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Loss of EGF Binding and Cation Transport Response During Differentiation of Mouse Neuroblastoma Cells

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Mouse neuroblastoma cells (clone N1E-115) differentiate in culture upon withdrawal of serum growth factors and acquire the characteristics of neurons. We have shown that exponentially growing N1E-115 cells possess functional epidermal growth factor (EGF) receptors but that the capacity for binding EGF and for stimulation of DNA synthesis is lost as the cells differentiate. Furthermore, in exponentially growing cells, EGF induces a rapid increase in amiloride-sensitive Na^+ influx, followed by stimulation of the $(Na^+\text{-}K^+)$ATPase, indicating that activation of the Na^+/H^+ exchange mechanism in N1E-115 cells [1] may be induced by EGF. The ionic response is also lost during differentiation, but we have shown that the stimulation of both Na^+ and K^+ influx is directly proportional to the number of occupied receptors in all cells whether exponentially growing or differentiating, thus only indirectly dependent on the external EGF concentration. The linearity of the relationships indicates that there is no rate-limiting step between EGF binding and the ionic response. Our data would suggest that as neuroblastoma cells differentiate and acquire neuronal properties, their ability to respond to mitogens, both biologically and in the activation of cation transport processes, progressively decreases owing to the loss of the appropriate receptors.

Key words: neuroblastoma, differentiation, EGF, binding assay, K^+ transport, Na^+ transport, amiloride, growth stimulation

During terminal differentiation most cells lose the capacity for division. In order to gain insight into the molecular mechanisms involved in this process, we have made use of mouse neuroblastoma cells (clone N1E-115). In tissue culture these cells can be induced to differentiate by the removal or depletion of serum from the culture medium. They then begin to acquire various differentiated neuronal properties, including the formation of neurites. With respect to understanding the growth control at the molecular level, the mechanism of action of epidermal growth factor (EGF), a potent mitogen for variety of epithelial and fibroblastic cells both in vivo and in vitro has been extensively studied. EGF initiates its action by binding to specific cell surface receptors; the receptors then aggregate and the complex is subsequently

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internalized and degraded [reviewed in 2]. Among the early events following addition of EGF to cells, tyrosine phosphorylation [3], stimulation in the rate of ion transport [4, 5], and nutrient uptake [6] have been reported, in addition to distinctive morphological changes [7].

In the present study, we show that this neuroblastoma cell line possesses specific receptors for EGF, and assess EGF binding, EGF-induced ionic events, and DNA synthesis. We show that in exponentially growing cells EGF stimulates unidirectional Na^+ influx, resulting in an acceleration of the pumping activity of the (Na^+-K^+)ATPase. The results show that as N1E-115 cells proceed along the differentiation pathway, both EGF and serum become less potent in their ability to stimulate DNA synthesis, and the cells in addition lose their capacity for binding EGF. Furthermore, the ability of EGF to induce the ionic events is diminished, the EGF dose-response curve in exponentially growing cells being significantly different from that in differentiating cells. However, we show that if the EGF dose-response curves for ion transport stimulation are considered in terms of the number of occupied receptors, points obtained from both exponentially growing and differentiating cells fall on the same line, so that there is a direct proportionality between the EGF bound and the flux response. There can therefore be no rate-limiting steps between EGF binding and the flux stimulation. The implications in terms of terminal differentiation to a neural cell type and possible mechanisms are discussed.

MATERIALS AND METHODS

Cell Culture

Mouse N1E-115 cells [8] provided by M. Nirenberg were routinely grown in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/liter glucose, buffered with 44 mM $NaHCO_3$, in a 10% CO_2 atmosphere at pH 7.4 and supplemented with 7.5% fetal calf serum (FCS; Flow Laboratories). Stock cultures were maintained at 37°C and were subcultured as described previously [9]. For EGF binding studies and Na^+, K^+ flux measurements cells were plated in 3.5-cm diameter Petri dishes (Costar) at 2.5×10^4 cells/cm^2, grown overnight in DMEM + 7.5% FCS and then, if required, incubated for various times in DMEM without FCS. Final density was $4–6 \times 10^4$ cells/cm^2.

DNA Synthesis

Cells were plated at 1.5×10^4/cm^2 in 1.6-cm diameter wells (Costar) in 1 ml DMEM containing 7.5% FCS. After 24 hr of incubation at 37°C, cultures were rinsed twice and incubated a further 24 or 48 hr in DMEM alone. The appropriate test medium—ie, DMEM, with or without FCS or with epidermal growth factor (EGF, 40 ng/ml)—was then added. At the required time the medium was replaced by 1 ml of DMEM, and ^{3}H-thymidine (spec. act. 55 Ci/mmole; 0.5 μCi/ml) incorporation into DNA, after a 30-min incubation at 37°C, was determined as described previously [9].

^{125}I-EGF Binding Assay

Cells in 3.5-cm dishes were washed twice with 1-ml aliquots of binding medium which consisted of DMEM (pH 7.4) containing 0.1% crystalline bovine serum albumin, buffered with 25 mM N-2-hydroxyethyl piperazine-N′-2-ethanesulphonic

acid (HEPES). For a time course of EGF binding the cells were incubated for various times on a gyratory shaker at 4°C, 18°C, or 37°C in 1 ml of binding medium containing ^{125}I-EGF (1.5 ng/ml, 200,000 cpm/ng). After incubation, unbound radioactivity was removed by washing the cells five times in ice-cold phosphate-buffered saline (PBS). The cells were then solubilized in 0.1% Triton, and radioactivity was determined by counting in Lumagel scintillation fluid in a liquid scintillation counter (Packard, Tricarb 2450).

The values for ^{125}I-EGF binding were corrected for nonspecific binding by subtracting the amount of radioactivity bound in the presence of 500 ng/ml unlabeled EGF. At equilibrium this was ~ 40% of the total binding at an ^{125}I-EGF concentration of 1.5 ng/ml. For the effect of EGF concentration on ^{125}I-EGF binding and Scatchard analysis, various concentrations (1.0–150 ng/ml) of unlabeled EGF were added with the binding medium and the cells incubated for 1 hr at 18°C before removing unbound radioactivity as above. All data points were taken in triplicate.

K^+ Influx Measurements

Cells were grown in Petri dishes and incubated for 1 or 48 hr in serum-free DMEM prior to experimentation. In determining K^+ influx ^{86}Rb was used as a tracer for K^+ for experimental convenience since the results obtained are not significantly different from those using $^{42}K^+$. The difference between measurements in the presence and absence of ouabain (5 mM) represent the (Na^+-K^+)ATPase-mediated or active K^+ influx. At the required time after exposure of cells to the test medium, ^{86}RbCl was added (spec. act. 1 Ci/mmol K^+, final concentration 2–5 mM) and incubated for 5 min at 37°C. The cells were then washed and radioactivity determined as described previously [9, 10]. Since the initial rate of uptake of the isotope is linear (not shown), the mean influx of K^+ per min could be estimated from the isotope content after 5 min. Data points were taken in triplicate.

Na^+ Influx Measurements

Na^+ influx was determined using methods described in detail previously [11] using ^{22}Na as a radioactive tracer in the presence of ouabain (5 mM) to eliminate "back flux" via the (Na^+-K^+)ATPase. The initial rates of uptake of the isotope in the presence and absence of ouabain are identical and in the presence of ouabain linear over a 5-min period (results not shown), so the mean influx of Na^+ per min could be estimated from the isotope content after 5 min as for $^{86}Rb^+$.

Materials

EGF was obtained from Collaborative Research, ouabain from Sigma ^{125}I-EGF from New England Nuclear, Boston, and the radiochemicals ^{86}RbCl, ^{22}NaCl, and ^{3}H-thymidine from Amersham International, Amersham. The diuretic amiloride (3,5-diamine-6-chlorpyrazinoylguanidine hydrochloride) was a gift from Merck, Sharp and Dohme.

RESULTS

DNA Synthesis

Upon removal of (serum) growth factors, N1E-115 cells show a characteristic flattening of the cell body followed by the formation of thin neurites, which attain a

length of several microns and eventually form a dense network between the cells. This is accompanied by a gradual decrease in the rate of DNA synthesis and cessation of division after 24 hr [9]. The differentiation process is reversible in the first 24 hr following serum deprivation so that readdition of (serum) growth factors results in retraction of neurites and in the reentry of the cells into S phase after an 8- to 10-hr lag period [9], with a peak in the stimulation ^{3}H-thymidine incorporation into DNA

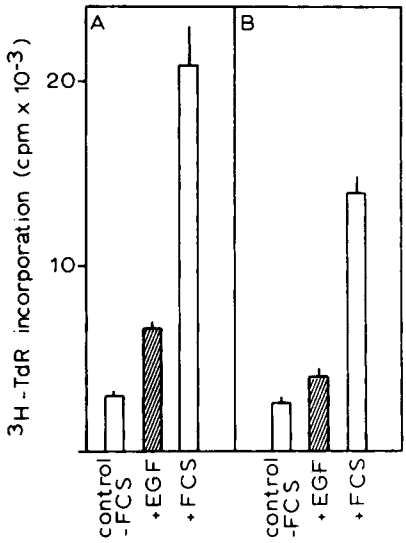

Fig. 1. Stimulation of ^{3}H-thymidine incorporation into DNA of N1E-115 cells by EGF (40 ng/ml) and fetal calf serum (10%) 16 hr after addition to cells deprived of serum for (A) 24 hr and (B) 48 hr. Data expressed as mean ± SEM (n = 3).

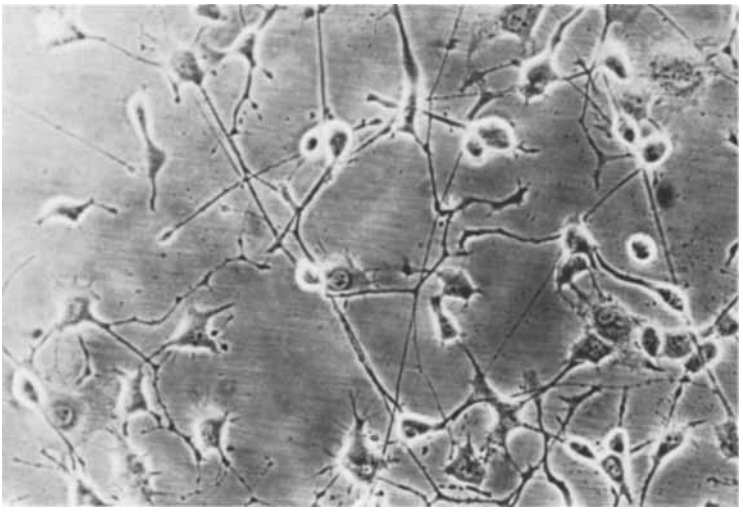

Fig. 2. Neuroblastoma cells (clone N1E-115) deprived of serum for 48 hr.

after 16–20 hr. A similar effect is observed if a purified growth factor such as EGF is added instead of serum, although the effect is severalfold lower in magnitude as illustrated in Figure 1A, where the maximum stimulation of ^{3}H-thymidine incorporation into DNA is shown for serum and EGF. The concentration of EGF required for half-maximal stimulation was ~ 20 ng/ml, whereas at 40 ng/ml the effect reached a maximum (not shown). If the cells were deprived of serum for 48 hr before readdition of growth factors, neither EGF nor serum was as potent in stimulating ^{3}H-thymidine incorporation into DNA in the same period of time (Fig. 1B), suggesting that after 48 hr there is greater commitment to differentiation. Morphologically the cell population appears homogeneously differentiated, since after 48 hr without serum almost all of the cells have long neurites (Fig. 2).

^{125}I-EGF Binding

Understanding the mechanism by which N1E-115 cells lose their ability to respond to EGF during differentiation necessitated an investigation of the epidermal growth factor receptor; thus the kinetics of binding of ^{125}I-EGF were characterized. Initially, the rate of specific ^{125}I-EGF binding was measured as a function of temperature in cells deprived of serum for 24 hr, in order to determine the conditions under which binding is at equilibrium (Fig. 3). At 37°C and 18°C binding was rapid, reaching equilibrium between 0.5 and 1 hr; at 18°C this value was maintained for at least 5 hr, whereas at 37°C binding decreased between 3.5 and 5 hr, presumably as internalization occurred [2]. At 4°C equilibrium was only reached after 1.5 hr, but a subsequent decrease in binding was not apparent.

On the basis of Figure 3, equilibrium binding studies were carried out using an incubation time of 1 hr at 18°C on cells in three different growth states—namely, exponentially growing cells deprived of serum for 0.5 hr and cells deprived of serum

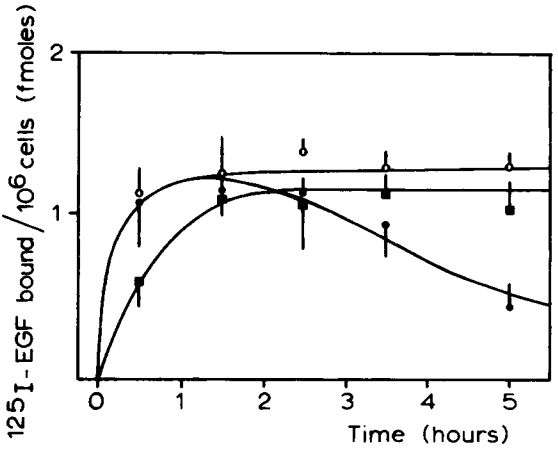

Fig. 3. Time course of (^{125}I)-EGF binding to N1E-115 cells deprived of serum for 24 hr at 37°C (●—●), 18°C (○—○), and 4°C (■—■). ^{125}I-EGF (1.5 ng/ml, 100,000 cpm/ng was added in 1 ml of standard binding medium. Values were corrected for non-specific binding, determined as the amount of radioactivity bound in the presence of 500 ng/ml unlabeled EGF. Data presented as mean ± SEM (n=3).

for 24 or 48 hr. The binding of EGF to the cells was concentration-dependent and saturable but, as is illustrated in Figure 4, significantly different in each state of growth. Least-squares linear regression analysis of Scatchard plots constructed from the data of Figure 4 are shown in Figure 5. They give good fits to a straight line, indicating only one class of receptor in all three states of growth, but there is clearly a significant loss in the number of binding sites per cell as differentiation proceeds. The results are summarized in Table I. The decrease in the number of binding sites in the first 24 hr following removal of serum is approximately equal to that in the second 24 hr, although the fractional decrease is, of course, greater. In addition, the apparent dissociation constant decreases (ie, affinity increases) as differentiation progresses although, in contrast to the decrease in the number of binding sites, both the absolute and fractional decreases in the first 24 hr are considerably less than those in the second 24 hr.

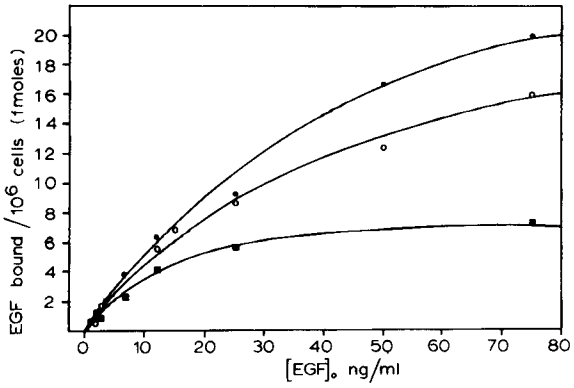

Fig. 4. Effect of EGF concentration on binding to cells deprived of serum for 48 hr (■—■), 24 hr (○—○), or exponentially growing (●—●). Unlabeled EGF was added to 1 ml of binding medium containing 1 ng/ml (^{125}I)-EGF (~ 200,000 cpm/ml). Each point was obtained in threefold from the separate experiments.

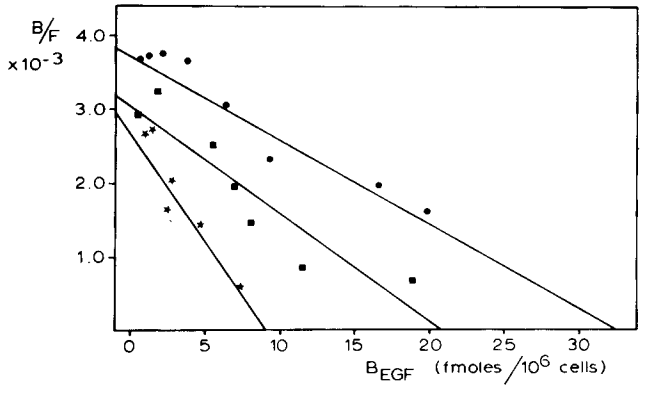

Fig. 5. The data shown in Figure 4 analyzed according to the method of Scatchard for cells deprived of serum for 48 hr (★—★), 24 hr (■—■), or exponentially growing (●—●).

TABLE I. EGF Binding Characteristics During Differentiation of N1E-115 Cells

	Molecules EGF bound/cell (mean ± SEM)	K_d (nM) (mean ± SEM)
Exponentially growing	19,500 ± 1,100	8.4 ± 0.3
24 hr—FCS	12,500 ± 1,500	6.7 ± 0.5
48 hr—FCS	5,300 ± 500	3.1 ± 0.3

Data derived from Scatchard analysis of binding shown in Figure 5.

EGF Stimulates Active K^+ Influx and Unidirectional Na^+ Influx

In view of the rapid ionic events known to occur when serum growth factors are added to N1E-115 cells and their possible role in the initiation of the mitogenic response [9], the ability of EGF to stimulate two selected, but related, ionic events was investigated as a function of differentiation—ie, as a function of the characteristics of the receptor for EGF. Initial experiments in exponentially growing cells, deprived of serum for 1 hr, showed that EGF (25 ng/ml) significantly stimulated (Na^+-K^+)ATPase-mediated K^+ influx, reaching a maximum value ~ 45% higher than control within 30 min (not shown). This compares, for example, with a transient, 100% stimulation induced by serum in the same time in cells deprived of serum for 24 hr [9]. The stimulation of active K^+ influx by EGF as a function of its concentration in the extracellular medium after a 30-min incubation at 37°C was then determined in exponentially growing cells (Fig. 6A) and in cells deprived of serum for 48 hr (Fig. 6B). The stimulation in both cases was half-maximal at an EGF concentration of approximately 6 ng/ml, and within error the basal rates of active K^+ uptake in the resting state were the same (4.83 ± 0.25 nmol/min/10^6 cells in exponentially growing cells compared with 5.08 ± 0.30 nmol/min/10^6 cells in differentiating cells), but the extent of the stimulation was significantly less (41% and 13.5% in exponentially growing and differentiating cells, respectively). This was specifically due to a decrease in the ability of EGF to stimulate the (Na^+-Na^+)ATPase since the Na^+

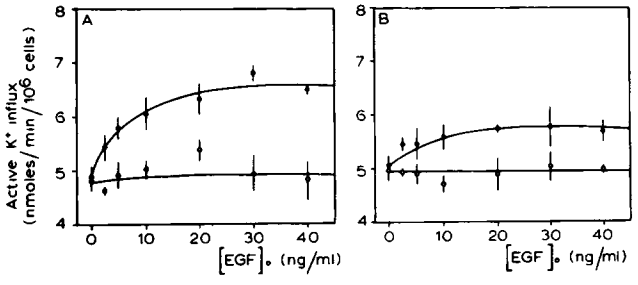

Fig. 6. Dose-response curve for stimulation of active K^+ influx by EGF in the presence (○—○) and absence (●—●) of amiloride (0.2 mM) in exponentially growing cells (A) and cells deprived of serum for 48 hr (B). Measurements were made as described in Materials and Methods after a 30-min incubation in EGF. Data presented as mean ± SEM (n=3).

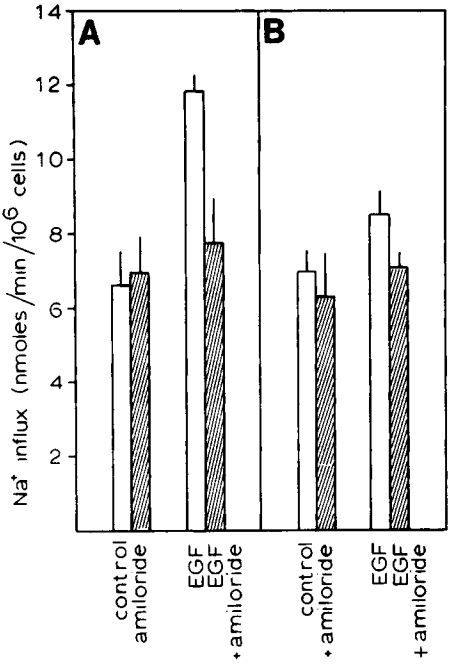

Fig. 7. Stimulation of Na^+ influx by EGF (40 ng/ml) after a 30-min incubation at 37°C in exponentially growing cells (A) and cells deprived of serum for 48 hr (B) in the presence and absence of amiloride (0.2 mM).

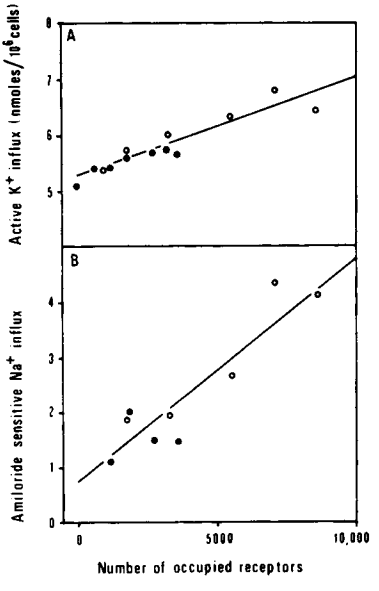

Fig. 8. Relationship between the number of occupied EGF receptors, active K^+ influx, and amiloride-sensitive Na^+ influx in exponentially growing cells (○—○) and cells deprived of serum for 48 hr (●—●).

inophore monensin [9] increased pumping activity 3- to 4-fold in both exponentially growing and differentiating cells (not shown). A similar difference between exponentially growing and differentiating cells was also found in the stimulation of Na^+ influx induced by EGF (Fig. 7). In this case the basal rates of Na^+ influx were again, within error, the same whereas the maximal stimulation was 78% in exponentially growing but only 21% in differentiating cells. In all cases and all concentrations of EGF, the stimulation in both Na^+ influx and active K^+ influx were completely inhibited by the amiloride (0.2–0.3 mM), an inhibitor of Na^+/H^+ exchange in N1E-115 cells [1, 9], suggesting that EGF may activate this mechanism while the increased intracellular availability of Na^+, acting as substrate for the (Na^+-K^+)ATPase, leads to increased active K^+ influx.

From the binding studies, the number of occupied binding sites can be calculated for each external EGF concentration in exponentially growing and differentiating cells. When either the active K^+ influx or Na^+ influx is then plotted as a function of the number of occupied binding sites, then both the points derived from exponentially growing cells and differentiating cells fall on the same regression line (Fig. 8A and B, respectively). There is therefore a direct correlation between the number of occupied EGF receptors and the measured response of Na^+ and K^+ transport to EGF addition.

DISCUSSION

In the present paper we have demonstrated that mouse N1E-115 neuroblastoma cells possess functional EGF receptors. We have shown that as the cells increasingly exhibit the properties characteristic of neural differentiation, such as electrical excitability, cessation of division, and neurite formation [for review, see 12], the growth response to EGF is progressively lost. The reason for this was a decrease in the number of EGF binding sites as cells differentiated which, despite increased affinity of the sites remaining, was apparently not sufficient to retain the growth response. Interpretation of binding data obtained on whole cells and analyzed using the Scatchard plot must, however, always be tempered with caution since its use, particularly with respect to the affinity constant, is open to question.

Nonetheless, a similar loss of EGF binding capacity with terminal, neuronal differentiation has also been reported by Huff et al [13] in rat pheochromocytoma PC12 cells where the fraction of receptors lost was of the same order of magnitude and occurred over approximately the same period of time as in N1E-115 cells. There was in this case, however, no change in the affinity of the receptors for EGF as differentiation proceeded. Such effects during terminal differentiation are thus in direct contrast with those during early differentiation. In embryocarcinoma cells, for example, EGF binding increases with differentiation, but in this case cell growth continues after the differentiated phenotype has appeared [32]. Among the numerous other examples of cells that have lost epidermal growth factor receptors for a variety of reasons and exhibit a parallel loss of biological response to the factor [14–16], the affinity of the receptor for EGF has also been observed to change [17].

In N1E-115 cells, aspecific binding is a relatively high percentage of total binding compared with a number of other cell types—eg, 5% in 3T3 cells [18]. However, in absolute terms it is comparable—ie, 3 pg/10^6 cells in N1E-115 compared to 2–15 pg/10^6 cells in 3T3, depending on cell density [18]. In addition, N1E-115

cells are typical of a number of cell types of neural origin where EGF binding is characteristically low [33].

In view of the multitude of studies showing that an early response in many cell types to a number of mitogens, including EGF, is a stimulation in both Na^+ influx and (Na^+-K^+)ATPase-mediated K^+ influx [for review, see 19], we investigated this response in N1E-115 cells as a function of the ability of cells to bind EGF. The results showed a direct correlation between the magnitude of the stimulation in Na^+ influx and active K^+ influx, and the absolute number of binding sites for EGF that were occupied. The ion flux responses were thus only indirectly dependent on the state of growth or differentiation and the extracellular EGF concentration. That the relationship was linear for both Na^+ and K^+ would suggest that even though a multitude of (un)known events occur between initial binding of EGF to its receptor and the stimulation of ion transport, none is a rate-limiting step.

It has previously been shown that N1E-115 cells possess an amiloride-sensitive, electrically silent, Na^+-H^+ exchange mechanism [1] that may have an important function in the regulation of intracellular pH [21]. Furthermore, it has been shown indirectly that activation of the Na^+-H^+ exchanger may be an important early event in the response of these cells to serum [9]. Direct evidence for activation of the Na^+-H^+ exchanger by growth factors was obtained from the effect of EGF on quiescent human fibroblasts [5]. The amiloride sensitivity of the EGF-induced ionic events in N1E-115 cells in the present study would suggest that activation of the Na^+-H^+ exchanger follows addition of EGF to these cells as well. This suggestion is supported by the failure to observe changes in the membrane potential of N1E-115 cells after EGF addition, the possibility of opposing potential changes (eg, P_K increase) having been ruled out [21]. Furthermore, no change in the intracellular content of Na^+ occurs in N1E-115 cells following either addition of serum [9] or EGF (not shown). The increase in the pumping activity of the (Na^+-K^+)ATPase, because of the increased intracellular availability of Na^+ following growth factor addition, is clearly sufficient to regulate intracellular Na^+ levels. This reflects the suboptimal intracellular availability of Na^+ for the (Na^+-K^+)ATPase that has been observed in neuroblastoma cells previously [9, 11, 30].

Maximum stimulation of DNA synthesis in N1E-115 cells occurs at relatively high external concentrations of EGF, where approximately 25% of receptors are occupied. This is comparable with the 20% receptor occupancy required for maximum mitogenic effect on 3T3 cells [35]. In many other cell types, however, only ~ 1% of receptors need be occupied for maximum mitogenic response, although higher concentrations, of the same order as those effective in N1E-115 cells, are required for stimulation of ion transport [see eg, 5]. Although the stimulation of DNA synthesis in N1E-115 cells by EGF appears relatively small compared with that of serum (a factor of 2 compared with 7 for serum in Fig. 1A, for example), it was observed in the complete absence of any medium supplements with which it might act synergistically. In most cases the level of EGF-mediated stimulation of DNA synthesis has been shown to be dependent on small amounts (1–2%) of serum in the medium or agents such as insulin, thrombin, etc [reviewed in 2].

It is plausible that cells of neural origin, such as N1E-115, represent one end of the spectrum with respect to the number of EGF receptors on its surface [33], that cells such as human fibroblasts with 3–5 times more receptors [22] represent a middle point in the spectrum and that cells such as the A431 epidermoid carcinoma cells,

with ~ 2.6×10^6 receptors per cell, represent the other end of the spectrum. In the last case, a number of events such as phosphorylation [23] are induced within seconds of EGF addition, when presumably only very few receptors are occupied. There is, however, no stimulation of ion transport (Moolenaar and Mummery, unpublished) or DNA synthesis [24], and indeed DNA synthesis may even be inhibited [31].

It is clearly not necessary, therefore, that a cell possess either a large number of receptors for EGF or that all available receptors be occupied for the induction of significant biological and cation transport responses. In this respect, it is also of interest to note that, as in a number of other cell types, following stimulation with EGF, the time at which a maximum occurs in ion flux stimulation, generally between 30 and 60 min, is similar to the times for equilibrium with respect to EGF binding to be reached [see eg, 25]. This would again illustrate the possibility of close coupling between the number of occupied receptors and ion flux stimulation.

At present, we cannot exclude the possibility that the cell population responds heterogeneously to EGF and that some cells lose all their receptors during serum deprivation while others lose none. Autoradiographic studies are in progress to investigate this question, and the extent to which EGF binding is restored to a population following serum readdition will give an indication of whether all or only part of the population are involved in the effects described. In this respect, however, it has been shown in fibroblasts that even though homogeneous binding to all cells is observed when fluorescent EGF is added to a confluent population, only about 10% of cells are observed to respond mitogenically in autoradiographic analysis following EGF addition [34].

In contrast to EGF, which binds to a wide variety of cell types in culture, nerve growth factor (NGF) binds to specific membrane receptors on the surface of relatively few cells [33]. It has been shown to stimulate the outgrowth of axons from sympathetic ganglia, providing the basis for its biological activity [36]. A number of neuroblastoma cell lines have been reported to have specific NGF binding sites, however, although the biological response has been varied in nature. Human neuroblastoma clone IMR-32 [37] and mouse neuroblastoma clone NB6R [38] are induced to differentiate whereas mouse clone NB1R are stimulated to DNA synthesis and proliferation [38].

A significant biological response may again be induced with relatively few specific receptors. Clone IMR-32, for example, binds only 50% of the amount of NGF as EGF [33], itself already at low levels. Although NGF binding to N1E-115 cells was not measured in the present study, preliminary experiments have suggested that specific receptors may be present since DNA synthesis stimulation of the same order of magnitude as with EGF is induced and cation transport is rapidly increased following NGF addition [21]. The stimulation of cation transport by both NGF and EGF in one cell line has also recently been reported in PC12, rat pheochromocytoma cells [20, 39].

At present, it is possible only to speculate on the mechanisms controlling the loss of receptors for EGF and the change in their affinity during neuroblastoma cell differentiation. It may be that cells destined to become neurons respond to a lack of external growth factors directly by a transcriptional alteration in the synthesis of the receptor, and in its internalization either in the occupied or unoccupied mode so that the whole recycling process is disturbed. Alternatively, regulation may be of an indirect nature via the plasma membrane. Physicochemical properties of the plasma

membrane have been shown in a variety of cell types to modulate binding of a number of ligands [27–29] by changes in the number and/or affinity of the receptors. In addition, we have for the first time correlated the activity of a membrane-associated enzyme, the (Na^+-K^+)ATPase, during the cell cycle of another mouse neuroblastoma clone, Neuro-2A, with membrane protein lateral mobility [30]. Furthermore, the initiation of morphological differentiation in this clone by dibutyryl cAMP and 3-isobutyl-1-methylxanthene results in both changes in membrane fluidity and in the lateral mobility of lipid and proteins, specifically in the neurite membrane [26]. It is also conceivable that the changes in the affinity and in the number of copies of the EGF receptor are independently regulated, since the changes in their properties with serum deprivation occur at different rates. It may therefore be that modulations occur in the physicochemical properties of the plasma membrane of N1E-115 cells during differentiation that regulate EGF receptor expression and hence the ability to respond to the growth factor. These possibilities are at present under investigation.

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