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The influence of nerve growth factor on the activities of adenylate cyclase and high-affinity GTPase in pheochromocytoma PC12 cells

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The influence of nerve growth factor (NGF) on the activities of adenylate cyclase and high-affinity GTPase in pheochromocytoma PC12 cells was studied. Incubation of cells with nerve growth factor led to a rapid activation of adenylate cyclase accompanied by an inhibition of high-affinity GTPase. By the 10th min of incubation the activity of adenylate cyclase had been reduced 2-fold when compared to the control. The activity of GTPase, however, increased. No significant changes in the cAMP level were detected. The data obtained indicate that NGF interaction with PC12 cells induces changes in the adenylate cyclase system and this process involves G-proteins that regulate the adenylate cyclase activity.

Nerve growth factor; Adenylate cyclase; GTPase; (PC12 cell)

1. INTRODUCTION

Nerve growth factor (NGF) is a protein determining the normal development and functioning of sympathetic, sensory and a number of central neurons (for review see [1]). The mechanism of NGF action on target cells is unknown at present despite the fact that specific receptors of the factor have been discovered and characterized and its effect on several systems (the systems of phosphoinositide turnover [2], calcium regulation [3], phospholipid methylation [4], and the guanylate cyclase system [5]) of second messengers has been demonstrated. The data on the effect of NGF on the adenylate cyclase system are rather contradictory. In some studies [6] NGF was shown to increase the intracellular level of cAMP, in others no increase was observed [7]. A variety of substances increasing the intracellular cAMP level acted synergistically with NGF [8]. In some studies [9] these substances were shown to cause NGF-like morphological differentiation.

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The objective of the present work is to study the influence of NGF on the activity of adenylate cyclase in membrane preparations of pheochromocytoma PC12 cells which are widely used for studying the mechanism of NGF action [10]. Besides, we studied the activity of high-affinity GTPase of PC12 cells and its changes under the action of NGF. GTPase activity with a high affinity for substrate is characteristic of G-proteins which include regulatory subunits of the adenylate cyclase enzymatic complex [11].

It is shown here that NGF is able to cause a rapid activation of adenylate cyclase with a subsequent reduction of the activity to a level lower than in the control experiments. The activity of high-affinity GTPase changed in the opposite way: inhibition was followed by an increase in the activity.

2. MATERIALS AND METHODS

Pheochromocytoma PC12 cells were cultured in DMEM as described previously [12]. NGF was isolated from bovine seminal plasma by the method of Kondratyev et al. [12] and added to living cells or to a membrane preparation in a final concentration of 1.9 nM.

To isolate the membrane fraction, the culture medium was removed and the cells were washed twice in cold (4°C) Hank's

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balanced salt solution. All subsequent procedures were performed at 4°C. The cells were placed in a cold lysing buffer containing 10 mM Tris-HCl, pH 7.0, 1 mM EDTA, 0.1 mM dithiothreitol and 4 mM $MgCl_2$ and then homogenized in a Teflon-glass homogenizer (B. Braun). The homogenate was centrifuged at 10000 × g for 4 min in a Biofuge A centrifuge (Heraeus). The supernatant was centrifuged at 100000 × g for 60 min on a Beckman L8-55 ultracentrifuge. The precipitate was resuspended in the lysing buffer and the enzyme activities were determined.

The activity of adenylate cyclase was determined by the previously described procedure [13,14]. The reaction was initiated by adding 20 μl of membrane suspension (10–40 μg of protein) to 50 μl of incubation buffer containing 50 mM imidazole-HCl, pH 6.7, 0.1 mM EGTA, 6 mM $MgCl_2$, 1 mM dithiothreitol, 3 U of creatine phosphokinase, 12 mM creatine phosphate and 0.3 mM [α-^{32}P]ATP (410 Ci/mmol) (Amersham). The mixture was incubated for 10 min at 37°C, then it was supplemented with 200 μl of 0.5 M HCl and boiled for 6 min in a water bath. The acid was neutralized by imidazole and the mixture was applied to a column with aluminium oxide (1 g). Radioactive cAMP was removed with distilled water and the radioactivity was measured on a Rackbeta (LKB) spectrometer.

The activity of high-affinity GTPase was determined according to Cassel and Selinger [15]. The reaction was initiated by adding 20 μl of membrane suspension (10–20 μg of protein) to 100 μl of incubation buffer containing 12.5 mM Tris-HCl, pH 7.0, 5 mM $MgCl_2$, 0.1 mM EDTA, 2 mM dithiothreitol, 5 U of creatine phosphokinase, 2 mM creatine phosphate, 0.1 mM ATP, 0.1 mM β,γ-imidoadenosine-5′-triphosphate and 0.5 μM [γ-^{32}P]GTP (>10 Ci/mmol) (Amersham). The mixture was incubated for 10 min at 37°C, placed on paper filters (Whatman 3 MM) which were subsequently washed, and the radioactivity was measured on a Rackbeta (LKB) spectrometer.

The level of cAMP was measured according to Gilman [16].

Protein concentration was determined by the method of Bradford [17].

3. RESULTS AND DISCUSSION

The activity of adenylate cyclase was determined in membrane preparations of pheochromocytoma PC12 cells. The effect of NGF on the adenylate cyclase activity was assessed in two ways (see section 2). In the in vivo experiments (fig.1) PC12 cells were first incubated with NGF for the required period of time, then the incubation was stopped by cooling the cells (for details see section 2), and the membrane fraction was isolated. In the in vitro experiments the membrane fraction was first isolated, and then NGF was added to the reaction mixture to determine the activity of adenylate cyclase. Fig.1 presents the results of the in vivo experiment which show that after a 1-min incubation of the cells with NGF an activation of adenylate

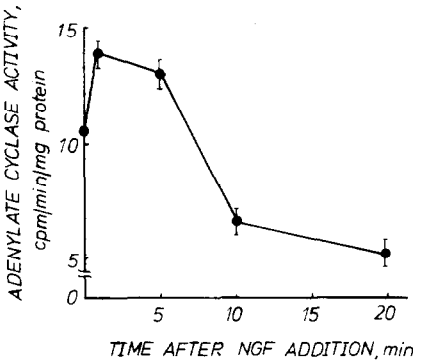

Fig.1. Relationship between the time of incubation of PC12 cells with NGF (1.9 nM) and the activity of adenylate cyclase in a membrane preparation (in vivo experiment). The control activity was 10.7 ± 0.5 cpm/min per mg of protein and remained unchanged throughout the experiment.

cyclase occurred. Then the activity gradually decreased until after the 10th min when the enzyme turned out to be inhibited nearly 2-fold. Thereafter the level of adenylate cyclase activity did not change for up to 24 h (data not shown). In the in vitro experiments we also observed 56.5 ± 1.4% inhibition of adenylate cyclase which, however, was independent of the time of incubation in the presence of NGF. In this case, no activation of the enzyme was observed, which may be due either to the time of reaction (not less than 10 min) or to disturbances in the interaction between NGF and adenylate cyclase upon the destruction of the cell and isolation of membranes. It should be noted that in none of the experiments were significant changes in the cAMP level observed.

It was shown earlier [12] that NGF reduces the level of endogenous ADP-ribosylation of a number of membrane proteins of PC12 cells with MM close to MM of adenylate cyclase regulatory G-proteins. These findings agree with the results of the present work since a decrease in the adenylate cyclase activity in PC12 cells is probably due to a decrease in endogenous ADP-ribosylation of adenylate cyclase G-proteins. To check the involvement of G-proteins in the regulation of NGF-dependent rapid events including modulation of adenylate cyclase activity we determined the activity of high-affinity GTPase characteristic of G-proteins. The activity of high-affinity GTPase in PC12 cells was determined in the presence of NGF under conditions similar to those used for measur-

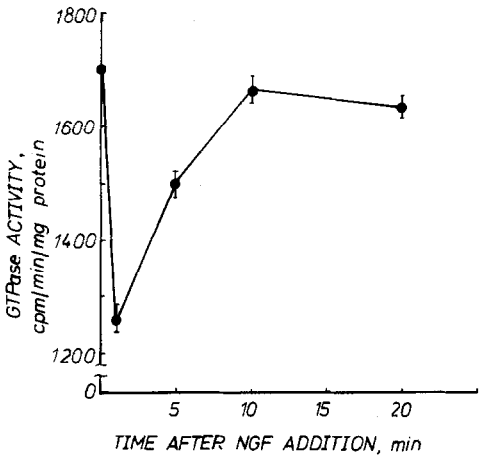

Fig.2. Relationship between the time of incubation of PC12 cells with NGF (1.9 nM) and the activity of high-affinity GTPase in a membrane preparation (in vivo experiment). The control activity was 1690 ± 12 cpm/min per mg of protein and remained unchanged throughout the experiment.

ing the activity of adenylate cyclase (in vivo and in vitro).

Fig.2 presents the data on the effect of NGF on the activity of high-affinity GTPase in PC12 cells depending on the time of incubation in vivo. These data indicate that the character of changes in the activity of high-affinity GTPase is opposite to that of changes in the adenylate cyclase activity under the same conditions. After 1 min of incubation, the activity increased to a level close to the control (i.e. to the level observed in cells not incubated with NGF). In the case of direct addition of NGF to the membrane preparation of PC12 cells, 17.6 ± 0.1% inhibition of the activity of high-affinity GTPase was observed irrespective of the time of incubation with NGF. These differences in the in vivo and in vitro experiments can be attributed to changes in the interaction between NGF receptor and GTPase in the course of membrane isolation.

The data obtained indicate that NGF causes a rapid activation of adenylate cyclase occurring simultaneously with a reduction in the activity of high-affinity GTPase. The activation of adenylate cyclase is followed by its inhibition accompanied by the restoration of the high-affinity GTPase activity. These results in combination with the previously obtained data [12] suggest that the NGF receptor interacts with adenylate cyclase through G-proteins that are the substrates of endogenous NGF-dependent ADP-ribosylation. The absence of activation of high-affinity GTPase may be the result of cumulative action on a number of other G-proteins unrelated to adenylate cyclase. The data obtained indicate that NGF causes changes in the adenylate cyclase system of PC12 cells. However, in combination with the data on the absence of detectable changes in the cAMP level under the action of NGF they do not allow us to regard cAMP as a second messenger through which the effect of NGF on target cells is mediated.

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