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Modulation of the Epidermal Growth Factor Receptor by Brain-Derived Growth Factor in Swiss Mouse 3T3 Cells

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Incubation of Swiss mouse 3T3 cells at 37°C with bovine brain-derived growth factor (BDGF) decreased the cell surface ^{125}I-EGF binding activity of these cells by 70–80%. This down-modulation of the EGF receptor by BDGF was time, temperature, and dose dependent. Scatchard plot analysis indicated that BDGF binding led to a selective decrease in the number of high-affinity EGF receptors. The BDGF-induced down-modulation of the EGF receptor was completely blocked by protamine, a potent inhibitor of receptor binding and mitogenic activities of BDGF.

BDGF down-modulated the EGF receptor in phorbol myristic acetate (PMA)-pretreated cells, as well as in control cells. Furthermore, PMA-pretreated cells responded mitogenically to BDGF, whereas PMA itself failed to stimulate the mitogenic response of PMA-pretreated cells. This BDGF-induced down-modulation of the EGF receptor in PMA-desensitized cells suggests that BDGF down-regulates the EGF receptor by a mechanism distinct from that of PMA.

Incubation of cells with compounds which are known to inhibit pinocytosis blocked the down-modulation induced either by BDGF or by platelet-derived growth factor (PDGF) but had no effect on the PMA-induced down-modulation. Incubation of cells with inhibitors of receptor recycling enhanced the BDGF-induced down-modulation of the EGF receptor. These results suggest that BDGF and PDGF induce down-modulation of the EGF receptor by increasing the internalization of cell surface high-affinity receptors and that the internalization process may not be required for down-modulation induced by PMA.

Key words: receptor modulation, internalization, EGF receptor

Several lines of evidence suggest that growth factors stimulate cell growth through interaction with specific receptors in responsive cells: 1) The optimal concen-

Abbreviations used: EGF, epidermal growth factor; PDGF, platelet-derived growth factor; BDGF, brain-derived growth factor; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate; DMEM, Dulbecco's modified Eagle's medium; PMA, phorbol myristic acetate.

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trations of growth factors to stimulate DNA synthesis are very close to the K_d of binding of these growth factors to their receptors [1–4]. 2) Cells which lack specific receptors do not respond to the respective growth factors [1,3,4]. 3) Specific binding inhibitors show inhibition of the mitogenic activity of the respective growth factors [5–8]. It is believed that the growth factor receptor on the cell surface plays a key role in transmission of the mitogenic signal to the nucleus following growth factor-receptor interactions [3,9,10]. Modulation of growth factor receptor on the cell surface should, therefore, influence the response of cells to growth factors.

Several polypeptide factors, including platelet-derived growth factor (PDGF) [11–16], fibroblast-derived growth factor (FDGF) [17], vasopressin [18], bomesin [19,20], and PMA [21–26], have been found to modulate the EGF receptor by decreasing the high-affinity receptor number for EGF. Basic fibroblast growth factor (FGF) was also found to inhibit EGF binding to receptor, but no details of the inhibition were present [13].

Recently, we have purified brain-derived growth factor (BDGF) to homogeneity [7]. BDGF is a 16–17-kilodalton (kDa) protein whose biochemical properties resemble those reported for endothelial cell growth factors [27], brain-derived acidic fibroblast growth factors [28,29], and heparin-binding growth factor α [30]. BDGF is a potent mitogen with a broad spectrum of cell specificity, being highly active in stimulation of DNA synthesis of fibroblasts, endothelial cells, osteoblasts, chondrocytes, glial cells, smooth muscle cells, and epithelial cells [7]. BDGF also appears to be a potent chemotactic factor for fibroblasts and astroglial cells but not for peripheral monocytes [31]. BDGF receptor has been shown to be a 135-kDa protein associated with a protein tyrosine kinase activity [32]. The broad spectrum of cell specificity of BDGF and the similarity of the BDGF receptor to EGF and PDGF receptors with respect to protein tyrosine kinase activity prompted us to investigate the modulation of the EGF receptor by BDGF in Swiss mouse 3T3 cells. In this communication, we show that, as in the cases of PDGF and PMA, BDGF transmodulates the EGF receptor by decreasing the high-affinity receptor number in a dose-dependent manner.

EXPERIMENTAL PROCEDURES

Materials

Swiss mouse 3T3 cells (CLL92) were obtained from the American Type Culture Collection (Rockville, MD) and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum. Cell culture cluster plates (24 wells, 16-mm well diameter; and 48 wells, 11.3-mm well diameter) were obtained from Costar (Cambridge, MA). $Na^{125}I$ (17 Ci/mg), and [methyl-^{3}H] thymidine (79.4 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Bovine BDGF was prepared as previously described [7]. The homogeneity of BDGF preparation was judged by the criteria of SDS polyacrylamide gel (15% gel) electrophoresis [7]. Human PDGF was prepared from human platelet-rich plasma according to the procedure described previously [6]. Mouse EGF was obtained from Collaborative Research (Bedford, MA). PMA (phorbol myristic acetate), methylamine HCl, chloroquine diphosphate salt, NaCN, Na azide, NaF, chloramine T, protein markers (low molecular weights), and salmon protamine sulfate were obtained from Sigma (St. Louis, MO). Human platelet-rich plasma and platelet-poor plasma were obtained from the American Red Cross (St. Louis, MO).

Preparation of ^{125}I-EGF

^{125}I-EGF was prepared by iodination of EGF in the presence of chloramine T and sodium ^{125}I [33]. Free iodide was removed by chromatography on a Sephadex G-25 column [33]. The incorporation efficiency of iodination was 90%, and the specific radioactivity of ^{125}I-EGF was $\sim 0.4 \times 10^6$ cpm/ng.

Binding Assay

Swiss mouse 3T3 cells were grown to confluency in Costar 24-well or 48-well cluster dishes in DMEM containing 10% fetal calf serum. The cells were pretreated with or without growth factors or PMA in DMEM at 37°C for 10 min. The cell-surface-bound ^{125}I-EGF was then determined by incubation of pretreated cells with ^{125}I-EGF (1 ng/ml) in DMEM containing 0.1% human serum albumin at 0°C for 2½ hr. Nonspecific binding was measured in the presence of 100-fold excess of unlabeled EGF. For Scatchard plot analysis, various concentrations of ^{125}I-EGF (0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 ng/ml) were used. The assays were performed in duplicate. Confluence monolayers of 3T3 cells contained approximately 3.3×10^4 cells/cm^2. More than 95% of ^{125}I-EGF specifically bound was removed with 0.2 M acetic acid, 0.5 M NaCl, pH 3.0.

Mitogenic Activity Assay

Mitogenic activity was assayed in serum-free or plasma-derived serum containing medium by growth factor-dependent incorporation of [methyl-^{3}H]thymidine into DNA of 3T3 cells [7].

RESULTS

Swiss mouse 3T3 cells have previously been used for studying the transmodulation of the EGF receptor by growth factors [11,17–20]. These cells also express a significant quantity of BDGF receptor on the cell surface [7] and are, therefore, suitable for studying the effect of BDGF on the EGF receptor. As shown in Figure 1, preincubation of cells at 37°C with BDGF (1 ng/ml) diminished ^{125}I-EGF binding in a time-dependent manner with a $t_{½}$ of ~1.5 min. After 5-min preincubation, ~20% of the ^{125}I-EGF binding remained, compared with control cells which had not been preincubated with BDGF. The ^{125}I-EGF binding was performed by incubation of 3T3 cells with ^{125}I-EGF (1 ng/ml) at 0°C for 2½ hr. BDGF (1 ng/ml) did not have an effect on ^{125}I-EGF binding to EGF receptor at 0°C over a 2½-hr incubation period. Incubation with BDGF at 37°C for up to 2 hr failed to decrease further the level of the ^{125}I-EGF binding observed at 5-min incubation. The level of inhibition of the ^{125}I-EGF binding by BDGF was intermediate between those observed for PDGF (60 ng/ml) and PMA (100 ng/ml) (Fig. 1). It is interesting that the rate of down-modulation of the ^{125}I-EGF binding activity by BDGF is faster than that of PDGF. The reason for this is not known. It is possible that the faster down-modulation rate is due to the rapid equilibrium of BDGF binding to the BDGF receptor [7]. Preincubation of 3T3 cells with BDGF (1 ng/ml) at 0°C for 40 min did not have any influence on the ^{125}I-EGF binding, suggesting that BDGF down-modulates the EGF receptor in a temperature-dependent manner as observed in the down-modulation of the EGF receptor by PDGF and PMA [16].

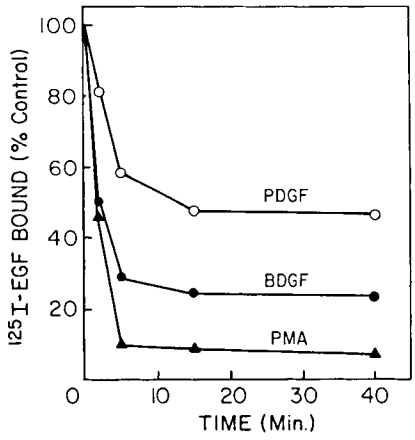

Fig. 1. Time course of down-modulation of the EGF receptor by BDGF, PDGF, and PMA. Confluent Swiss mouse 3T3 cells were incubated with BDGF (1 ng/ml), PDGF (60 ng/ml), and PMA (100 ng/ml) at 37°C in DMEM. At indicated time intervals, cell-surface-bound ^{125}I-EGF was measured by incubation of cells with ^{125}I-EGF (1 ng/ml) at 0°C for 2½ hr. Nonspecific binding was determined in the presence of 100-fold excess of unlabeled EGF. The specific binding of ^{125}I-EGF in control cells treated without mitogens did not show a significant difference at each time interval and was taken as 100% binding. The specific binding of ^{125}I-EGF in control cells was 2,500 ± 300 cpm/well.

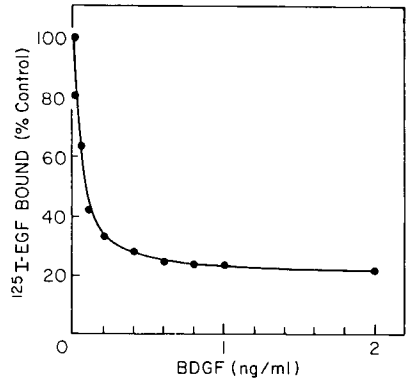

Fig. 2. Effect of BDGF concentrations on the down-modulation of EGF receptor. Confluent Swiss mouse 3T3 cells were incubated with various concentrations of BDGF in DMEM at 37°C. After 10 min, the cell-surface-bound ^{125}I-EGF was determined by incubation of cells with ^{125}I-EGF (1 ng/ml) at 0°C for 2½ hr. The specific binding in control cells treated without BDGF was taken as 100% binding.

The down-modulation of the EGF receptor by BDGF was also dependent on BDGF concentration (Fig. 2). The half-maximum concentration (ED_{50}) of BDGF to down-modulate the EGF receptor is very close to the K_d of BDGF binding to its receptor [7], suggesting that BDGF down-modulates the EGF receptor through binding to the BDGF receptor.

Scatchard plot analysis of EGF binding indicates that Swiss mouse 3T3 cells express both high-affinity and low-affinity EGF receptors [17]. To further define the inhibition by BDGF of ^{125}I-EGF binding to the EGF receptor, we examined the binding of various concentrations of ^{125}I-EGF to 3T3 cells following preincubation of cells with and without BDGF (1 ng/ml). Scatchard plot analysis of this binding

data indicates that preincubation of BDGF at 37°C selectively diminished the number of high-affinity EGF receptors (Table I). BDGF did not affect the number of low-affinity EGF receptors.

Protamine has been shown to be a potent inhibitor of receptor binding activity and mitogenic activity of BDGF and PDGF [6,7]. The specificity of down-modulation of the high-affinity EGF receptor induced by BDGF was also examined. As shown in Table II, protamine completely inhibited the down-modulation of the EGF receptor induced either by BDGF or PDGF, while protamine did not influence the down-modulation of the EGF receptor induced by PMA. The effect of protamine on BDGF-induced down-modulation of the EGF receptor was dose dependent (Fig. 3), and the dose dependence is very similar to, if not identical with, that for inhibition of ^{125}I-BDGF binding to receptor [7]. In control experiments, preincubation of cells with 0–10 μg/ml of protamine did not show any effect on ^{125}I-BDGF binding to receptor [7].

TABLE I. Binding Characteristics of ^{125}I-EGF in Swiss Mouse 3T3 Cells Treated With or Without BDGF*

Cells	Dissociation constant (K_d*) (nM)	Receptor No. (receptor/cell)
Control cells	0.3 (high affinity)	3.5×10^4
	2.4 (low affinity)	1.6×10^5
BDGF (1 ng/ml)-treated cells	0.2 (high affinity)	1.1×10^4
	4.9 (low affinity)	1.6×10^5

*Swiss mouse 3T3 cells were treated with or without BDGF (1 ng/ml) at 37°C for 10 min in DMEM. The binding of ^{125}I-EGF to these cells was then carried out by incubation of the cells with various concentrations (0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 ng/ml) of ^{125}I-EGF in DMEM containing 0.1% bovine serum albumin. After 2½ hr at 0°C, the assay was stopped by washing three times with 1 ml of cold HEPES, 0.15 NaCl, pH 7.4. The nonspecific binding at each concentration was measured in the presence of 100-fold excess of unlabeled EGF. The dissociation constants and receptor numbers in control cells treated without BDGF and BDGF-treated cells were estimated by Scatchard plot analysis of these binding data, using a linear least squares program. The Scatchard plot indicated two classes of the EGF receptor including high-affinity and low-affinity receptors.

TABLE II. Effects of Protamine on the Down-Modulation of the EGF Receptor Induced by BDGF, PDGF, and PMA*

	^{125}I-EGF bound			
	− Protamine		+ Protamine (10 μg/ml)	
Medium	cpm	%	cpm	%
Control	2,810 ± 185	100	2,824 ± 126	100
BDGF (1 ng/ml)	686 ± 74	24	2,830 ± 106	100
PDGF (60 ng/ml)	1,094 ± 138	39	2,784 ± 151	99
PMA (100 ng/ml)	205 ± 25	7	169 ± 20	6

*Swiss mouse 3T3 cells were incubated at 37°C for 10 min with mitogens in the presence and absence of protamine (10 μg/ml) in DMEM. The cell-surface-bound ^{125}I-EGF was then determined by incubation of cells with ^{125}I-EGF (1 ng/ml) in the presence and absence of 100-fold excess unlabeled EGF at 0°C for 2½ hr.

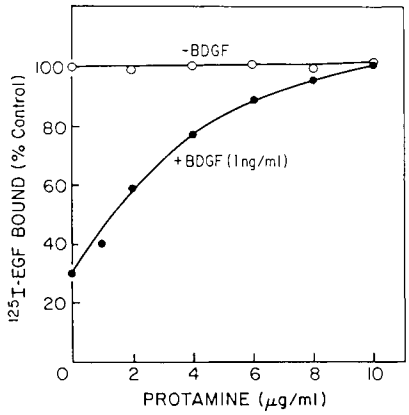

Fig. 3. Inhibition of BDGF-induced down-modulation of the EGF receptor as a function of protamine concentration. Monolayers of Swiss mouse 3T3 cells were incubated with or without BDGF (1 ng/ml) in the presence of different concentrations of protamine in DMEM. After 10 min at 37°C, cell-surface-bound ^{125}I-EGF was determined as described in the legend of Figure 1. The specific binding in control cells treated without BDGF was taken as 100% binding.

These results suggest that protamine may inhibit down-modulation of the EGF receptor by blocking BDGF binding to the BDGF receptor.

Recently, Olashaw et al [16] suggested that PDGF and PMA down-modulate the EGF receptor through different mechanisms following demonstration of down-modulation of the EGF receptor in PMA-pretreated cells by PDGF, but not by PMA [16]. PMA pretreatment down-regulates the protein kinase C activity and desensitized the response of cells to subsequent stimulation with PMA [16]. To see whether BDGF also down-modulates the EGF receptor by a mechanism distinct from that of PMA, we performed similar experiments. Swiss mouse 3T3 cells were pretreated with or without PMA (400 ng/ml) for 24 hr. The down-modulation of the EGF receptor induced by BDGF and PMA was then investigated in these cells. As shown in Figure 4, the down-modulation of the EGF receptor induced by BDGF is observed either in PMA-pretreated or control cells, whereas the down-modulation induced by PMA was found in control cells but not in PMA-pretreated cells. These results suggest that PMA pretreatment desensitized the response of cells to PMA but not to BDGF and that the down-modulation of the EGF receptor induced by BDGF is independent of protein kinase C whose activity is down-regulated by PMA pretreatment [16,19]. Since both BDGF and PMA are mitogenic for mouse 3T3 cells, the effect of PMA pretreatment on the mitogenic response of cells to BDGF and PMA was investigated. As shown in Figure 5B, PMA-pretreated cells remained mitogenically responsive to BDGF, although the level of mitogenic response was somewhat lower than that observed in untreated cells (Fig. 5A). In contrast, PMA-pretreatment completely abolished the mitogenic response of cells to PMA. It is interesting that the level of the mitogenic response to PDGF seems to be unaltered after pretreatment with PMA. These results suggest that the mechanism of mitogenic stimulation by BDGF differs at least in part from that stimulated by PMA.

The BDGF-induced loss of high-affinity EGF receptor from the cell surface of 3T3 cells might be a result of internalization of the receptor. To test this hypothesis, cells were preincubated with energy inhibitors (10 mM Na azide, 10 mM NaF, and 1

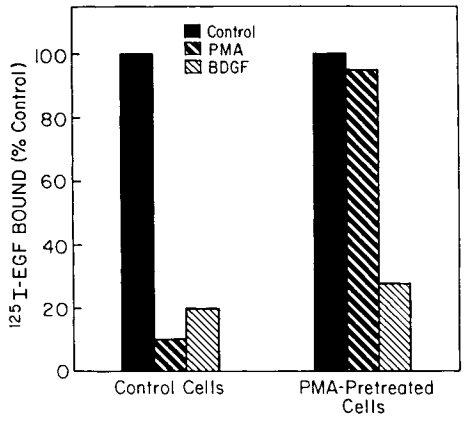

Fig. 4. Down-modulation of the EGF receptor by BDGF and PMA in PMA-pretreated and control Swiss mouse 3T3 cells. Swiss mouse 3T3 cells were treated with or without PMA (400 ng/ml) in DMEM at 37°C for 24 hr. Cells were then washed and incubated with BDGF (1 ng/ml) or PMA (100 ng/ml) in DMEM at 37°C for 10 min. The cell-surface-bound EGF was determined as described in the legend of Figure 1. The specific binding of ^{125}I-EGF in control cells without pretreatment with PMA and in cells pretreated with PMA did not show a significant difference and were taken as 100% binding.

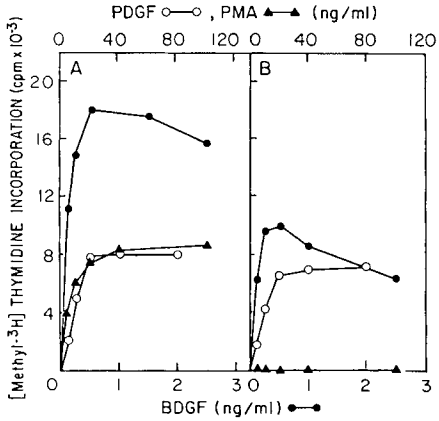

Fig. 5. Effect of PMA pretreatment on the mitogenic response of Swiss mouse 3T3 cells to BDGF, PDGF, and PMA. Swiss mouse 3T3 cells were pretreated without **(A)** or with PMA (400 ng/ml) **(B)** in DMEM. After 24 hr at 37°C, cells were washed and incubated with various concentrations of BDGF, PDGF, or PMA in DMEM containing 5% plasma-derived serum and [methyl-^{3}H]thymidine (0.3 μCi/ng/ml). The mitogenic activity assay was stopped by washing monolayers sequentially with 10% trichloroacetic acid and ethanol:ether (2:1, v/v).

mM NaCN), which have been shown to inhibit the process of pinocytosis in fibroblasts [34,35] and Swiss mouse 3T3 cells (our unpublished results). The ability of BDGF to down-modulate the EGF receptor in these cells was then examined. As shown in Table III, preincubation of cells with energy inhibitors blocked the down-modulation of EGF receptor induced either by BDGF or by PDGF, whereas the preincubation failed to inhibit the down-modulation induced by PMA. The effect of these energy inhibitors was reversible (Table IV); following removal of the inhibitors and subsequent incubation of the cells in DMEM for 2 hr, the EGF receptor could be

TABLE III. Effect of Preincubation of Swiss Mouse 3T3 Cells With a Mixture of Energy Inhibitors (NaCN, Na Azide, and NaF) on Down-Modulation of the EGF Receptor Induced by BDGF, PDGF, and PMA*

	^{125}I-EGF bound (cpm/well)	
Medium	Control cells	Cells preincubated with energy inhibitors
No addition	2,187 ± 222 (100%)	2,325 ± 195 (100%)
BDGF (1 ng/ml)	349 ± 75 (16%)	2,083 ± 141 (90%)
PDGF (40 ng/ml)	1,269 ± 125 (58%)	2,439 ± 178 (105%)
PMA (100 ng/ml)	241 ± 40 (11%)	382 ± 110 (16%)

*The monolayers of Swiss mouse 3T3 cells in Costar 24-well cluster dishes were preincubated with or without a mixture of inhibitors, 10 mM Na azide, 10 mM NaF, and 1 mM NaCN in 5 mM HEPES (pH 7.4), 0.15 M NaCl, at 37°C for 10 min. BDGF (1 ng/ml), PDGF (40 ng/ml), or PMA (100 ng/ml) was then added to these wells. Cells were incubated at 37°C for a further 10 min. Afterward, the specific cell surface ^{125}I-EGF binding was measured by incubation of cells with ^{125}I-EGF (1 ng/ml) in the presence and absence of 100-fold excess of unlabeled EGF at 0°C for 2½ hr.

TABLE IV. Reversibility of the Inhibition of BDGF-Induced Down-Modulation of the EGF Receptor by Energy Inhibitors in Swiss Mouse 3T3 Cells*

	^{125}I-EGF bound (cpm/well)	
	− BDGF	+ BDGF (1 ng/ml)
Cells preincubated with energy inhibitors at 37°C for 10 min	2,810 ± 250 (100%)	2,010 ± 192 (92%)
Cells preincubated with energy inhibitors at 37°C for 20 min, followed by removal of energy inhibitors and subsequent incubation in DMEM at 37°C for 2 hr	2,280 ± 195 (100%)	510 ± 75 (22%)

*Swiss mouse 3T3 cells were preincubated with energy inhibitors (10 mM Na azide, 10 mM NaF, and 1 mM NaCN) in 5 mM HEPES buffer (pH 7.4), 0.15 M NaCl at 37°C for 10 min. BDGF (0 and 1 ng/ml) was then added to the buffer medium, and the cells were incubated at 37°C for a further 10 min. The specific cell surface ^{125}I-EGF binding was then determined. In a parallel experiment, 3T3 cells were incubated with energy inhibitors at 37°C for a further 10 min. The specific cell surface ^{125}I-EGF binding was then determined. In a parallel experiment, 3T3 cells were incubated with energy inhibitors at 37°C for 20 min. The buffer medium was replaced by DMEM. After 2 hr at 37°C, DMEM was changed to 5 mM HEPES (pH 7.4), 0.15 M NaCl. The cells were then treated with or without BDGF (1 ng/ml) at 37°C for 10 min. The specific cell surface ^{125}I-EGF binding was determined.

down-modulated by BDGF. These results suggest that BDGF and PDGF induce down-modulation of the EGF receptor by stimulating receptor internalization. Furthermore, it appears that PMA induces down-modulation through a different mechanism.

It has been shown that amino compounds such as methylamine and chloroquine, which raise the intraendosomal and intralysosomal pH [36], inhibit recycling of the EGF receptor [37] and of the phosphomannosyl receptor [34] but not the internalization of these receptors. If BDGF induces the down-modulation of EGF receptor by increasing the rate of internalization of the receptor, then these amino compounds might augment the down-modulation. To test this possibility, 3T3 cells were preincubated with or without 10 mM of methylamine at 37°C for 10 min and then subjected to treatment with different concentrations of BDGF for a further 10 min at 37°C. Following this, the cell surface high-affinity receptors were measured by incubation of cells at 0°C with ^{125}I-EGF (1 ng/ml) for 2½ hr. As shown in Figure 6, methylamine indeed enhanced the down-modulation of the EGF receptor induced by BDGF. A similar effect was also observed when the cells were preincubated with 50 μM of chloroquine (Table V). These results are consistent with BDGF-stimulated internalization of the EGF receptor.

With respect to mitogenesis, the functional relevance of transmodulation of the EGF receptor by growth factors [11–17] and biologically active ligands [18–20] is not clear. Rozengurt and his coworkers have correlated the relative capacities of vasopressin- or bomesin-related peptides to down-modulate the EGF receptor with their ability to stimulate DNA synthesis in Swiss mouse 3T3 cells [18–20]. As demonstrated by several investigators [16,38], the ligands that down-modulate EGF receptor can act synergistically with EGF to promote cell proliferation. To define the functional relevance of down-modulation of the EGF receptor by BDGF in terms of mitogenic

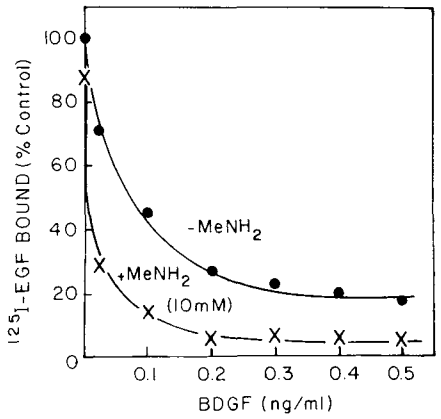

Fig. 6. Effect of methylamine on down-modulation of the EGF receptor induced by various concentrations of BDGF. The monolayers of Swiss mouse 3T3 cells were preincubated with and without 10 mM of methylamine ($MeNH_2$) in 5 mM HEPES (pH 7.4), 0.15 M NaCl. After 10 min at 37°C, various concentrations of BDGF were added to the buffer medium. The cells were then incubated at 37°C for a further 10 min. The specific cell surface ^{125}I-EGF binding was measured by incubation of cells with ^{125}I-EGF (1 ng/ml) in the presence and absence of 100-fold excess of unlabeled EGF. The specific cell surface ^{125}I-EGF binding of cells preincubated without BDGF and methylamine was taken as 100% binding.

TABLE V. Effect of Preincubation of Swiss Mouse 3T3 Cells With Methylamine or Chloroquine on Down-Modulation of the EGF Receptor Induced by BDGF*

	^{125}I-EGF bound (cpm/well)	
	− BDGF	+ BDGF (1 ng/ml)
Control cells	2,280 ± 125 (100%)	478 ± 45 (21%)
Cells preincubated with methylamine (10 mM)	2,075 ± 140 (91%)	116 ± 20 (5%)
Cells preincubated with chloroquine (50 μM)	2,052 ± 180 (89%)	126 ± 25 (6%)

*Swiss mouse 3T3 cells were preincubated with or without methylamine (10 mM) or chloroquine (50 μM) in 5 mM HEPES (pH 7.4), 0.15 M NaCl at 37°C for 10 min. BDGF (1 ng/ml) was then added to the buffer medium, and the cells were incubated at 37°C for a further 10 min. The specific cell surface ^{125}I-EGF binding was then measured by incubation of cells with ^{125}I-EGF (1 ng/ml) at 0°C for 2½ hr.

TABLE VI. Additive Effects of BDGF and EGF on the Stimulation of DNA Synthesis in Swiss Mouse 3T3 Cells*

	[Methyl-^{3}H]thymidine incorporation (cpm/well)	
Medium	Observed	Expected
Serum-free medium assay		
BDGF (1 ng/ml)	17,402 ± 1,072	—
EGF (1 ng/ml)	5,176 ± 310	—
BDGF (1 ng/ml) + EGF (1 ng/ml)	21,542 ± 2,466	22,578
Plasma-derived serum medium assay		
BDGF (1 ng/ml)	16,336 ± 1,066	—
EGF (1 ng/ml)	6,580 ± 902	—
BDGF (1 ng/ml) + EGF (1 ng/ml)	19,416 ± 1,782	22,916

*Swiss mouse 3T3 cells were preincubated with or without BDGF (1 ng/ml) in serum-free medium or plasma-derived serum containing medium. After 10 min at 37°C, EGF (1 ng/ml) was added as indicated above. The mitogenic activity assay in serum-free medium or in plasma-derived serum containing medium was then carried out as described in Experimental Procedures.

activity, the interaction of BDGF and EGF in stimulation of mitogenesis in Swiss mouse 3T3 cells was investigated. In our assay conditions of 20-hr incubation in human plasma-derived serum containing medium or serum-free medium, an additive effect of stimulation of DNA synthesis was observed at 1 ng/ml of BDGF and 1 ng/ml of EGF (Table VI). This result is consistent with our earlier observation [4] and suggests that the down-modulation of EGF receptor by BDGF does not have an effect on the DNA synthesis stimulated by EGF.

DISCUSSION

In this communication, we have demonstrated that BDGF induces the down-modulation of high-affinity EGF receptors in a time, temperature, and concentration-dependent manner. BDGF appears to induce the down-modulation through interaction with BDGF receptor. This conclusion is based on the following observations: 1)

Protamine, a potent inhibitor of BDGF receptor binding and mitogenic activities, completely inhibited the down-modulation induced by BDGF. 2) The concentration dependence profile of protamine inhibition of BDGF-induced down-modulation is very similar to that of protamine inhibition of BDGF binding to its receptor [7,32]. 3) The half-maximal concentration of BDGF to induce the down-modulation is very close to the K_d of BDGF binding to receptor.

PDGF and PMA have previously been shown to induce down-modulation of the high-affinity EGF receptor in Swiss mouse 3T3 cells [11–16]. We, therefore, compared the down-modulation induced by these different mitogens. BDGF appears to be more potent than PDGF but weaker than PMA in terms of the rate and magnitude of down-modulation induced. The potency of these three mitogens may correlate with their binding kinetics rather than their receptor number in Swiss mouse 3T3 cells [6,7,39]. The BDGF receptor number is estimated to be about 1/10 to 1/20 of that of the PDGF receptor in these cells [6,7].

The down-modulation of the high-affinity EGF receptor by BDGF did not seem to be the result of the conversion of the high-affinity form to the low-affinity form of the receptor as has been suggested to occur in the case of PMA-induced down-modulation [40]. It seems that the BDGF-induced down-modulation was due to a selective disappearance of the high-affinity EGF receptor from the cell surface. This disappearance could be the result of either increased internalization of the EGF receptor or the inhibition of recycling of constitutively internalized receptor. It has been proposed that EGF receptors on the cell surface of KB (human epidermoid carcinoma) cells undergo continuous internalization and recycling even in the absence of ligand [37]. The fact that BDGF or PDGF-induced down-modulation of the EGF receptor was blocked by inhibitors of the pinocytosis process clearly suggests that both BDGF and PDGF induce down-modulation by stimulating the internalization of the receptor. This is supported by the observation that receptor recycling inhibitors, such as methylamine and chloroquine, augment the down-modulation induced by BDGF.

The presence of pinocytosis inhibitors had no effect on down-modulation of the EGF receptor induced by PMA, suggesting that the internalization process may not be required for the PMA down-modulation activity. Further evidence that BDGF and PMA cause down-modulation of the EGF receptor by different mechanisms came from the study of PMA-pretreated cells. BDGF was capable of down-modulating the EGF receptor in PMA-pretreated cells, whereas PMA had no effect on the cell surface EGF receptor of these cells.

The functional relevance of down-modulation of the EGF receptor by BDGF is not known. The additive effect of BDGF and EGF on stimulation of DNA synthesis in 3T3 cells suggests that the rapid down-modulation of the EGF receptor by BDGF does not affect EGF-stimulated DNA synthesis which begins at 12 hr after stimulation of cells by growth factor. It has been reported that the stimulation of Na^+/H^+ antiporter is one of the early biochemical reactions occurring following growth factor-cell surface receptor interaction, but that this is also not necessary for induction of DNA synthesis by EGF [41]. Since cell growth is a complex biological process, the rapid down-modulation of the EGF receptor and other early biochemical reactions may be required for the complete process of cell growth stimulated by growth factors.

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