. $*P<0.05$ *vs* control (without drug) in each cell.

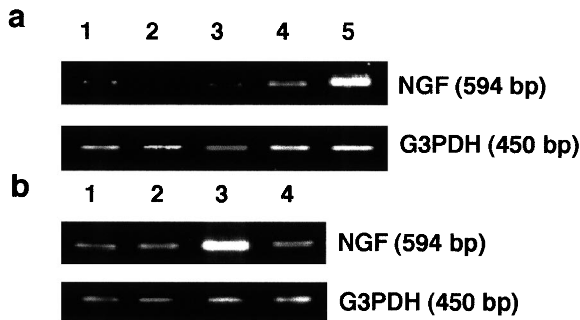

Figure 5 (a) NGF mRNA expression by maitotoxin. The cells were stimulated by maitotoxin for 3 h under various concentrations of extracellular Ca^{2+}, then total RNA from C6-BU-1 cells was reverse transcribed followed by PCR as described before. Lane 1, control in 1.8 mM $CaCl_2$; 2, control in 1.8 mM $CaCl_2$ + 3.6 mM EGTA; 3, maitotoxin (10 ng ml^{-1}) in 1.8 mM $CaCl_2$ + 3.6 mM EGTA; 4, maitotoxin (10 ng ml^{-1}) in 1.8 mM $CaCl_2$ + 1.7 mM EGTA; 5, maitotoxin (10 ng ml^{-1}) in 1.8 mM $CaCl_2$. (b) NGF mRNA expression by A-23187. The cells were stimulated by A-23187 for 3 h in the presence or absence of 1.8 mM EGTA. Lane 1, control in 1.8 mM $CaCl_2$; 2, control in 1.8 mM $CaCl_2$ + 1.8 mM EGTA; 3, A-23187 (1 μM) in 1.8 mM $CaCl_2$; 4, A-23187 (1 μM) in 1.8 mM $CaCl_2$ + 1.8 mM EGTA.

in the non-excitable plasma membranes of glioma cells, and that maitotoxin increases the Ca^{2+} permeability through these Ca^{2+} channels (Konoki *et al*., 1998; Murata *et al*., 1992). Recent lines of evidence suggest that maitotoxin activates non-selective cation channels (Bielfeldackermann *et al*., 1998; Dietl & Volkl, 1994; Estacion *et al*., 1996; Leech & Habener, 1997).

We demonstrated that maitotoxin and A-23187 as well as by dbcAMP caused NGF synthesis and secretion. In addition, the increase of NGF secretion by maitotoxin was initiated by its gene expression. Maitotoxin-induced NGF mRNA expression was completely reversed by the removal of extracellular Ca^{2+}, indicating that Ca^{2+} influx is essential for NGF synthesis by maitotoxin. There have previously been a few reports regarding NGF mRNA expression enhanced by increasing $[Ca^{2+}]_i$. It was shown that N-methyl-D-aspartic acid (NMDA)-induced NGF production was reversed under the extracellular Ca^{2+}-free condition in C6-BU-1 cells (Amano *et al*., 1992), suggesting that Ca^{2+} is necessary for NGF production. However, Jehan *et al*. (1995) showed that A-23187 inhibited the phorbol ester-induced production of the NGF mature protein in mouse primary astrocytes in spite of causing a weak NGF mRNA expression. It was shown that phorbol ester, cyclic AMP and Ca^{2+} ionophore expressed proto-oncogenes such as c-*jun* and c-*fos* (Jehan *et al*., 1995), the products of which might regulate the NGF mRNA expression. The Fos/Jun heterodimer complex known as activator protein-1 (AP-1), one of the major targets of protein kinase A (PKA) and PKC, could be a regulatory transcription factor for the expression of the NGF mRNA *in vitro*, which was supported by an identification of an AP-1 consensus sequence within downstream of the TATA box at the junction of the exon I/intron I region of rat and mouse NGF gene (D'Mello *et al*., 1991). In addition, it has been shown that the increase in AP-1 activity correlates with the induction of NGF mRNA (Colangelo *et al*., 1996).

It is reported that thapsigargin induces c-*fos* and c-*jun* expression mediated by increasing $[Ca^{2+}]_i$ (Schonthal *et al*., 1991). On the other hand, it has been shown that PKA phosphorylates cyclic AMP responsive element (CRE)-binding protein (CREB) (Hagiwara *et al*., 1993). Since calmodulin kinase also activates CREB (Sheng *et al*., 1990), Ca^{2+} is assumed to cause the gene expression of proto-oncogenes such as c-*fos via* a CRE promoter. Therefore, it is supposed that the transcription activity of AP-1 is increased by the *de novo* synthesis of proto-oncogenesis *via* those pathways. In addition, since calmodulin kinase can activate adenylyl cyclase type I by phosphorylation (Impey *et al*., 1994), there is a possibility that increased $[Ca^{2+}]_i$ by maitotoxin can activate PKA or CREB by cyclic AMP accumulation. Therefore, although further study is necessary, Ca^{2+} influx by maitotoxin causes NGF synthesis possibly through the expression of those proto-oncogenes or phosphorylation of AP-1 by PKA.

Exogenously administered NGF can serve as a neurotrophic factor in the brain, preventing neuronal death and activating neuronal function. However, it is difficult and inconvenient to administer NGF by intracerebral infusion, and peripherally administered NGF does not cross the blood–brain barrier. This is why the clarification of the mechanism of NGF synthesis in glial cells is indispensable for the development of new NGF inducers which pass through blood–brain barrier. These drugs will be useful for serious neuronal disorders such as Alzheimer's disease.

In conclusion, the present results suggest that maitotoxin activates the voltage-insensitive Ca^{2+} channel and accelerates NGF production in non-excitable C6-BU-1 glioma cells. Because maitotoxin is a powerful activator of voltage-insensitive Ca^{2+} channels, we believe that it will be a beneficial drug to clarify the role of Ca^{2+} in NGF synthesis and secretion.

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