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NERVE GROWTH FACTOR-INDUCED REDUCTION IN EPIDERMAL GROWTH FACTOR RESPONSIVENESS AND EPIDERMAL GROWTH FACTOR RECEPTORS IN PC12 CELLS: AN ASPECT OF CELL DIFFERENTIATION

Kenneth R. Huff and Gordon Guroff

Section on Intermediary Metabolism
Laboratory of Developmental Neurobiology
National Institute of Child Health and Human Development
National Institutes of Health
Bethesda, MD 20205

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Summary

The rat pheochromocytoma clone PC12 responds to nerve growth factor through the expression of a number of differentiated neuronal properties. One of the most rapid changes is a large, transient increase in the activity of ornithine decarboxylase. These cells also show an increase in ornithine decarboxylase activity in response to the mitogen, epidermal growth factor, but do not respond morphologically as they do to nerve growth factor. Specific, high-affinity epidermal growth factor receptors are present on the cells. When the cells are differentiated with nerve growth factor, the response to epidermal growth factor is markedly diminished and there is a marked reduction in the binding of epidermal growth factor to the cells.

Introduction

The rat pheochromocytoma clone PC12 is a neural crest-derived adrenergic tumor cell line which has been studied recently as a model of neuronal development (1). This cell demonstrates many aspects of morphological, biochemical, and electrical differentiation in response to nerve growth factor (NGF) (2). Certain non-neural systems respond to a related hormone, epidermal growth factor (EGF), which has some biochemical and biological similarities to NGF, but is mitogenic in many systems and elicits responses in a broader range of cell types than does NGF (3). We and others have recently demonstrated trophic responses of PC12 cells to EGF (4,5), which are also known for NGF (6), and among which is a several-fold increase in the activity of ornithine decarboxylase (ODC). This activity is a nuclear marker in many systems (7), including the NGF-responsive cells of the superior cervical ganglia (8), for impending increases in cellular anabolism. The effect of EGF on ODC activity in PC12 cells is less

Abbreviations: NGF, nerve growth factor; EGF, epidermal growth factor; ODC, ornithine decarboxylase.

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than that produced by NGF, but is not inhibited by antibody to NGF, and appears to be additive to the effect of NGF. Further, EGF binds to specific receptors on PC12 cells and this binding is not inhibited by the presence of high concentrations of NGF. The influence of EGF on thymidine incorporation is opposite to that of NGF, and, although EGF produces changes in the surface properties of PC12 cells it leads to only minimal neurite production. Thus, EGF, like NGF, produces an increase in ODC activity in these cells after binding to specific membrane receptors, but appears to have different effects on other developmental parameters (4).

Because of the implications to cell division, metabolic maturation, and cellular differentiation of the responses of the PC12 clone to these two different growth factors, it seemed appropriate to examine further the developmental roles and the possible interactions of the factors. We now report a reduction of the response to EGF in ODC activation after NGF treatment and a concomitant reduction of receptor binding for EGF, indicating a modulatory effect of NGF over the ability of the cell to respond to EGF after NGF-induced differentiation *in vitro*.

Materials and Methods

PC12 cells were cultured in Falcon flasks in Dulbecco's Modified Eagle Medium (Grand Island Biological Company) containing 15% fetal calf serum and treated with 2.5 S NGF (9) and EGF (10) as described in Table 1. Ornithine decarboxylase activity was measured on the supernatant fraction of cell homogenates using [1-^{14}C]ornithine (11). EGF was iodinated using chloramine T and talcum (12). Binding studies were done as described in Table 2 (13). Protein was measured by using appropriate modifications of the Lowry method (14).

Results

Table 1 shows that pretreatment of the PC12 cells with NGF for 3 days largely prevents the response in ODC activity 5 hours after EGF addition to the cultures. By this time profuse neurite outgrowth can be seen in the treated cultures, and the activity of ornithine decarboxylase, increased by the addition of nerve growth factor, has returned to base-line (6). The results shown in Table 2 indicate a possible reason for this lack of response. Binding of ^{125}I-labeled EGF to untreated control cells, which is specific and inhibited by unlabeled EGF but not by high concentrations of NGF, is markedly reduced in PC12 cells grown in the

TABLE 1. The effect of epidermal growth factor on ornithine decarboxylase levels in nerve growth factor-treated and control PC12 cells.

Cells	Treatment	Ornithine decarboxylase activity
		nmoles $^{14}CO_2$ released/mg protein per h
Control	None	1.22
	EGF	7.56
NGF-treated	None	1.01
	EGF	2.31

PC12 cells were plated in 25 cm^2 Falcon flasks in 7 ml of DMEM + 15% fetal calf serum. Three days later the medium was changed; the control cells received fresh DMEM-15% fetal calf serum, the treated cells received comparable medium containing 2.5 S nerve growth factor (10 ng/ml). This medium was removed and fresh control or NGF-containing medium added two days later. Twenty-four hours later epidermal growth factor was added (100 ng/ml). The flasks were kept at 37° for 5 hours and then the medium was removed. The cells were collected in 4.5 ml of cold 0.32 M sucrose - 0.01 M Tris pH 7.4. They were centrifuged, resuspended in 4.5 ml of fresh sucrose-Tris, centrifuged again, and finally dispersed in 0.2 ml of ornithine decarboxylase homogenizing buffer and frozen on dry ice. The next day the cells were thawed, homogenized in an all glass homogenizer, centrifuged, and the supernatant fraction assayed for ornithine decarboxylase activity and for protein. The data presented are the results of a single experiment; five independent experiments with slight differences in amount of growth factor added or time of treatments gave comparable results.

TABLE 2. The binding of ^{125}I-epidermal growth factor to nerve growth factor-treated and control PC12 cells

Cells	Specific binding of epidermal growth factor	
	cpm/μg protein	
	Experiment 1	Experiment 2
Control	762 (448)	416 (244)
NGF-treated	131 (131)	81 (81)

PC12 cells were grown as described in Table 1. Epidermal growth factor (4μg) was iodinated by the chloramine T-talcum procedure (12). Labeled epidermal growth factor (6 x 10^6 cpm per flask, 1.6 ng/ml) was added to the cells in fresh medium. The cells were kept at 24° for 40 minutes, rinsed rapidly once with cold DMEM and once with cold phosphate-buffered saline and then detached from the surface by vigorous agitation in 5 ml of phosphate-buffered saline. Specific binding was evaluated by subtracting the counts bound to the cells using ^{125}I-epidermal growth factor after a 10 minute preincubation in the presence of 350 ng/ml of unlabeled epidermal growth factor from those bound in its absence. In the presence of this excess of epidermal growth factor control cells bound 265 cpm/μg protein and NGF-treated cells bound 254 cpm/μg protein. The numbers in parentheses indicate the cpm/10^3 cells.

presence of NGF for 3 days. EGF receptor-mediated activation of ODC and any related or ensuing transcriptional or mitogenic nuclear effects would thus not be possible in these differentiated cells.

Discussion

The presence of receptors for such a ligand as EGF does not always imply a biologic response (15), but in this system we have found a correlation between ODC activation following EGF treatment and the level of specific EGF receptors. It could be that the ODC induction system is simply refractory, but this seems unlikely since ODC levels rise normally upon addition of dibutyryl cyclic AMP. The full significance of ODC activation at the molecular level is still unknown, and it may not be an essential intermediate in all cellular responses to a hormone. Indeed, it has recently been demonstrated in the PC12 cells that ODC activation can be dissociated from the NGF-dependent survival of cells and from neurite outgrowth by ODC inhibitors (6), and ODC induction requires protein synthesis while some cellular responses do not (16). Nevertheless, the tremendous increase in ODC enzyme activity is often correlated with dramatic changes in cell metabolism including increases in cyclic AMP content of the cytoplasm and RNA synthesis in the nucleus (7) and at present it is a useful marker for hypertrophic responses in the cell.

It is interesting to speculate as to how the EGF receptors and the later response in ODC activity are lost following NGF treatment. It is known that EGF has complex specific interactions with the fibroblast cell membrane including its binding to receptors, the aggregation of the receptors into "patches", the internalization of the complexes within endocytic vesicles, and finally the degradation of the complex internally (17,18). It is also known that EGF receptor levels can be "down-regulated" in this fashion by EGF pretreatment (19), can be enhanced by glucocorticoids (20), or can be influenced in a specific manner by lectins (21). Binding of lectins to undifferentiated neural crest cells (22) and of hormones in other systems (23) change with differentiation, and NGF-induced differentiation in PC12 cells alters such surface properties as cellular adhesion

and lectin binding (24), as well as neurite outgrowth. Thus, although NGF does not interfere with EGF binding through direct immediate interaction, it could influence it later by blocking internalization of the receptors or by other physicochemical alterations in the cell membrane which would reduce the affinity of the receptor for EGF.

Alternatively, the lack of response to EGF could be influenced at a more central level, perhaps by direct action at the nucleus. It is known that NGF can interact with specific nuclear receptors (25), can act on the transcription of specific genes, e.g., tyrosine hydroxylase (26), ODC (8), and can influence other specific nuclear events (27,28). Clearly, NGF could directly or indirectly repress the synthesis of the EGF receptor.

A reciprocal interaction between NGF and EGF could be an important aspect of neuronal differentiation. If indeed EGF acts in this system to promote cell division, then one aspect of the differentiating action of NGF could be to prevent EGF action through the reduction of EGF receptors. Such a reciprocal inter action of hormones and growth factors may also be a more general phenomenon. It has been seen in adrenal cell cultures in which fibroblast growth factor enhances mitogenesis and inhibits steroidogenesis, the differentiated response, while ACTH inhibits cell proliferation and stimulates steroid production (29). The finding of changes in receptors and responsiveness of cells to EGF with NGF-induced cell differentiation in the PC12 cell system may thus have general implications for the study of the developmental interactions of hormones, in addition to importance for investigations into the mechanism of action of these hormones and growth factors and possibly for investigations into the controlling factors in cell proliferation in the area of tumor cell biology.

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