Calcium induces cyclic GMP formation in *Dictyostelium*

N.V. Small, G.N. Europe-Finner and P.C. Newell

Department of Biochemistry, South Parks Road, Oxford OX1 3QU, England

Received 16 May 1986

Cyclic GMP is rapidly formed a few seconds after binding of chemotactic signalling molecules to specific receptors on the cell surface of *Dictyostelium* amoebae. This phenomenon could be mimicked by addition of a pulse of Ca^{2+} to permeabilised amoebae. The concentration of Ca^{2+} for half-maximal response was 60 μM. Other ions (K^+, Na^+, Mg^{2+} or Mn^{2+}) had no effect. A pulse of 5 μM IP_3 produced a cyclic GMP response of similar magnitude but IP_2 elicited no response. The data provide strong support for the hypothesis that cell surface receptor binding induces cyclic GMP formation by liberating Ca^{2+} from internal stores.

(Dictyostelium) *cyclic GMP* *Chemotaxis* *Ca^{2+}*

1. INTRODUCTION

Amoebae of the cellular slime mould, *Dictyostelium discoideum*, respond chemotactically to signalling molecules such as folate during the food-seeking phase [1], and cAMP during the subsequent starvation phase [2] when they aggregate to form motile multicellular organisms [3]. These chemoattractants bind to separate cell surface receptors, yet appear to induce a group of internal responses common to both types of stimulus [4].

Following a pulse of exogenous cAMP or folate, two of the initial responses identified are the polymerisation of actin associated with the cytoskeleton, peaking 3–5 s after stimulation [5–10], and the transient formation of cyclic GMP, which peaks 9–12 s after cAMP or folate binding [8–10], and is rapidly degraded by a specific cGMP phosphodiesterase [11,12].

This cGMP formation has been implicated in chemotactic movement by studies which indicate its rapid accumulation following pulsing of several species of cellular slime mould (including *D. discoideum*, *D. lacteum*, and *Polysphondylium violaceum*) with their specific chemoattractants [13,14]. It is also strongly implicated in chemotaxis by the finding that streamer F mutants of *D. discoideum* (which lack the specific cGMP phosphodiesterase and hence produce a large peak of cGMP in response to cAMP-receptor binding that persists for at least 1 min at elevated levels) show a chemotactic movement period that is greatly lengthened compared to the parental wild type [10,15,16].

Recently, studies in this laboratory have produced indirect evidence for the involvement of Ca^{2+} in chemotaxis in *D. discoideum* [17–19] and have revealed that IP_3, when added to permeabilised amoebae, can mimic the action of chemoattractants on normal intact amoebae in inducing cGMP formation [20]. As IP_3 in mammalian systems acts via release of intracellular Ca^{2+} [21], it seemed worthwhile to study the direct effect of pulses of Ca^{2+} on the formation of cGMP in permeabilised amoebae.

Abbreviations: IP_3, inositol 1,4,5-trisphosphate; IP_2, inositol 1,4-bisphosphate

2. MATERIALS AND METHODS

2.1. *Materials*

IP_3 (potassium salt) and IP_2 (potassium salt) were obtained from Amersham. Saponin and EGTA were obtained from Sigma.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

2.2. *Harvesting and permeabilisation of amoebae*

D. discoideum strain NC4 was grown in association with *Klebsiella aerogenes* strain OXF1 on SM nutrient agar [22]. Amoebae were prepared by growth as mass plates on SM agar until uniform clearing of the bacterial lawn by the feeding amoebae occurred. Harvesting and permeabilisation of the amoebae were performed as in [20] except that 1 mM EGTA (final concentration) was included in the incubation with saponin.

2.3. *Stimulation of amoebae and cGMP assay*

Stimulation using Ca^{2+}, IP_3, and (for controls) water and IP_2, was as described for IP_3 in [20]. The final Ca^{2+} concentrations ranged from 0 to 2 mM and the final concentration of IP_3 and IP_2 was 5 μM. For stimulation of amoebae with K^+, Na^+, Mg^{2+} and Mn^{2+}, the final ion concentration was 1.87 mM. Reactions were stopped by rapid addition of an equal volume of 3.5% perchloric acid, and cGMP assayed by radioimmunoassay as described in [23].

3. RESULTS

To test the ability of Ca^{2+} to stimulate cGMP formation, pulses of Ca^{2+} at a final concentration of 1.87 mM were added to freshly permeabilised amoebae. The results (fig.1) revealed that Ca^{2+} induced a rapid accumulation of cGMP, peaking at 9 s, with a maximum value of 15 pmol cGMP $\cdot 10^7$ cells^{-1}. In contrast, addition of an equal volume of water to permeabilised amoebae gave no increase in cGMP formation, other than random fluctuations near the baseline.

To confirm that 1.87 mM was an appropriate final Ca^{2+} concentration for stimulation, a range of Ca^{2+} concentrations from 0 to 2 mM final concentration were added to permeabilised amoebae. For these experiments the reaction was stopped at a standard time of 10 s which numerous experiments indicated was around the peak time of formation. An assay of cGMP formation, as before, yielded the dose-response curve (fig.2), showing a Ca^{2+} concentration for half-maximal response of 60 μM.

For reasons that have not been determined, considerable variation in the maximal response to Ca^{2+} was observed from experiment to experiment (as shown by the error bars). This was particularly

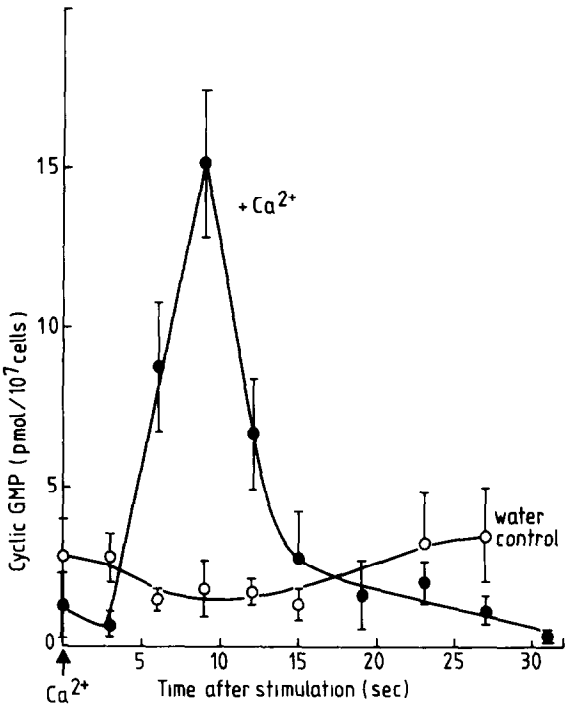

Fig.1. Time course of cGMP formation in response to a pulse of 1.87 mM Ca^{2+} (●) or water (○) given at time = 0 s. Error bars indicate SE.

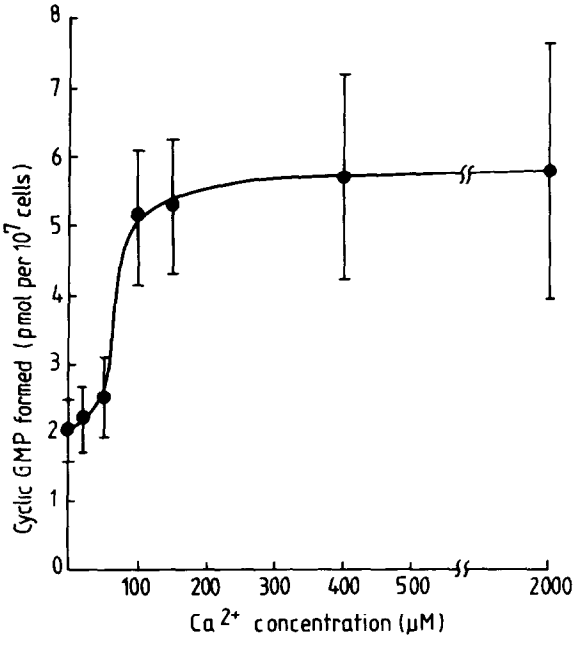

Fig.2. Dose-response curve for cGMP formation, 10 s after pulsing with a range of Ca^{2+} concentrations. Error bars indicate SE.

noticeable between different batches of amoebae grown at different times (compare, e.g. the mean peak height of 15 pmol·10^7 cells^{-1} in fig.1 with 6 pmol·10^7 cells^{-1} in fig.2).

To test the specificity of Ca^{2+} in eliciting the cGMP response, experiments were conducted in which K^+, Na^+, Mg^{2+} and Mn^{2+} were added to permeabilised amoebae at a final concentration of 1.87 mM. The results indicated that these ions induced no significant formation of cGMP above background.

To compare the effects of Ca^{2+} with those of IP_3 previously reported [20], IP_3 (and as a control, its breakdown product, IP_2) was used to induce cGMP formation under identical conditions to those used above for Ca^{2+}. The results (fig.3) revealed a peak of 14.7 pmol cGMP·10^7 cells^{-1} occurring 9 s after stimulation with 5 μM IP_3, whereas IP_2 produced no measurable response.

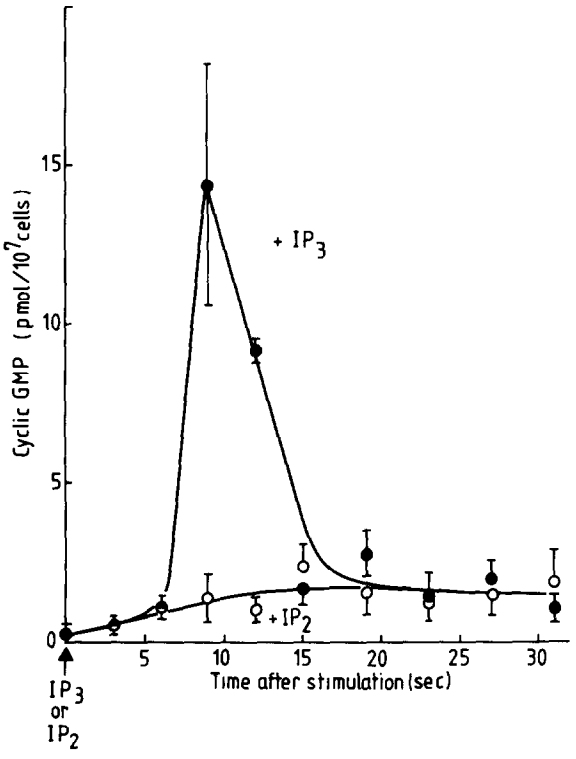

Fig.3. Time course of cGMP formation in response to a pulse of 5 μM IP_3 (●) or 5 μM IP_2 (○). Error bars indicate SE.

4. DISCUSSION

Our results demonstrate that addition of Ca^{2+} but not K^+, Na^+, Mg^{2+}, Mn^{2+} or IP_2 to permeabilised amoebae stimulates the rapid formation of a transient peak of cGMP comparable with that elicited by addition of 5 μM IP_3. This strongly supports the hypothesis that signal transmission in *D. discoideum* operates via IP_3 and Ca^{2+}. Concurrent studies in this laboratory showing intracellular release of Ca^{2+} by IP_3 from non-mitochondrial stores [24] reinforce this viewpoint.

How cGMP formation is regulated by the Ca^{2+} is as yet unclear. Guanylate cyclase has been reported not to be activated in vitro by added Ca^{2+} [25], so the observed stimulation in vivo seems likely, from this evidence, to involve yet another intermediate signalling molecule, as yet undefined.

The precise role of the cGMP in chemotactic signalling is also as yet unknown. It does not appear to control the rapid actin polymerization changes associated with chemotaxis, as the actin response is normal in streamer F mutants which show highly abnormal cGMP metabolism [10]. However, these streamer mutants are very abnormal in their subsequent movement phase (starting about 60 s after chemotactic stimulation) during which the amoebae elongate and cell translocation occurs, and we suggest that it is this phase of chemotaxis that is regulated by the level of cGMP.

ACKNOWLEDGEMENTS

We wish to thank Frank Caddick for his help in drawing the figures, Jon Shatwell and Ian Crandall for reading the manuscript and the SERC for financial assistance.

REFERENCES

[1] Pan, P., Hall, E.M. and Bonner, J.T. (1972) Nat. New Biol. 237, 181–182.

[2] Konijn, T.M., Van de Meene, J.G.C., Bonner, J.T. and Barkley, D.S. (1967) Proc. Natl. Acad. Sci. USA 58, 1152–1154.

[3] Newell, P.C. (1981) in: Biology of the Chemotactic Response. Society for Experimental Biology Seminar Series vol.12 (Lackie, J.M. and Wilkinson, P.C. eds) pp.89–114, Cambridge University Press, Cambridge.

[4] Van Haastert, P.J.M. and Konijn, T.M. (1982) Mol. Cell Endocrinol. 26, 1–17.
[5] McRobbie, S.J. and Newell, P.C. (1983) Biochem. Biophys. Res. Commun. 115, 351–359.
[6] McRobbie, S.J. and Newell, P.C. (1984) J. Cell Sci. 68, 139–151.
[7] McRobbie, S.J. and Newell, P.C. (1985) Exp. Cell Res. 160, 275–286.
[8] Mato, J.M., Van Haastert, P.J.M., Krens, F.A., Rhijnsburger, E.H., Dobbe, F.C.P.M. and Konijn, T.M. (1977) FEBS Lett, 79, 331–336.
[9] Wurster, B., Schubiger, K., Wick, U. and Gerisch, G. (1977) FEBS Lett. 76, 141–144.
[10] Ross, F.M. and Newell, P.C. (1981) J. Gen. Microbiol. 127, 339–350.
[11] Bulgakov, R. and Van Haastert, P.J.M. (1983) Biochim. Biophys. Acta 756, 56–66.
[12] Coukell, M.B., Cameron, A.M., Pitrie, C.M. and Mee, J.D. (1984) Dev. Biol. 103, 246–257.
[13] Mato, J.M. and Konijn, T.M. (1977) in: Development and Differentiation of the Cellular Slime Moulds (Cappucinelli, P. and Ashworth, J.M. eds) pp.93–103, Elsevier/North-Holland, Amsterdam, New York.
[14] Wurster, B., Bozzaro, S. and Gerisch, G. (1978) Cell. Biol. Int. Rep. 2, 61–69.
[15] Van Haastert, P.J.M., Van Lookeren Campagne, M.M. and Ross, F.M. (1982) FEBS Lett. 147, 149–152.
[16] Coukell, M.B. and Cameron, A.M. (1986) Dev. Genet. 6, 163–177.
[17] Europe-Finner, G.N., McClue, S.J. and Newell, P.C. (1984) FEMS Microbiol. Lett. 21, 21–25.
[18] Europe-Finner, G.N. and Newell, P.C. (1984) FEBS Lett. 171, 315–319.
[19] Europe-Finner, G.N., Tillinghast, H.S. jr, McRobbie, S.J. and Newell, P.C. (1985) J. Cell Sci. 79, 151–160.
[20] Europe-Finner, G.N. and Newell, P.C. (1985) Biochem. Biophys. Res. Commun. 130, 1115–1122.
[21] Berridge, M.J. (1984) Biochem. J. 220, 345–360.
[22] Sussman, M. (1966) Methods Cell Physiol. 2, 397–410.
[23] Van Haastert, P.J.M., Van der Meer, R.C. and Konijn, T.M. (1981) J. Bacteriol. 147, 170–175.
[24] Europe-Finner, G.N. and Newell, P.C. (1986) Biochim. Biophys. Acta, in press.
[25] Padh, H. and Brenner, M. (1984) Arch. Biochem. Biophys. 229, 73–80.