Volume 235, number 1,2, 141–143 FEB 06131 August 1988

Nerve growth factor induces rapid redistribution of F-actin in PC12 cells

Heiti Paves, Toomas Neuman, Madis Metsis and Mart Saarma

Laboratory of Molecular Genetics, Institute of Chemical Physics and Biophysics, Estonian Academy of Sciences, Akadeemia 23, 200026 Tallinn, Estonian SSR, USSR

Received 23 May 1988

Nerve growth factor (NGF) induces the redistribution of F-actin in rat pheochromocytoma PC12 cells within 2–10 min, whereas epidermal growth factor (EGF) has no effect on microfilament organization. This redistribution of F-actin in PC12 cells is not protein synthesis dependent, but can be blocked by methyltransferase inhibitors.

Nerve growth factor; F-Actin; Methyltransferase; Enzyme inhibitor; Insulin; (PC12 cell)

1. INTRODUCTION

Nerve growth factor is a target-derived trophic protein which acts as a regulator of the survival, development, and maintenance of sympathetic and some sensory neurons [1]. In the presence of NGF, the pheochromocytoma PC12 cell line [2] acquires some of the properties of sympathetic neurons, such as neurite outgrowth [2], increased electric excitability [2], and increased levels of acetylcholine receptors [2]. All of these changes occur slowly over a period of several days. In contrast, NGF also induces some rapid changes in PC12 cells. Within several minutes the morphology of the cell surface [2] and the level of phosphorylation of several proteins are changed [3]. In addition, the genes of c-*fos* and β-actin are also induced within several minutes [4]. Similar changes are also induced by EGF [2–4].

In almost all cell lines, EGF and other mitogenic growth factors decrease the adhesion properties of the cells, accompanied by the disappearance of actin-containing stress fibres [5]. Since NGF has mitogenic activity on PC12 cells before differentiation and cessation of the division of these cells [6,7] and increases the adhesion properties of the target cells [8], it was of interest to study the state of the actin skeleton.

We report here that NGF causes rapid formation of F-actin bundles in PC12 cells, whereas EGF has no similar effects on the redistribution of F-actin.

Correspondence address: M. Saarma, Laboratory of Molecular Genetics, Institute of Chemical Physics and Biophysics, Akadeemia 23, 200026 Tallinn, Estonian SSR, USSR

2. MATERIALS AND METHODS

PC12 cells (kindly provided by Dr J. Patrick, Salk Institute) were grown in DMEM medium (Flow Labs, Scotland) containing 10% horse serum. For staining with rhodamine-phalloidin [5] the cells were grown on glass coverslips coated with poly(L-lysine) (Serva, FRG). Mouse 7 S NGF (provided by Dr V. Kaljunov, Minsk), mouse 2.5 S NGF (Sigma, USA), bovine NGF (gift from Dr E. Severin) and *Vipera lebetina* venom NGF [9] were diluted in serum-free medium to the required final concentration. Insulin, 5′-deoxy-5′-methylthioadenosine (MTA) and adenosyl-D-homocysteine (SAH) were also used and were obtained from Sigma. All experiments were carried out in serum-free medium.

3. RESULTS AND DISCUSSION

Since the discovery that phalloidin binds specifically to F-actin, fluorochrome-conjugated phalloidins have been widely used to stain F-actin in different cells.

In untreated PC12 cells, F-actin shows diffuse

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

staining with punctuate spots in the periphery of cells (fig.1A). 2–3 min after NGF addition F-actin shows rosette-like staining in the periphery of cells (fig.1B). During the next 5–7 min F-actin forms 2–4 groups (patches) of bundles (fig.1C). This distribution of F-actin remains unchanged for at least 2 days, even if NGF is excluded from growth media after 10 min.

Several days after NGF treatment, when neurite outgrowth appears in PC12 cells, F-actin has the same distribution in cell bodies as above. In addition, F-actin bundles are visible in growth cones and in the branching points of neurites (fig.1D), i.e. at the points of contact of neurite with substratum.

To examine the dose dependence of the redistribution of F-actin cells were treated with various concentrations of 7 S NGF for 10 min.

It is evident from table 1 that the minimal concentration of NGF required to initiate redistribution of F-actin is 2.5 ng/ml. In our experiments, neurite outgrowth in PC12 cells appears at 2-fold higher NGF concentration. NGF-induced F-actin redistribution is not specific for mouse 7 S NGF. Mouse 2.5 S NGF, bovine βNGF and *V. lebetina* venom NGF induce identical, rapid changes in distribution of F-actin in PC12 cells.

Bundling of microfilaments is also induced by insulin (table 1). It is possible to suggest that insulin acts through binding to the NGF receptor [10], and activates at least some second messenger systems common to NGF.

Treatment of PC12 cells with different concentrations of EGF does not induce any redistribution of F-actin (table 1).

The addition of cycloheximide, at 1 mM for 30 min before NGF addition, which completely inhibits protein synthesis in PC12 cells, has no effect

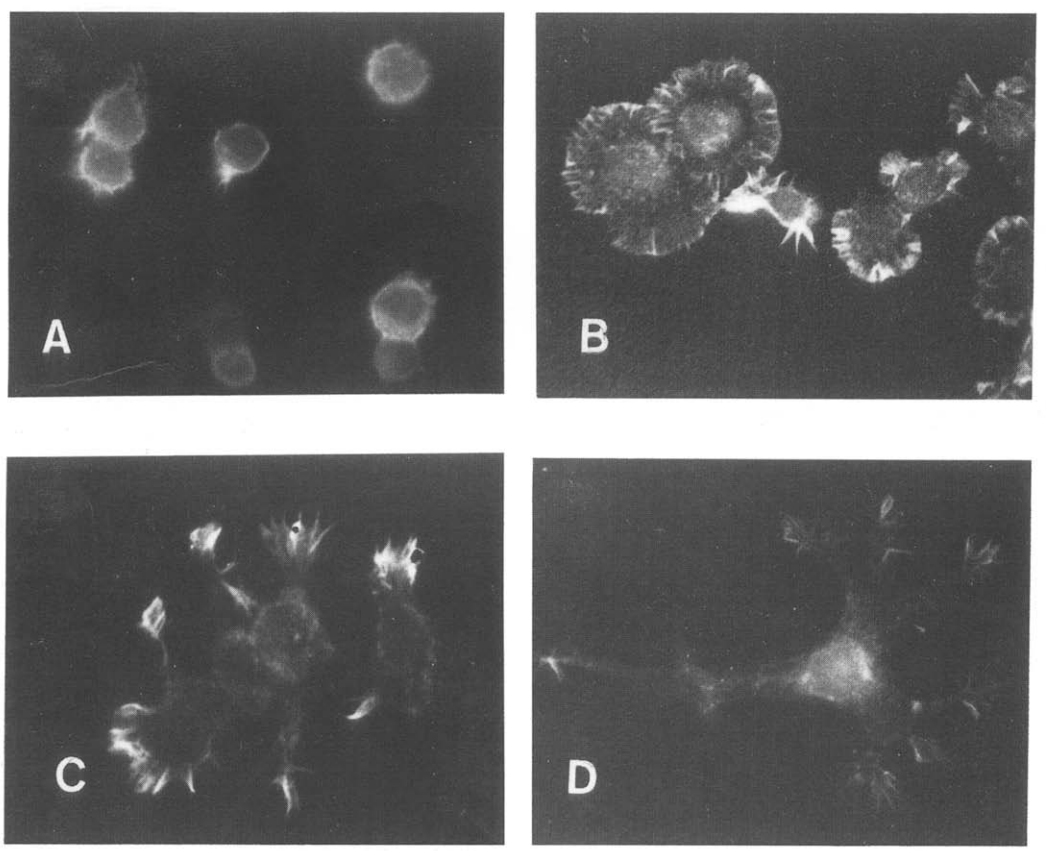

Fig.1. PC12 cells staining with rhodamine-phalloidin during NGF treatment. (A) Untreated; (B) treated with 50 ng/ml mouse 7 S NGF for 2 min, (C) 10 min, (D) 2 days. Magnification × 900.

Table 1

Redistribution of F-actin staining in PC12 cells 10 min after ligand addition

Ligand	Dose (ng/ml)	Appearance of F-actin bundles
None (control)	–	–
Mouse 7 S NGF	2.5–500	+
	1	–
Mouse 2.5 S NGF	50[a]	+
Bovine βNGF	500[a]	+
V. lebetina venom NGF	500[a]	+
EGF	0.1–100	–
Insulin	500	+
Mouse 7 S NGF after 1 mM cycloheximide treatment for 30 min	50	+
Mouse 7 S NGF with 2.5–20 mM SAH	50	–
Mouse 7 S NGF with 3 mM MTA	50	–

[a] Other NGF concentrations not tested

on the redistribution of F-actin (table 1). Therefore, the NGF-induced rapid changes in F-actin distribution are not protein synthesis dependent.

Seeley et al. [11] have reported differential inhibition of NGF and EGF effects by several methylation inhibitors. In this respect, the rapid inhibition of NGF-induced F-actin bundling by MTA and SAH (table 1) indicates that methylation might be the earliest molecular event triggered by NGF in PC12 cells.

The present data allow us to propose that the well-established NGF-induced rapid changes in the PC12 cell surface [2] are related with the rapid redistribution of F-actin. EGF has no influence on the F-actin distribution over a wide range of concentrations studied. EGF and NGF induce many similar changes in PC12 cells (surface changes, c-*fos* activation, etc.), which require similar sets of second messengers. The difference in action of NGF and EGF on F-actin redistribution allows us to assume that this process is triggered by different second messengers. This hypothesis is supported by the observation that the inhibition of methyltransferases in some cases blocks the effect of NGF, but not the action of EGF on PC12 cells [11].

The involvement of different second messenger systems in triggering the redistribution of F-actin is under investigation using specific inhibitors.

Acknowledgements: The authors are indebted to T. Espe and U. Järv for preparation of the manuscript.

REFERENCES

[1] Vinores, S. and Guroff, G. (1980) Annu. Rev. Biophys. Bioeng. 9, 223–257.
[2] Greene, L.A. (1978) Adv. Pharmacol. Ther. 10, 197–206.
[3] Halegoua, S. and Patrick, J. (1980) Cell 22, 571–581.
[4] Greenberg, M.E., Greene, L.A. and Ziff, E.B. (1985) J. Biol. Chem. 260, 14101–14110.
[5] Herman, B. and Pledger, W.J. (1985) J. Cell Biol. 100, 1031–1040.
[6] Burstein, D.E. and Greene, L.D. (1982) Dev. Biol. 94, 477–482.
[7] Saarma, M., Timmusk, T., Paves, H., Metsis, M., Arumäe, U., Kelve, M. and Neuman, T. (1988) in: Macromolecules in the Functioning Cell (Ruffo, A. et al. eds) vol.17, pp.67–76, Consiglio Nazionale delle Ricerche Quadermi de La Ricerca Scientifica.
[8] Schubert, D. and Whitlock, C. (1977) Proc. Natl. Acad. Sci. USA 74, 4055–4058.
[9] Siigur, E., Neuman, T., Järve, V., Tara, A. and Siigur, J. (1985) Comp. Biochem. Physiol. 81B, 211–215.
[10] Frazier, W.A., Hogue-Angeletti, R. and Bradshaw, R.A. (1972) Science 176, 482–488.
[11] Seeley, P.J., Rukenstein, A., Connolly, J.L. and Greene, L.A. (1984) J. Cell Biol. 98, 417–426.