Volume 287, number 1,2, 15–18 FEBS 09956 August 1991

ADONIS 001457939100666Y

Mast cell degranulating (MCD) peptide and its optical isomer activate GTP binding protein in rat mast cells

Ichiro Fujimoto[1], Kazuhiro Ikenaka[1], Tetsuro Kondo[1], Saburo Aimoto[1], Miyuki Kuno[2] and Katsuhiko Mikoshiba[1,3]

[1] *Institute for Protein Research, Osaka University, 3-2 Yamadaoka Suita 565, Japan,* [2] *Department of Physiology, Osaka City University Medical School, Abeno-ku, Osaka 545, Japan* and [3] *The Institute of Medical Science, University of Tokyo, 4-6-1 Shiroganedai, Minato-ku, Tokyo 108, Japan*

Received 29 April 1991; revised version received 20 May 1991

The MCD peptide in bee venom induces degranulation in mast cells. The internal calcium concentration of mast cells increased and remained high following MCD stimulation. This calcium increase was blocked by pertussis toxin (Ptx) treatment, suggesting that MCD peptide activates Ptx-sensitive G-protein. Even in the absence of external calcium in the incubation medium, the calcium concentration increased by MCD treatment, but soon returned to the original level. D-MCD, the optical isomer of the MCD peptide, also increased the internal calcium concentration through a Ptx-sensitive pathway. We suggest that cationic clusters at one side of the surface are more important in activating the G-protein than the α-helix conformation.

Mast cell degranulating peptide; Mast cell; G-protein; α-Helix; Optical isomer

1. INTRODUCTION

Histamine release by rat peritoneal mast cells has been extensively used as a model of exocytosis [1]. The introduction of non-hydrolyzable analogs of GTP that persistently activate GTP-binding proteins into mast cells induces exocytosis [2]. This suggests that G-proteins play a role in the cascade of events leading to histamine secretion [1,2]. The early events after stimulation of mast cells include the breakdown of phosphoinositides (PIP_2), a rise in the internal Ca^{2+} concentration and the generation of arachidonic acid [1–3]. However, these signaling pathways and their relative role in secretion remain to be clarified.

A wasp venom, mastoparan, is known to induce histamine release from mast cells [4,5]. It has previously been shown that mastoparan activates G-proteins and stimulates their GTPase activity, leading to histamine release [6]. When mastoparan is bound to a phospholipid bilayer, it forms an α-helix that lies parallel to the plane of the membrane, with its hydrophobic face within the bilayer and its four positive charges facing outward; thus, this peptide has an amphiphilic property [7,8]. This conformation is thought to mimic the structure of the interaction site of agonist-liganded receptors with the G-protein [9,10]. Disruption of the α-helix conformation and the amphiphilic property by replacing the amino acid residues of mastoparan results in the loss of the ability to activate the G-protein [11]. However, it is not clear which is more important for the activation of the G-protein, α-helix formation or cationic clusters at one side of the surface.

We have been studying the molecular mechanism that underlies the induction of long-term potentiation (LTP) of synaptic transmission efficacy induced by the mast-cell-degranulating (MCD) peptide in bee venom [12,13]. This MCD peptide is a highly basic toxin of 22 amino acids with two disulfide bonds [14]. It also causes mast cells to release histamine, as does mastoparan [15,16]. The structure of the MCD peptide has been elucidated through NMR studies: it was shown to contain an α-helix structure with an amphiphilic property, fixed by two disulfide bonds at its C-terminal [17]. Thus, it was intriguing to investigate whether histamine release caused by the MCD peptide also involves the activation of G-protein. G-protein activation has been shown to play an important role in long-term potentiation (LTP) formation in the hippocampus [18,19], and therefore, it would also aid in understanding the mechanism underlying MCD-induced LTP if this were the case. In this paper, we show that the MCD peptide causes an increase in the intracellular calcium level of mast cells via a G-protein-dependent pathway. Moreover, the complete optical isomer of MCD, which was synthesized by replacing all amino acid residues of the MCD peptide with D-amino acids, also activates the G-protein in mast cells. This indicates that cationic clusters at one

Correspondence address: K. Ikenaka, Division of Regulation of Macromolecular Function, Institute for Protein Research, Osaka University, 3-2 Yamadaoka Suita 565, Japan. Fax: (81) (6) 8762533

side of the molecular surface are more important than the α-helical conformation of the backbone in activating the G-protein in mast cells.

2. MATERIALS AND METHODS

2.1. *Synthesis of the MCD peptide and the D-MCD peptide*

Peptide-chain elongation was carried out on an Applied Biosystem peptide synthesizer Model 430A. Two S-S bonds were selectively formed by air oxidation and iodide oxidation. They were purified by SP-Sephadex C-25 column chromatography (2.8 × 30 cm Pharmacia, Uppsala, Sweden), reversed-phase HPLC (1.9 × 15.0 cm μBondasphere 5μ C8-300A, Waters, Tokyo, Japan) and ion-exchange HPLC (0.75 × 7.5 cm TSK SP-5PW, Tosoh, Tokyo, Japan) as described in detail [13].

2.2. *Isolation and pertussis toxin (Ptx) treatment of rat mast cells*

Mast cells were obtained from the peritoneal cavity of Wistar rats and purified through metrizamide gradient centrifugation as described [20]. Mast cells were collected from the pellet at a purity of >90%. The cells were incubated for 2 h at 37°C with pertussis toxin at 10 ng/ml or 100 ng/ml in Ringer solution as described previously [1,20]. Control cells were incubated without Ptx for the same period.

2.3. *Measurement of internal Ca^{2+} concentrations*

Ptx-treated and control cells were incubated with 1 μM Fura2/AM (Dojindo, Japan) in Ringer solution at 37°C for 15 min, resuspended in 1.5 ml of fresh Ringer solution at 0.3 × 10^6 cells/ml in a starring cuvette and measured by F-2000 fluorescence spectrophotometer (Hitachi, Japan). Emission was monitored at 510 nm after excitation with two different wavelengths (340 nm and 380 nm): F_{340}, F_{380}. The switching time for the two wavelengths was 0.5 s. The concentration of Ca^{2+} was calculated by the following equations: $R_{min} = \{F_{min(340)} - Z_1\}/\{F_{min(380)} - Z_2\}$, $R_{max} = \{F_{max(340)} - Z_1\}/\{F_{max(380)} - Z_2\}$, $R = \{F_{340} - Z_1\}/\{F_{380} - Z_2\}$, $[Ca^{2+}] = 224\ nM\{(R - R_{min})/(R_{max} - R)\}\{F_{min(380)}/F_{max(380)}\}$.

Z_1 and Z_2 are the autofluorescence intensities at 340 nm and 380 nm before Fura2 was loaded. $F_{min(380)}$ is the fluorescence intensity for free dye at 380 nm and $F_{max(380)}$ is that for Ca^{2+}-bound dye at 380 nm. $F_{min(340)}$ and $F_{max(340)}$ are the fluorescence intensities for free dye and Ca^{2+}-bound dye at 340 nm.

3. RESULTS AND DISCUSSION

MCD peptide has been characterized by its potent ability to induce degranulation in mast cells [15,16]. When mast cells loaded with Fura2 were stimulated with MCD peptide (1 μM), the internal calcium concentration increased and reached the maximum level soon after stimulation (Fig. 1A). The calcium level declined slowly thereafter, reaching a constant level substantially higher than the original level. G-protein is known to play a crucial role in the degranulation process induced by various chemoattractants, e.g. mastoparan and Compound 48/80 [1,7,20].

To examine the possible involvement of G-protein in this process, the internal calcium concentration was analyzed in mast cells that were pretreated with 10 ng/ml or 100 ng/ml pertussis toxin (Ptx) for 2 h (Fig. 1B,C). The increase in the calcium level after stimulation with 1 μM MCD peptide was suppressed in a dose-dependent manner. Therefore, inactivation of G-protein(s) sensitive to Ptx treatment reduced the

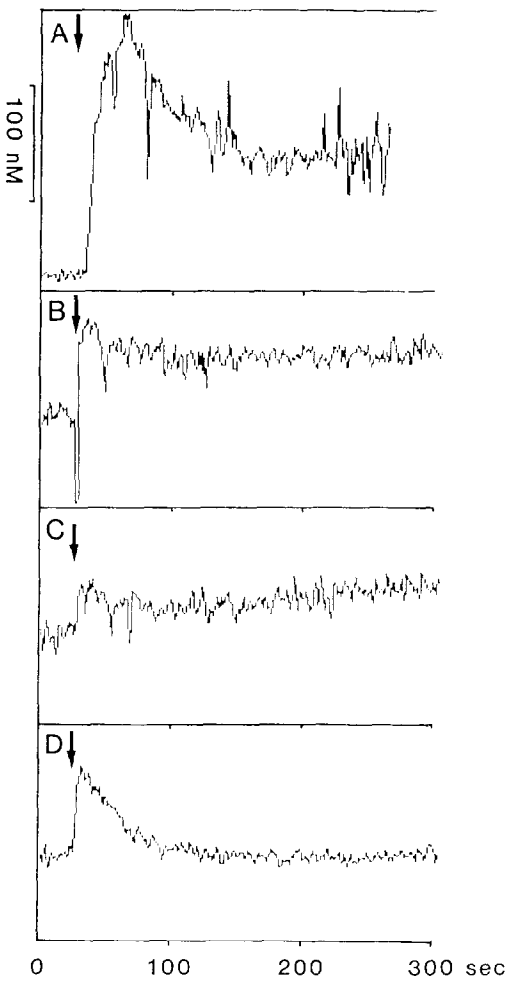

Fig. 1. Intracellular calcium concentration in mast cells stimulated with MCD. MCD (1 μM) was added to 3~5 × 10^5 cells/ml of mast cells loaded with Fura2 at the point indicated by arrows. The calcium concentration was measured as described in section 2. A, control; B and C, the cells were preincubated for 2 h at 37°C with 10 ng/ml and 100 ng/ml, respectively, of pertussis toxin; D, calcium was removed from the incubation medium by adding EGTA.

response of mast cells toward MCD peptide treatment.

We then investigated the source of the calcium. When external calcium was removed from the incubation medium 1 min before MCD peptide treatment by adding EGTA (2 mM), the initial rise in calcium concentration still occurred, however, it soon returned to the original level (Fig. 1D). These results indicate that the MCD peptide increases the intracellular calcium concentration by activating the G-protein and that the initial transient increase in calcium concentration is independent of external calcium, while the latter sustained increase is dependent. The initial small peak might be induced through an inositol 1,4,5-trisphosphate (IP_3)-dependent pathway [1,3,21,22]. These effects of the MCD peptide toward mast cells resemble those of mastoparan.

The relationship between structure and function has been well studied in mastoparan, and it has been shown

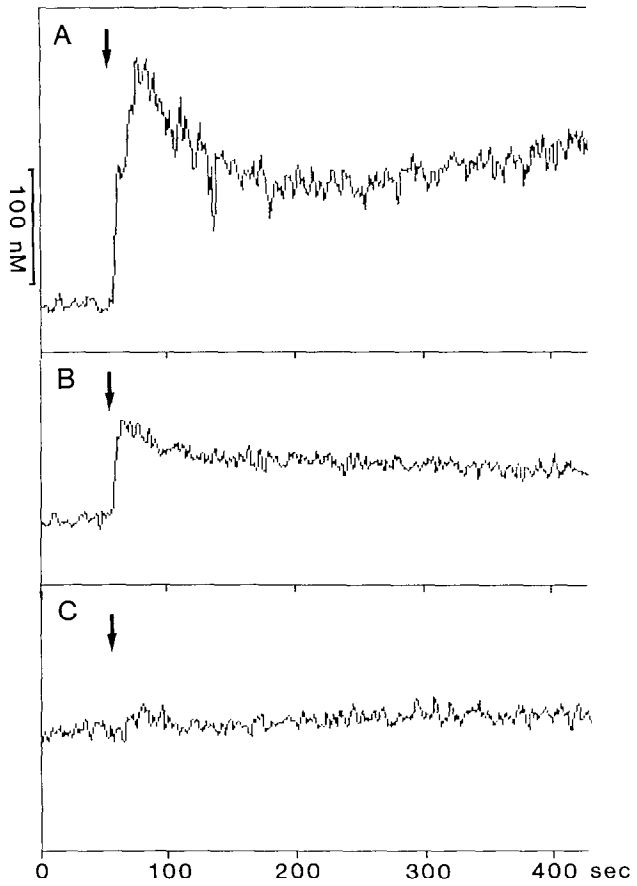

Fig. 2. Intracellular calcium concentration in mast cells stimulated with D-MCD, the optical isomer of MCD. D-MCD (1 μM) was added to 3~5 $\times$ 10^5 cells/ml of mast cells loaded with Fura2 at the point indicated by arrows. The calcium concentration was measured as described in section 2. A, control; B and C, the cells were preincubated for 2 h at 37°C with 10 ng/ml and 100 ng/ml, respectively, of pertussis toxin.

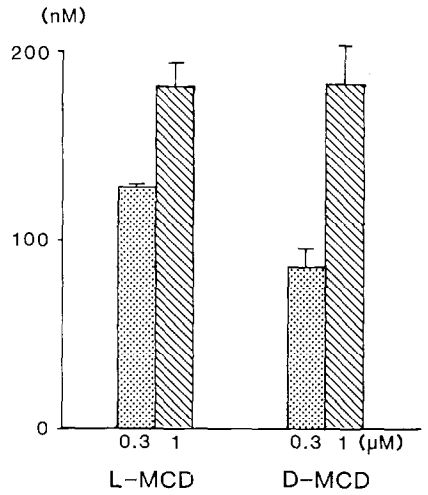

Fig. 3. Dose–response of MCD- and D-MCD-induced increases in the intracellular calcium concentration. The calcium concentration was measured at 24°C in assay medium that contained 2 mM calcium. Each point represents the average of the maximum calcium increase after 0.3 μM or 1 μM of MCD or D-MCD stimulation of more than 3 independent experiments.

that disrupting the α-helix conformation by replacing amino acid residues results in the loss of the ability to activate the G-protein [11]. When the α-helix conformation with its amphiphilic property is disrupted, however, cationic clusters at one side of the surface will also be disrupted [11]. We have shown that the MCD peptide has an α-helical structure at its C-terminal, which is fixed by two disulfide bridges, and that it also displays an amphiphilic property [17]. We have also synthesized a complete optical isomer of the MCD peptide by replacing all the amino acid residues with their D-isomers (D-MCD). Protein–protein (peptide) recognition would no longer exist if the α-helical conformation were changed to its optical isomeric form, while the ionic clusters on the surface of the two optical isomers should be the same. Therefore, this peptide provides a unique opportunity to investigate which property of the peptide is essential for G-protein activation, the α-helical conformation or cationic clusters at one side of the molecule. The D-MCD-induced increase in the internal calcium level, and the time course of the response was quite similar to that induced by the MCD peptide (Fig. 2A). This process was also inhibited by Ptx treatment (Fig. 2B,C). Fig. 3 shows the maximum increase in calcium levels after treatment with the designated concentrations of MCD or D-MCD peptide. D-MCD peptide activated the G-protein almost to the same extent as the MCD peptide, although it was slightly less effective at a lower concentration (0.3 μM). These results suggested that cationic clusters at one side of the surface were more important in activating the G-protein than the α-helix conformation.

Since the G-protein(s) is (are) known to play an important role in LTP induction, it is necessary to study the interaction of the MCD peptide with the G-protein in brain.

Acknowledgements: We are grateful to Dr. Hiroshi Kato for helpful discussions and Junko Kawaguchi for technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Japan.

REFERENCES

[1] Nakamura, T. and Ui, M. (1985) J. Biol. Chem. 260, 3584–3593.
[2] Ardor, M., Traub, L.M. and Sagi-Eisenberg, R. (1990) J. Cell Biol. 111, 909–917.
[3] Penner, R., Matthews, G. and Neher, E. (1988) Nature 344, 499–504.
[4] Hirai, Y., Yasuhara, T., Yoshida, H., Nakajima, T., Fujino, M. and Kitada, C. (1979) Chem. Pharm. Bull. 27, 1942–1944.
[5] Mousli, M., Bronner, C., Bueb, J.L., Tschirhart, E., Gies, J.P. and Landry, Y. (1989) J. Pharmacol. Exp. Ther. 250, 329–335.
[6] Higashijima, T., Uzu, S., Nakajima, T. and Miyazawa, T. (1986) Peptide Chemistry, 75–78.
[7] Higashijima, T., Wakamatsu, K., Takemitsu, M., Fujino, M., Nakajima, T. and Miyazawa, T. (1983) FEBS Lett. 152, 227–230.

[8] Wakamatsu, K., Higashijima, T., Fujino, M., Nakajima, T. and Miyazawa, T. (1983) FEBS Lett. 162, 123–126.
[9] Higashijima, T., Uzu, S., Nakajima, T. and Ross, E.M. (1988) J. Biol. Chem. 263, 6491–6494.
[10] Weingarten, R., Ransnas, L., Mueller, H., Sklar, L.A. and Bokoch, G.M. (1990) J. Biol. Chem. 265, 11044–11049.
[11] Higashijima, T., Burnier, J. and Ross, E.M. (1990) J. Biol. Chem. 265, 14176–14186.
[12] Cherubini, E., Ari, Y.B., Gho, M., Bidard, J.N. and Lazdunski, M. (1987) Nature 328, 70–73.
[13] Kondo, T., Ikenaka, K., Kato, H., Ito, K., Aimoto, S., Hojo, H. and Mikoshiba, K. (1990) Neurosci. Res. 8, 147–157.
[14] Gauldie, J., Hanson, J.M., Shipolini, R.A. and Vernon, C.A. (1978) Eur. J. Biochem. 83, 405–410.
[15] Breithaupt, H. and Habermann, E. (1968) Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmacol. 261, 252–270.
[16] Jasani, B., Kreil, G., Mackler, B.F. and Stanworth, D.R. (1979) Biochem. J. 181, 623–632.
[17] Kobayashi, Y., Sato, A., Takashima, H., Tamaoki, H., Nishimura, S., Kyogoku, Y., Ikenaka, K., Kondo, T., Mikoshiba, K., Hojo, H., Aimoto, S. and Moroder, L. (1990) Neurosci. Int., in press.
[18] Ito, I., Okada, D. and Sugiyama, H. (1988) Neurosci. Lett. 90, 181.
[19] Goh, J.W. and Pennefather, P.S. (1989) Science 244, 980–983.
[20] Kuno, M., Kawaguchi, J., Mukai, M. and Nakamura, F. (1990) Am. J. Physiol. 259, C715–C722.
[21] Okano, Y., Takagi, H., Tohmatsu, T., Nakashima, S., Kuroda, Y., Saito, K. and Nozawa, Y. (1985) FEBS Lett. 188, 363–366.
[22] Perianin, A. and Snyderman, R. (1989) J. Immunol. 143, 1669–1673.