

Extracellular Calcium Changes the Morphology of Induced Pinocytosis in *Amoeba proteus*

Wanda KŁOPOCKA, Anna WASIK and Lucyna GRĘBECKA

Department of Cell Biology, Nencki Institute of Experimental Biology, Warszawa, Poland

Summary. The morphology of pinocytosing *Amoeba proteus* induced by two monovalent cations: Na⁺ and K⁺ were examined at different calcium concentration. It was demonstrated that pinocytotic response of amoeba (number, size and shape of pinocytotic pseudopodia) was related to the amount of Ca²⁺ accumulated on the cell surface.

Key words: amoebae, [Ca²⁺]_o, endocytosis.

INTRODUCTION

In *Amoeba proteus* a typical induced pinocytosis is manifested after external application of different agents: aminoacides, proteins, monovalent cations. This pinocytosis is a kind of fluid-phase endocytosis driven by cortical cytoskeleton and may be compared to macropinocytosis in *Dictyostelium discoideum* in which large fluid-filled vesicles are formed (Maniak 2002). During induced pinocytosis *A. proteus* attains a rosette form with specialized pinocytotic pseudopodia containing channel-like invaginations. The morphology of pinocytosing amoebae: number, shape, and size of pinocytotic pseudopodia and polarity of their distribution on the cell surface vary depending on the kind of

inducer, as it was demonstrated for two monovalent cations: Na⁺ and K⁺ (Grębecka and Kłopocka 1986). Since Na⁺ and K⁺ can also exert variable effects on the pattern of Ca²⁺ shifting between the surface of amoeba and the medium during induction of pinocytosis (Kłopocka *et al.* 2000), it seems possible that there is a relationship between the type of pinocytotic reaction and the behaviour of calcium at the cell surface.

MATERIALS AND METHODS

Amoeba proteus was cultured in Pringsheim medium with standard concentration of Ca²⁺ (0.85 mM) and fed twice a week on *Tetrahymena pyriformis*. Before experiments cells were starved for 2 days. All experiments were carried out at room temperature.

The course of pinocytosis was compared in amoebae exposed to different concentrations of usually used pinocytotic inducers: KCl and NaCl, in the standard Pringsheim medium, and in Pringsheim solution with modified [Ca²⁺]. Pinocytosis was induced by 30 mM KCl, 125 mM KCl and 125 mM NaCl in standard concentration of

Address for correspondence: Wanda Kłopocka, Department of Cell Biology, Nencki Institute of Experimental Biology, ul. Pasteura 3, 02-093 Warszawa, Poland; E-mail: aniak@nencki.gov.pl

Ca^{2+} (0.85 mM), by 125 mM KCl in $[\text{Ca}^{2+}]_e$ increased up to 4.85 mM, and by 30 mM KCl and 125 mM NaCl in the presence of 10 mM EGTA. Calcium concentration either was changed before application of the inducer or during pinocytosis.

Changes in the amount of cell-associated Ca^{2+} during pinocytotic induction were assessed by adding $^{45}\text{Ca}^{2+}$ (0.1 mCi/ml, Amersham Life Science, Little Chalfont, Buckinghamshire, England) according to the procedure described earlier (Kłopocka *et al.* 2000). Calcium shifts between the cell surface and the surrounding medium are shown as per cent increase or decrease of cell-associated radioactivity during the experiment. Pinocytosis was controlled 7, 12 and 17 min after application of the inducer.

Observations were carried out in the differential interference contrast (Pluta system, PZO Warsaw) and recorded on the tape with a Panasonic wv BL 600 camera and NV-8051 Panasonic time-lapse video recorder. Selected frames of various pinocytotic forms were stored in an IBM PC compatible computer memory. The morphology of pinocytosis is shown by scanning electron microscopy (SEM) of amoebae fixed and processed as it was previously described elsewhere (Grębecki *et al.* 2001).

RESULTS AND DISCUSSION

It was the purpose of present investigations to reveal whether there is a relationship between the decrease or increase of calcium amount on the cell surface and the pinocytotic response induced by two different monovalent cations: K^+ and Na^+ .

Pinocytotic reaction of amoeba was graduated and characterised by a distinct polarity (the first pinocytotic structures appeared in the uroidal region, next ones at the former fronts) and by the development of numerous small pseudopodia with thin channels, after application of 125 mM NaCl (Fig. 1) or 30 mM KCl (Fig. 2) under standard medium conditions, and during pinocytosis induced by 125 mM KCl in the medium with $[\text{Ca}^{2+}]_e$ increased up to 4.85 mM (not shown). It means that this kind of pinocytosis occurred, when Ca^{2+} was binding to amoebae during stimulation, more or less similar as to the control cells (Fig. 5).

Stimulation by 125 mM K^+ applied in standard Pringsheim medium caused immediate cell contraction, the first pinocytotic pseudopodia were formed in any place of strongly deformed cells and, as a result, amoebae produced few large pseudopodia with very wide channels (Fig. 3). This was accompanied with a decrease of $[\text{Ca}^{2+}]_e$ associated to the surface of amoeba (Fig. 5). Similar coincidence between the alteration of the morphological features of pinocytosis and the displacement of calcium from the cell surface could be produced as well during pinocytosis induced by 125 mM

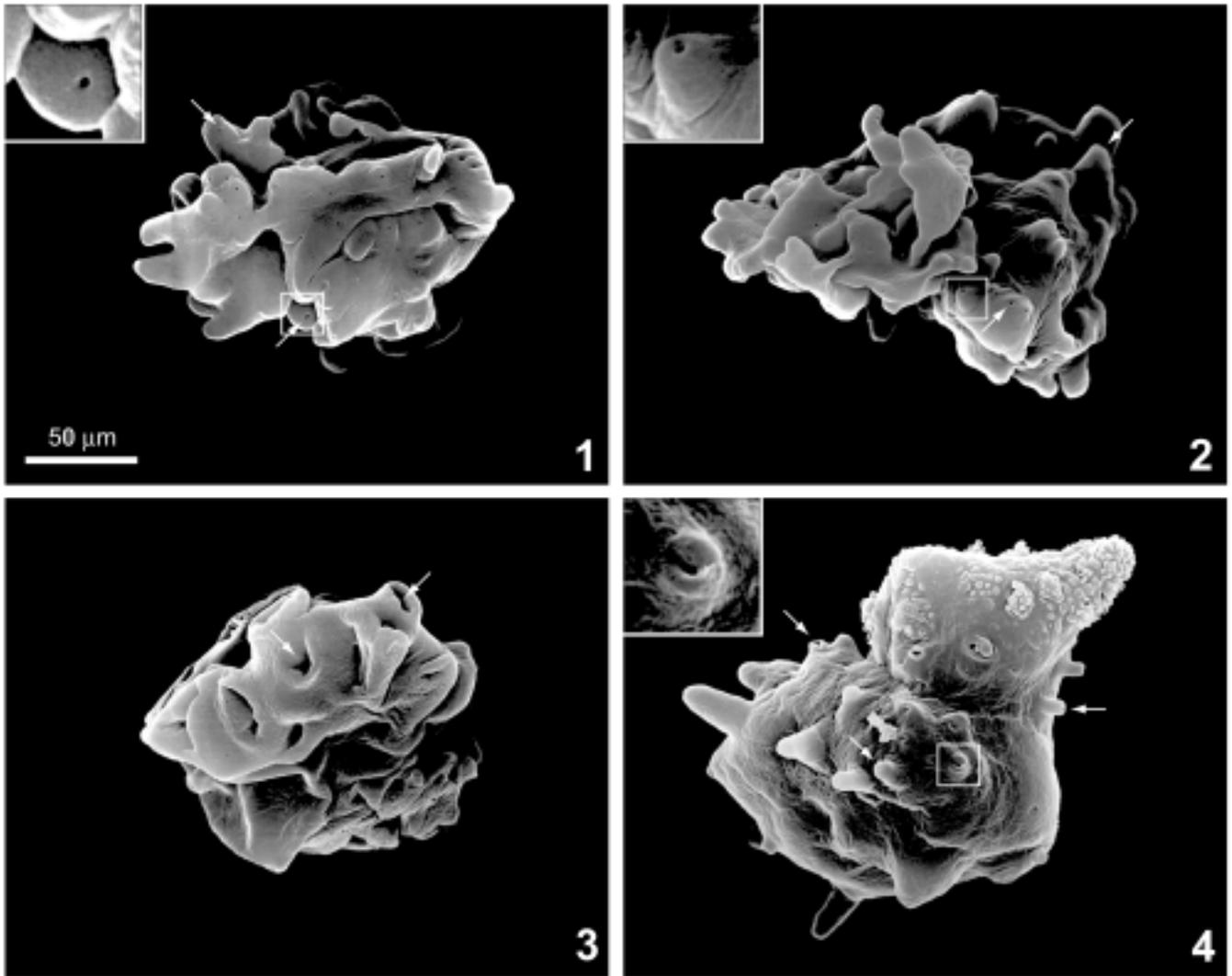
NaCl and 30 mM KCl by Ca^{2+}_e chelating by EGTA (not shown).

On the contrary, addition of 4 mM CaCl_2 during pinocytosis induced by 125 mM K^+ , that is increasing $[\text{Ca}^{2+}]_e$ from 0.85 mM up to 4.85 mM, caused immediate association of more calcium with the cell surface (Fig. 5) accompanied by changes in the morphology of pinocytosis. Within 30s after application of calcium, the pinocytosing cells with few and large pseudopodia (such as shown in Fig. 3) became rosettes with numerous, small pseudopodia with thin channels (Fig. 4).

In general, it may be concluded that changes in the concentration of extracellular calcium associated during pinocytosis with the cell membrane outer surface and/or with glycocalyx caused immediate alterations of size and number of existing pinocytotic structures.

One can suggest that the course of events during macropinocytosis in *Amoeba proteus* depends on the direction of extracellular calcium shifting between amoebae and the medium during cells stimulation. It was postulated that extracellular calcium plays an important role in controlling the physiological state of the plasma membrane in amoebae (Brandt and Freeman 1967, Brandt and Hendil 1972, Kukulis *et al.* 1986). Low concentration of Ca^{2+}_e in the presence of other cations can induce such cellular activities as endocytotic membrane internalisation (Marshall and Nachmias 1965, Hendil 1971, Braatz-Schade and Haberey 1975, Josefsson 1975, Stockem 1977), whereas high concentration of Ca^{2+}_e has a stabilising effect on the membrane (Gingell 1972). In our experiments neither 125 mM Na^+ nor 30 mM K^+ could not induce pinocytosis in *A. proteus* in $[\text{Ca}^{2+}]_e$ increased up to 4.85 mM.

The polarity of pinocytosis is probably related to stability of the cell membrane. When externally bound Ca^{2+} is substituted by the inducer, as it takes place in 125 mM K^+ in standard medium, the membrane potential immediately decreases around the whole cell (Braatz-Schade and Haberey 1975, Josefsson *et al.* 1975) and the first pinocytotic pseudopodia can develop in any place of the surface of amoeba. If calcium is not displaced from the cell surface by the inducing agent, it is in 125 mM Na^+ or 30 mM K^+ in the standard medium and 125 mM K^+ in the increased $[\text{Ca}^{2+}]_e$, the first channels are formed at the uroid where suitable conditions for a permanent pinocytosis exist (Wohlfarth-Bottermann and Stockem 1966), because of the strong membrane folding (Czarska and Grębecki 1966) and its low stability in this area (Batueva 1965a, b; Bingley



Figs 1-4. Morphology of *Amoeba proteus* pinocytosis shown in SEM. In all pictures arrowheads indicate pinocytotic structures. Scale bar on Fig. 1 applies to all figures. **1**- pinocytosis 7 min after induction by 125 mM NaCl introduced to the standard Pringsheim medium. **2** - pinocytosis induced for 7 min by low (30 mM) concentration of KCl in the culture medium. **3** - pinocytosis stimulated 7 min by high (125 mM) concentration of KCl in the culture medium. **4** - pinocytosing amoeba initially stimulated 7 min by 125 mM KCl, and then followed by addition of 4 mM CaCl_2 . Insets in Figs 1, 2, 4 are digitally enlarged 3.5 x.

1966). Next channels are formed at the front when it begins to contract (Grębecka and Kłopocka 1985, Grębecka 1988) and similar conditions as in uroid are established.

Calcium also controls cortical cytoskeleton functions that are responsible for cell shape changes and pseudopodia formation: reorganisation of cortical cytoskeleton in amoebae (Hellewel and Taylor 1979, Taylor and Fehheimer 1982, Bray 1992) and interactions of actin microfilaments with the plasma membrane (Taylor *et al.* 1980, Kawakatsu *et al.* 2000), and thus it may influence the course of pinocytosis. According to the view of

Maeda and Kawamoto (1986) a low $[\text{Ca}^{2+}]$ activates pinocytosis in *Dictyostelium discoideum* because under such conditions the content of actin in the cell cortex and consequently the number of membrane-associated microfilaments are reduced, which may restrict the plasma membrane flexibility, necessary for pinocytotic structures formation. Our results seem to confirm this hypothesis and indicate that membrane flexibility in *A. proteus* may influence the number and size of pinocytotic structures.

According to Klein *et al.* (1988) the size of pinocytotic pseudopodia and the diameter of channels are

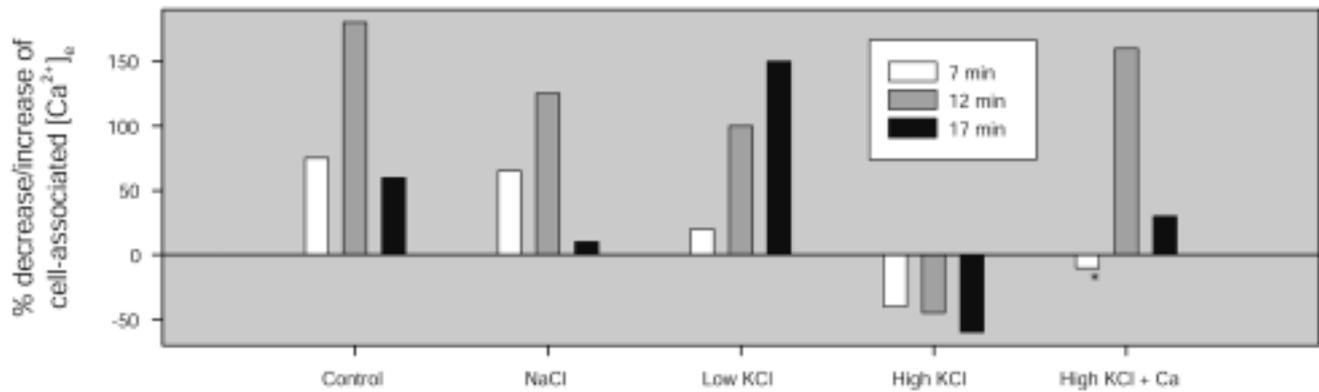


Fig. 5. Per cent changes in the increase or decrease of $[Ca^{2+}]_0$ associated with control cells, during pinocytosis induced by 125 mM NaCl, 30 mM KCl and 125 mM KCl under standard conditions in Pringsheim medium, and 125 mM KCl followed by application of 4 mM $CaCl_2$ (asterisk indicates the moment of calcium application).

regulated by the mode of membrane-microfilament interaction. Pinocytotic pseudopodia are developed due to the circular detachment of the microfilament layer from the plasma membrane with the exception of a central region of fast contact (Stockem *et al.* 1983, Grębecki 1991). The diameter of the channels depends on the extent of the areas of contact between the microfilament layer and the internal face of the plasma membrane (Klein *et al.* 1988). The size of pseudopodia is related to the area of detachment and probably the contraction degree. It seems possible that Ca^{2+}_0 can control, *via* the membrane of amoeba, the rearrangement of actin cortical network and its attachment to the cell surface.

Conclusions:

1. The pinocytotic response of *Amoeba proteus* is related to the amount of Ca^{2+} accumulated on the cell surface during the induction of this phenomenon.
2. The direction of calcium shifting during the initiation of pinocytosis depends on the strength of inducer, its concentration, and the concentration of extracellular calcium.

Acknowledgement. Authors wish to express their sincere gratitude to Prof. A. Grębecki for his constructive suggestions for improvement and modification of the manuscript. Authors also thank to Dr. P. Pomorski for his help in preparation of figures.

REFERENCES

- Batueva I. V. (1965a) The electrical parameter of *Amoeba proteus* I. Motionless amoebae. *Cytologia* **7**: 188-196
- Batueva I. V. (1965b) The electrical parameters of *Amoeba proteus*. II. The motile amoebae. *Cytologia* **7**: 553-561
- Bingley M. S. (1966) Membrane potentials in *Amoeba proteus*. *J. Exp. Biol.* **45**: 251-267
- Braatz-Schade K., Haberey M. (1975) Bioelectrical potentials and motile activity in *Amoeba proteus*. *Cytobiologie* **11**: 87-94
- Brandt P. W., Freeman A. R. (1967) Plasma membrane: Substructural changes correlated with electrical resistance and pinocytosis. *Science* **155**: 582-585
- Brandt P., Hendil K. B. (1972) Plasma membrane permeability and pinocytosis in *Chaos chaos*. *C. R. Trav. Lab. Carlsberg* **38**: 423-443
- Bray D. (1992) Integration of cell movement. In: Cell Movements (Ed. D. Bray) Garland Publishing, Inc. New York and London 309-375
- Czarska L., Grębecki A. (1966) Membrane folding and plasma-membrane ratio in the movement and shape transformation in *Amoeba proteus*. *Acta Protozool.* **4**: 201-239
- Gingell D. (1972) Cell membrane surface potential as a transducer. In: Membranes and Ion Transport **3**: 317-357
- Grębecka L. (1988) Polarity of the motor functions in *Amoeba proteus* I. Locomotory behaviour. *Acta Protozool.* **27**: 83-96
- Grębecka L., Kłopocka W. (1985) Relationship between the surface distribution of membrane reserves and the polarity of pinocytosis in *Amoeba proteus*. *Protistologica* **21**: 207-213
- Grębecka L., Kłopocka W. (1986) Morphological differences of pinocytosis in *Amoeba proteus* related to the nature of pinocytotic inducer. *Protistologica* **22**: 265-270
- Grębecki A. (1991) Participation of the contractile system in endocytosis demonstrated *in vivo* by video-enhancement in heat-treated amoebae. *Protoplasma* **160**: 144-158
- Grębecki A., Grębecka L., Wasik A. (2001) Minipodia, the adhesive structures active in locomotion and endocytosis of amoebae. *Acta Protozool.* **40**: 235-247
- Hellewell S. B., Taylor D. L. (1979) The contractile basis of amoeboid movement VI. The solution-contraction coupling hypothesis. *J. Cell Biol.* **83**: 633-648
- Hendil K. B. (1971) Ion exchange properties of the glycocalyx of the amoeba *Chaos chaos* and its relation to pinocytosis. *C. R. Trav. Lab. Carlsberg* **38**: 187-211
- Josefsson J.-O. (1975) Studies on the mechanism of induction of pinocytosis in *Amoeba proteus*. *Acta Physiol. Scand.* **97** (Suppl. **423**): 1-65
- Josefsson J.-O., Holmer N. G., Hansson S. E. (1975) Membrane potential and conductance during pinocytosis induced in *Amoeba proteus* with alkali metal ions. *Acta Physiol. Scand.* **94**: 278-288
- Kawakatsu T., Kikuchi A., Shimmen T., Sonobe S. (2000) Interaction of actin filaments with the plasma membrane in *Amoeba proteus*: studies using a cell model and isolated plasma membrane. *Cell Struct. Funct.* **25**: 269-277

- Klein H. P., Köster B., Stockem W. (1988) Pinocytosis and locomotion in amoebae. XVIII. Different morphodynamic forms of endocytosis and microfilament organization in *Amoeba proteus*. *Protoplasma* **2 (Suppl.)**: 76-87
- Kłopočka W., Balińska M., Kołodziejczyk J. (2000) Role of extracellular calcium in the induction of pinocytosis in *Amoeba proteus* by Na and K ions. *Acta Protozool.* **39**: 143-148
- Kukulis J., Ackermann G., Stockem W. (1986) Pinocytosis and locomotion of amoebae. XIV. Demonstration of two different receptor sites on the cell surface of *Amoeba proteus*. *Protoplasma* **131**: 233-243
- Maeda Y., Kawamoto T. (1986) Pinocytosis in *Dictyostelium discoideum* cells. A possible implications of cytoskeletal actin for pinocytotic activity. *Exp. Cell Res.* **164**: 516-526
- Maniak M. (2002) Conserved features of endocytosis in *Dictyostelium*. *Int. Rev. Cytol.* **221**: 257-287
- Marshall J. M., Nachmias V. T. (1965) Cell surface and pinocytosis. *J. Histochem. Cytochem.* **13**: 92-104
- Stockem W. (1977) Endocytosis. In: Mammalian Cell Membranes, (Eds. G. A. Jamieson and D. M. Robinson). Butterworths, London, **5**: 151-195
- Stockem W., Naib-Majani W., Wohlfarth-Bottermann K. E. (1983) Pinocytosis and locomotion in amoebae. XIX. Immunocytochemical demonstration of actin and myosin in *Amoeba proteus*. *Eur. J. Cell Biol.* **29**: 171-178
- Taylor D. L., Fechheimer M. (1982) Cytoplasmic structure and contractility: the solation-contraction coupling hypothesis. *Phil. Trans. R. Soc. Lond. B* **299**: 185-197
- Taylor D. L., Blinks J. R., Reynolds G. (1980) Contractile basis of ameboid movement VIII. Aequorin luminescence during ameboid movement, endocytosis, and capping. *J. Cell Biol.* **86**: 599-607
- Wohlfarth-Bottermann K. E., Stockem W. (1966) Pinocytose und Bewegung von Amöben II. Permanente und Induzierte Pinocytose bei *Amoeba proteus*. *Z. Zellforsch.* **73**: 444-474

Received on 28th March, 2003; accepted on 31st March, 2003