Somatostatin Analogues and Bombesin/Gastrin-releasing Peptide Antagonist

RC-3095 Inhibit the Growth of Human Glioblastomas in Vitro and in Vivo

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ABSTRACT

We investigated the effects of somatostatin analogues and a synthetic bombesin/gastrin-releasing peptide (GRP) antagonist on the growth of the human malignant glioma cell lines U-87MG and U-373MG transplanted into nude mice or cultured in vitro. Nude mice bearing s.c. implanted U-87MG or U-373MG tumors were treated for 4 and 6 weeks, respectively, with various somatostatin analogues or bombesin/GRP antagonist RC-3095. Somatostatin analogues RC-160, RC-160-II, and RC-101-I, given s.c. in doses of 100 μg/animal/day, inhibited the growth of U-87MG xenografts as shown by more than 60% reduction in tumor volumes and 45% reduction in tumor weights compared with the control group. Bombesin/GRP antagonist RC-3095, given s.c. at a dose of 20 μg/animal twice daily, had the greatest inhibitory effect and decreased tumor volumes and weights by approximately 79% and 72%, respectively. The growth of U-373MG xenografts was also significantly inhibited by treatment with analogue RC-160 or antagonist RC-3095. The mean survival time of nude mice, inoculated orthotopically with U-87MG cells into the brain, was significantly prolonged by 4.9 days by treatment with antagonist RC-3095.

Serum gastrin levels in animals bearing U-87MG tumors, treated with antagonist RC-3095 or somatostatin analogues, were decreased compared with controls. All three somatostatin analogues also reduced serum growth hormone levels. Receptor analyses demonstrated high-affinity binding sites for bombesin, somatostatin, and epidermal growth factor on membranes of U-87MG and U-373MG tumors. The concentration of binding sites for epidermal growth factor on both tumors was significantly decreased after in vivo treatment with antagonist RC-3095 or the somatostatin analogues. In studies in vitro, RC-3095, added to the culture medium, significantly inhibited the proliferation of U-87MG and U-373MG cells in the presence of GRP(14-27), as measured by cell number, but only a moderate suppression of growth of both cell lines was observed with somatostatin analogue RC-160. These results demonstrate that bombesin/GRP antagonist RC-3095 and somatostatin analogues such as RC-160 can inhibit the growth of human glioblastoma cell lines U-87MG and U-373MG in vitro as well as in vivo. Our work suggests the merit of further investigations of these analogues for the possible development of new approaches for treatments of patients with malignant gliomas.

INTRODUCTION

Malignant astrocytomas or glioblastomas represent the most common type of primary brain tumor in adults (1, 2). More than half of the gliomas of adults are malignant astrocytomas (1, 2). Surgery, radiation, and chemotherapy are of limited effectiveness in the treatment of malignant gliomas and other therapeutic approaches must be explored. That glioblastomas and other brain tumors are hormone sensitive is supported by epidemiological, clinical, and laboratory evidence (3–7). The presence of receptors for glucocorticoid, androgen, and progesterone in glioblastomas has been documented (4–7). The expression of receptors for EGF (3) and IGF-I and IGF-II in human brain tumors, including malignant gliomas, is also well established (4, 8–15). It thus appears that sex steroids and growth factors might be involved in the proliferation of brain tumors. Some types of brain tumors, such as astrocytomas and meningiomas, contain significant levels of high-affinity receptors for bombesin/GRP or somatostatin (16–23). These findings suggest the need for investigations to determine the effects of somatostatin analogues and bombesin/GRP antagonists on the growth of brain tumors.

Potent octapeptide analogues of somatostatin and pseudononapeptide antagonists of bombesin/GRP have been synthesized in our laboratory (24–26). Somatostatin analogues RC-160, RC-160-II, and RC-101-I are about 100 times more potent than somatostatin in tests for inhibition of GH release in rats, and possess a prolonged duration of action (25). [D-Tp6,Leu13][CH2NH]3 bombesin(6-14) (RC-3095) is a short-chain pseudononapeptide bombesin antagonist (26). This analogue blocks GRP-stimulated amylase release from rat pancreatic acini, binds to Swiss 3T3 fibroblasts and H-345 small-cell lung carcinoma cells, and inhibits GRP-stimulated growth of these cells in vitro (26). Various findings suggest that somatostatin analogues as well as bombesin/GRP antagonists may inhibit the growth of several cancers by interfering with the action, secretion, signal transmission, or receptors of endogenous growth factors (24–31). Previously, we have shown that both types of these peptide analogues can significantly inhibit the growth of gastric, pancreatic, mammary, prostatic, and lung cancers in vivo (27–31). This antitumor effect of bombesin/GRP antagonist and somatostatin analogues was associated with a significant down-regulation of EGF receptors on membranes of these tumors.

In this study, we have evaluated the effects of somatostatin analogues RC-160, RC-160-II, and RC-101-I and bombesin/GR antagonist RC-3095 on the growth of xenografts of the human malignant glioma cell lines U-87MG and U-373MG in athymic nude mice. To clarify the mechanisms of antitumor action of these agents, detailed receptor status and histological examinations were performed. We also examined the effects of RC-160 and RC-3095 on serum GH and gastrin levels because the growth of brain tumors may be promoted by GH through stimulation of IGF-1 (32), as well as by gastrin, since evidence exists for the presence of gastrin and gastrin receptors in human brain (33, 34). In addition, possible direct effects of RC-3095 and RC-160 on proliferation of U-87MG and U-373MG cells in vitro were evaluated in tissue cultures.

MATERIALS AND METHODS

Peptides

Somatostatin analogues D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH2 (RC-160), N-acetyl-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH2 (RC-160-II, corresponding to acetylated RC-160), and N-acetyl-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH2 (RC-101-I), originally synthesized by us (35), were made by classical synthesis by Novabiochem (Laufingen, Switzerland)
and supplied by Debiopharm S. A. (Lausanne, Switzerland). For the injections, the somatostatin analogues were dissolved in 0.1 N acetic acid and diluted with saline containing 0.1–0.5% bovine serum albumin.

Bombesin/GRP antagonist RC-3095 [D-Tpi⁶, Leu¹³ ψ(CH₂NH)]Leu¹⁴ bombesin (6–14), originally synthesized in our laboratory (26), was made by Asta Medica (Frankfurt/Main, Germany). Tpi is a conformationally constrained secondary amine derivate of tryptophan. GRP(14–27) was synthesized using solid-phase methods in our laboratory. RC-3095 and GRP(14–27) were dissolved in 0.1% dimethyl sulfoxide in saline.

Animals

Athymic male NCrnu/nu nude mice, approximately 6 weeks old on arrival, were obtained from the National Cancer Institute (Bethesda, MD) and maintained under pathogen-limited conditions.

Cell Lines

The U-87MG and U-373MG malignant glioma cell lines (astrocytomas, grade III; American Type Culture Collection, Rockville, MD) were grown as monolayers in Eagle’s basal medium supplemented with 10% fetal bovine serum, sodium pyruvate, and antibiotics and antimitotics. Cultures were incubated at 5% CO₂ in air at 37°C.

In Vivo Studies

Studies on Tumor Growth. In the first experiment, xenografts were initiated by s.c. injection of 1 × 10⁷ U-87MG cells into the right flanks of 5 male mice. Tumors resulting after 4 weeks were aseptically dissected and mechanically minced; 3-mm³ pieces of tumor tissue were transplanted s.c. by trocar needle into 60 male animals under methoxyflurane anesthesia. Two weeks after transplantation, when tumors had grown to a volume of approximately 25 mm³, the mice were randomized and divided into 5 experimental groups of 10 animals each and received the following treatment for 4 weeks: group 1, 0.1 M acetic acid; group 2, RC-101-I at a dose of 100 μg/day/animal; group 3, RC-160-II at a dose of 100 μg/day/animal; and group 4, RC-101-I at a dose of 100 μg/day/animal; and group 5, RC-3095 at a dose of 20 μg/animal, injected s.c. twice daily. In the second experiment, xenografts were initiated by s.c. injection of 1 × 10⁷ U-373MG cells into the right flanks of 5 male mice. Tumors resulting after 4 weeks were aseptically dissected and mechanically minced; 3-mm³ pieces of tumor tissue were transplanted s.c. by trocar needle into 40 male animals under methoxyflurane anesthesia. Two weeks after transplantation, when tumors had grown to a volume of approximately 7 mm³, the mice were randomized and divided into 3 experimental groups of 10 animals each and received the following treatment for 6 weeks: group 1, 0.1% dimethyl sulfoxide in saline; group 2, RC-160 at a dose of 100 μg/day/animal; and group 3, RC-3095 at a dose of 20 μg/animal, injected s.c. twice daily.

In both experiments, the tumors were measured once a week. The tumor volumes and weights were measured in a blinded manner. Tumor volume was calculated as length × width × height × 0.5236. Tumor volume doubling time was calculated between the start and the end of the treatment. At the end of the experiments, there were no significant differences in body weights between groups.

Radioimmunoassays. Serum levels of GH were determined by double-antibody radioimmunoassay using materials supplied by the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases. Interassay and intrainassay coefficients of variation were less than 15% and 10%, respectively. Serum gastrin levels were measured by double-antibody radioimmunoassay with a kit provided by Becton Dickinson (Orangeburg, NY). The interassay variation was less than 7.5%, and the intrainassay variation was less than 4.0%.

Receptor Assays. Receptors for bombesin, somatostatin, and EGF on the membranes of U-87MG and U-373-MG tumors were measured as described previously (28, 29, 35). The LIGAND PC computerized curve-fitting program of Munson and Rodbard (37) was used to determine the types of receptor binding, dissociation constant (Kd) values, and the maximal binding capacity (Bmax) of receptors.

In Vitro Studies

U-87MG and U-373MG cells from 70 to 80% confluent cultures were seeded into Costar 24-multiwell plates (Costar, Cambridge, MA) at a density of 5 × 10⁵ cells/well and grown in Eagle’s minimum essential medium supplemented with 10% fetal bovine serum, sodium pyruvate, and antibiotics and antimitotics. Cultures were incubated at 5% CO₂ in air at 37°C.

RESULTS

Effect of Peptide Analogues on Growth of Malignant Gliomas in Nude Mice. The effects of treatment with various peptide analogues on body and tumor weights in nude mice implanted s.c. with malignant gliomas as well as final tumor volumes in both experiments are shown in Table 1. At the end of the experiments, there were no significant differences in body weights between groups.

In experiment 1, all three somatostatin analogues significantly suppressed growth of U-87MG tumors. After 4 weeks, the mean tumor volume was significantly (P < 0.01) reduced in groups receiving RC-160, RC-160-II, and RC-101-I to 598.6 ± 123.9 mm³, 448.5 ± 91.2 mm³, and 398.0 ± 126.7 mm³, respectively, as compared with that of the control group (1599.0 ± 250.1 mm³) (Fig. 1; Table 1). Tumor volume doubling time was significantly prolonged by all three somatostatin analogues. Treatment with RC-101-I extended the tumor doubling time to 8.0 ± 1.6 days compared with that of the control group, which had a tumor doubling time of 4.6 ± 0.1 days. The final tumor weights were reduced by 45–61% in the groups treated with RC-160, RC-160-II, or RC-101-I as compared with those of the controls (Table 1).

Therapy with bombesin/GRP antagonist RC-3095 appeared to be the most effective and resulted in the greatest inhibition of tumor growth of both cell lines. 

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Bombesin/GRP antagonist RC-3095 inhibited the growth of cell lines U-87MG and U-373MG. The inhibition was significant after 2 weeks of treatment for RC-3095, whereas that for RC-160 was not statistically significant from that of controls. The ratio of apoptotic to mitotic indices was significantly (P < 0.01) higher only in the group receiving RC-101-I (2.67 ± 0.8 versus 0.84 ± 0.2 in controls).

The U-373-MG tumors consisted of elongated cells arranged in curving bundles. The cells had narrow eosinophilic cytoplasms and long, rod-shaped nuclei. The number of mitotic cells decreased and that of apoptotic cells increased in the treated tumors, but the difference was not statistically significant from that of controls. The ratio of apoptotic to mitotic indices was significantly (P < 0.01) higher only in the group receiving RC-101-I (2.67 ± 0.8 versus 0.84 ± 0.2 in controls).

Effect of RC-3095 and RC-160 on Survival Time. The life span of mice, inoculated orthotopically with U-87MG cells, was prolonged significantly (P < 0.01) by giving them bombesin/GRP antagonist RC-3095 (Fig. 3). The mean survival time of mice in the control and RC-3095-treated groups were 41.2 ± 0.7 and 46.1 ± 0.6 days, respectively. Thus, treatment with RC-3095 increased the mean survival time by 4.9 days. The treatment with somatostatin analogue RC-160 also prolonged the mean survival time to 44.2 ± 1.8 days (not shown), but this extension of survival time was not statistically significant compared to the control group.

Histological Findings. Histologically, the U-87MG tumors were highly cellular and contained very little stroma. The cells were polygonal or elongated and the oval-shaped and chromatin-rich nuclei were surrounded by broad, light eosinophilic and finely granular cytoplasm. Some of the tumors contained necrotic areas with inflammatory cell infiltration. The number of mitotic cells decreased and that of apoptotic cells increased in the treated tumors, but the difference was not statistically significant from that of controls. The ratio of apoptotic to mitotic indices was significantly (P < 0.01) higher only in the group receiving RC-101-I (2.67 ± 0.8 versus 0.84 ± 0.2 in controls).

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<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Experiment 1: (U-87MG)</th>
<th>Experiment 2: (U-373MG)</th>
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<tbody>
<tr>
<td></td>
<td>Body weight (g)</td>
<td>Tumor weight (g)</td>
</tr>
<tr>
<td>Control</td>
<td>24.1 ± 0.8a</td>
<td>1.8 ± 0.3a</td>
</tr>
<tr>
<td>RC-3095</td>
<td>22.0 ± 1.3</td>
<td>0.5 ± 0.2a</td>
</tr>
<tr>
<td>RC-160</td>
<td>22.3 ± 0.7</td>
<td>0.8 ± 0.2a</td>
</tr>
<tr>
<td>RC-160-II</td>
<td>21.0 ± 1.2</td>
<td>0.7 ± 0.3a</td>
</tr>
<tr>
<td>RC-101-I</td>
<td>23.0 ± 1.1</td>
<td>0.052 ± 0.01a</td>
</tr>
</tbody>
</table>

* a Mean ± SE.  
* b P < 0.01 vs. control.  
* c P < 0.05.

Table 1. Effect of treatment with various peptide analogues on body weight and tumor volume in nude mice with xenografts of human malignant glioma cell lines U-87MG and U-373MG.
that of apoptotic cells increased, but the ratio of apoptotic to mitotic indices was not significantly changed by the treatments.

**Serum Hormone Levels.** The levels of serum gastrin and GH in nude mice bearing U-87MG glioma treated with peptide analogues in the first experiment are shown in Table 2. Gastrin levels in animals treated with the somatostatin analogues or bombesin/GRP antagonist RC-3095 were significantly reduced compared with the levels of controls. Serum GH in animals treated with somatostatin analogues RC-160, RC-160-II, and RC-101-I was greatly decreased (P < 0.01) compared with that of controls. There were no changes in serum GH levels after treatment with RC-3095.

**Receptor Findings.** The binding characteristics of receptors for bombesin/GRP, somatostatin, and EGF in U-87MG and U-373MG tumors were analyzed following treatment with peptide analogues in both experiments, and the results on the EGF receptors are presented in Table 3. In experiment 1, receptor assays on U-87MG tumor membranes showed high-affinity binding sites for bombesin/GRP (Kd, 6.1 ± 0.5 nm), somatostatin (Kd, 7.1 ± 2.1 nm), and EGF (Kd, 0.9 ± 0.1 nm). The concentration of receptors for bombesin/GRP was significantly (P < 0.01) reduced by treatment with bombesin/GRP antagonist RC-3095 to 172.7 ± 10.3 fmol/mg protein as compared to that in the control group (392.7 ± 27.8 fmol/mg protein). This may be due to receptor occupation by this analogue. The binding capacity of EGF receptors was significantly (P < 0.01) decreased after treatment with somatostatin analogues RC-160, RC-160-II, and RC-101-I or bombesin/GRP antagonist RC-3095 (Table 3). Therapy with the somatostatin analogues somewhat increased the binding capacity of receptors for somatostatin on membranes of U-87MG tumors, but this increase was not statistically significant compared to that of the controls. The binding capacity (Bmax) of somatostatin receptors in the control group was 706.7 ± 155.0 fmol/mg protein. In experiment 2, receptor assays on membranes of U-373MG tumors also demonstrated high-affinity binding sites for bombesin/GRP (Kd, 1.3 ± 0.2 nm), somatostatin (Kd, 8.4 ± 1.1 nm), and EGF (Kd, 0.86 ± 0.1 nm) (Table 3). A significant (P < 0.01) reduction in binding capacity of bombesin/GRP receptors was again observed after treatment with RC-3095. The binding capacity (Bmax) of bombesin/GRP receptors in the control tumors was 788.0 ± 18.2 fmol/mg protein, but only 530.0 ± 24.7 fmol/mg protein in tumors treated with RC-3095. Somatostatin analogue RC-160 and bombesin/GRP antagonist RC-3095 significantly (P < 0.01) decreased the binding capacity of EGF receptors in membranes of this tumor, the latter analogue causing a greater reduction as in experiment 2 (Table 3). No changes in binding capacity and affinity of somatostatin receptors occurred after treatment with RC-3095 or RC-160 on U-373MG glioblastoma cells in vitro.

**In Vitro Studies.** The proliferation of U-373MG and U-87MG glioblastoma cells in vitro monitored by cell number could be only moderately stimulated by about 15% (P < 0.05) and 11%, respectively, as compared with the controls, by the addition of GRP(14-24) at 10-5 M concentration to the culture medium (not shown). This poor stimulation by exogenous GRP could be due to production of endogenous bombesin-like peptides by these tumors. Bombesin/GRP antagonist RC-3095, added alone to the culture medium at 10-7, 10-6, and 10-5 M concentrations, significantly (P < 0.01) decreased the number of U-87MG cells by 14%, 30%, and 37%, respectively, as compared to that of controls. At lower doses of RC-3095, the decrease in cell number was not statistically significant. In contrast, RC-3095 significantly (P < 0.01) suppressed the proliferation of U-373MG glioblastoma cells at concentrations of 10-11-10-8 M, but not at higher doses. In the presence of 10-11, 10-10, 10-9, and 10-8 M RC-3095, the number of U-373MG cells was reduced by 33%, 25%, 27%, and 21%, respectively, as compared to cells not exposed to this antagonist.

Bombesin antagonist RC-3095 significantly inhibited the proliferation of U-87MG and U-373MG cells in vitro at 10-11-10-5 M concentrations in the presence of (10-5 M) GRP(14-27) as shown in Fig. 4. At 10-8 M concentration of RC-3095, the number of U-87MG and U-373MG cells was decreased by about 40% and 61%, respectively (Fig. 4). In the presence of (10-5 M) RC-3095, the number of

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**Table 2. Serum gastrin and GH levels in nude mice with xenografts of the human malignant glioma cell line U-87MG after treatment with various peptide analogues.**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Gastrin (pg/ml)</th>
<th>Growth hormone (ng/ml)</th>
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<tbody>
<tr>
<td>Control</td>
<td>167±5 12.0a</td>
<td>17.0 ± 4.9a</td>
</tr>
<tr>
<td>RC-3095</td>
<td>130.2 ± 14.8b</td>
<td>19.5 ± 6.5</td>
</tr>
<tr>
<td>RC-160</td>
<td>99.5 ± 10.8</td>
<td>7.2 ± 2.4</td>
</tr>
<tr>
<td>RC-160-II</td>
<td>98.6 ± 3.4c</td>
<td>3.9 ± 0.2c</td>
</tr>
<tr>
<td>RC-101-I</td>
<td>95.8 ± 7.4c</td>
<td>3.8 ± 0.7c</td>
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* Mean ± SE.
*P* < 0.05.
*P* < 0.01 vs. control.

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**Table 3. Binding characteristics of receptors for EGF in membranes of U-87MG and U-373MG human malignant gliomas after in vivo treatment with various peptide analogues.**

<table>
<thead>
<tr>
<th>EGF</th>
<th>Group</th>
<th>Kd (nm)</th>
<th>Bmax (fmol/mg protein)</th>
</tr>
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<tbody>
<tr>
<td>Experiment 1: (U-87MG)</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>0.9 ± 0.1a</td>
<td>250.3 ± 14.6</td>
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<tr>
<td>RC-3095</td>
<td>0.4 ± 0.2b</td>
<td>69.7 ± 9.8</td>
<td></td>
</tr>
<tr>
<td>RC-160</td>
<td>1.0 ± 0.1</td>
<td>130.3 ± 14.1</td>
<td></td>
</tr>
<tr>
<td>RC-160-II</td>
<td>1.0 ± 0.2</td>
<td>139.7 ± 17.3</td>
<td></td>
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<tr>
<td>RC-101-I</td>
<td>1.1 ± 0.1</td>
<td>141.0 ± 8.1</td>
<td></td>
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<tr>
<td>Experiment 2: (U-373MG)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>0.86 ± 0.1</td>
<td>244.8 ± 3.9</td>
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<tr>
<td>RC-3095</td>
<td>0.58 ± 0.1</td>
<td>113.4 ± 1.6</td>
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<tr>
<td>RC-3095</td>
<td>0.69 ± 0.2</td>
<td>87.2 ± 12.2</td>
<td></td>
</tr>
</tbody>
</table>

* Binding characteristics were obtained from 10-point displacement experiments in triplicate tubes. Significance was calculated with Duncan’s new multiple range test. All values represent mean ± SE of 2–3 experiments, each done in triplicate.
*P* < 0.05.
*P* < 0.01 vs. control.
U-87MG and U-373MG cells was reduced by approximately 43% and 67%, respectively, as compared to cells cultures only with 10^{-5} \text{ M GRP}(14-27).

Somatostatin analogue RC-160 significantly suppressed the proliferation of U-87MG and U-373MG glioblastoma cell lines at 10^{-6} and 10^{-5} \text{ M concentrations (not shown). At lower doses of RC-160, the decrease in cell number was not statistically significant as compared to U-87MG and U-373-MG cells not exposed to this analogue. In the presence of 10^{-6} or 10^{-5} \text{ M RC-160, the number of U-373MG cells was significantly (P < 0.01) reduced by approximately 38% and 37%, respectively. In the case of the U-87MG cell line, a somewhat smaller but significant (P < 0.05) decrease in cell number of 13 and 15%, respectively, was found at both concentrations of RC-160.

DISCUSSION

In this study, we demonstrated a significant inhibitory effect of bombesin/GRP antagonist RC-3095 on proliferation of U-87MG and U-373MG cell lines in vitro as well as on the growth of xenografts of these malignant gliomas in athymic nude mice. RC-3095 also prolonged the survival time of nude mice inoculated orthotopically with U-87MG cells. We have shown that inhibition of the growth of both glioblastomas was accompanied by a decrease in the concentration of bombesin/GRP receptors on membranes of these tumors. Our findings, indicating the presence of high-affinity binding sites for bombesin/GRP on membranes of both tumors, are in agreement with results previously reported by other groups demonstrating the expression of high-affinity receptors for bombesin/GRP in rat and human glioblastoma cell lines (16–18). In our study, the proliferation of both glioblastoma cell lines was only moderately stimulated by addition of 10^{-5} \text{ M GRP}(14-27) to the culture medium. This observation could be tentatively explained by an autocrine or paracrine production of bombesin/GRP-like peptides by the glioblastoma cells, thereby causing a nearly maximum stimulation of cell growth. Bombesin/GRP-like peptides were shown to stimulate the growth of small-cell lung carcinoma, prostatic, mammary, and pancreatic cancer lines (38–41), and this growth could be inhibited by bombesin/GRP antagonists (38, 39, 42).

The inhibition of U-87MG and U-373MG tumor growth by bombesin/GRP antagonist RC-3095 might be partially brought about by blockade and/or down-regulation of bombesin/GRP receptors on U-87MG and U-373MG cells. We have also shown that inhibition of growth of various cancers, including pancreatic, prostatic, mammary, gastric, and lung by antagonist RC-3095, was associated with a major decrease in EGF receptor levels on tumor membranes (27–31). Thus, bombesin/GRP antagonists may act locally by various mechanisms which result in a reduction in the available binding sites for EGF. EGF receptors have been shown to be present in various brain tumors such as meningiomas and glia-derived tumors (4, 8, 11–15). A positive relationship was found between EGF receptor levels, phosphokinase activity, and the degree of malignancy of astrocytomas (11). In primary cell cultures, EGF was reported to stimulate the proliferation of rat astrocytic glial cells and human glioma cells derived from surgically obtained glioma specimens (13, 43).

The exact molecular mechanism of action of bombesin/GRP antagonists of EGF receptors is still not well understood. Bombesin initiates a series of intracellular signals which cause an increase in inositol 1,4,5-triphosphate, a mobilization of Ca^{2+}, and diacylglycerol production, leading to activation of protein kinase C (29, 42, 44). Activation of protein kinase C causes phosphorylation of EGF receptors on threonine residues. Bombesin and GRP were shown to enhance the phosphorylation of EGF receptors, and antagonist RC-3095 inhibited these effects in various cancer lines and cancer specimens (45). These results suggest that bombesin and GRP may function by up-regulating EGF receptors and that antagonist RC-3095 prevents this up-regulation (45). Bombesin/GRP antagonists may also block early cellular events that precede calcium mobilization and stimulation of mitogenesis (46). Chronic administration of our potent somatostatin analogues also significantly inhibited the growth of U-87MG and U-373MG cell lines xenografted into nude mice. The number of mitotic cells decreased and that of apoptotic cells increased in the treated tumors, but the ratio of apoptotic to mitotic indices was significantly higher only in the group receiving RC-101-I. Antineoplastic actions of somatostatin analogues appear to involve multiple mechanisms. A significant fall in GH levels induced by somatostatin analogues could, through mechanisms involving suppression of endogenous growth factors such as IGF-I and IGF-II, be of major importance for the inhibition of tumor growth (24). In our study, serum GH levels in mice treated with RC-160, RC-160-II, or RC-101-I were decreased by more than 58% as compared with those of control mice. Membrane receptors for IGF-I and specific mRNA for IGF-I and IGF-II were found in human CNS, suggesting that these growth factors may play a role in the growth and maturation of neural tissue (47–49). In addition, it has been shown that meningiomas and gliomas possess specific IGF receptors and that exposure to IGFs can promote the proliferation of these cells in culture (32, 50), suggesting that IGFs may play a role in the development and progression of certain CNS tumors. On the basis of our receptor assay results, which indicate the presence of high-affinity receptors for somatostatin on tumor membranes, analogues of somatostatin could also directly inhibit the growth of glioblastoma cells. Somatostatin and its analogues stimulate tyrosine phosphatase and promote the dephosphorylation of EGF receptors (19, 51). In our study, somatostatin analogues produced a great reduction in the concentration of EGF receptors on membranes of both glioblastoma tumors. Thus the sensitivity of U-87MG and U-373MG tumor cells to transforming growth factor \(\alpha\) and EGF could have been decreased in our study. Inhibition of tumor growth may also be brought about by the blockade of gastrin production, since gastrin and gastrin receptors were demonstrated in human brain (33, 34). However, the physiological role of gastrin in the brain, the presence of gastrin receptors in brain tumors, as well as the influence of gastrin on brain tumor growth remain to be elucidated in future studies.

In contrast to our data, Reubi et al. (22, 23) reported that highly undifferentiated glial tumors, such as glioblastomas, do not express somatostatin receptors. This discrepancy may be partially explained by more recent studies, which demonstrated the existence of several somatostatin receptor subtypes (19–21). Molecular cloning revealed the presence of five structurally related integral membrane glycoproteins, that are pharmacologically distinct high-affinity somatostatin receptors with different regional distributions and functions (19–21). Pharmacological studies on the characteristics of somatostatin receptors showed that the different subtypes exhibit major differences in their affinities for structural analogues of somatostatin (19–21). For example, SMS 201–995, a somatostatin analogue that is used to treat acromegaly as well as carcinoid syndrome, binds strongly to the somatostatin receptor subtype SSTR2, whereas receptor subtype SSTR1 has low affinity for this analogue (19, 21). The methodologies used by Reubi et al. (22, 23) in their studies on glioblastomas included in vitro receptor-binding analyses on homogenates and receptor autoradiography on tissue sections, using preferentially as radioligands a somatostatin-28 analogue or a somatostatin octapeptide analogue (125I-labeled Tyr\(^3\)-SMS 201–995) (22, 23). In our study, 125I-labeled RC-160 was used as a tracer in radioreceptor assays. Thus, the divergent results on the concentration of somatostatin receptors in glioblastomas between these two studies may be due to different
binding affinities of the somatostatin analogues used to various subtypes of somatostatin receptors.

Somatostatin analogue RC-160 was markedly less effective in suppressing the proliferation of U-87MG and U-373MG cells in vitro than the growth of xenografts of these cell lines in vivo. This phenomenon might be attributed to a reduction of binding sites for somatostatin on U-87MG and U-373MG cells in vitro. Both glioblastomas show high-binding capacity receptors for somatostatin on membranes of their xenografts but may have undergone changes in receptor content during long-term passages in vitro. Similar phenomena were observed by others. In a study on the growth-promoting action of gastrin on human colonic and gastric tumor cells, Watson et al. (52) found that the newly established cell lines were stimulated by pentagastrin at passage 2, but long-established cell lines did not respond to pentagastrin. Similarly, the colon adenocarcinoma DHD/K12 was inhibited by RC-160 when it grew as xenografts in BD IX rats, but was not responsive to RC-160 and somatostatin-14 in vitro (53). These findings, which indicate that tumor cell response to hormones could be decreased or lost during in vitro cultures, could explain the different extent of tumor growth inhibition obtained with RC-160 in vivo and in vitro in our study.

For many therapeutic agents, access to normal brain is retarded by the blood-brain barrier and to tumor tissues by a blood-brain tumor barrier. Many chemotherapeutic agents effective against peripheral cancers cannot be used for tumors of the CNS because the blood-brain barrier, comprised primarily of the capillary bed, often remains intact experimental investigations followed by clinical trials are necessary to point out content during long-term passages in vitro. Similar phenomena were observed by others. In a study on the growth-promoting action of gastrin on human colonic and gastric tumor cells, Watson et al. (52) found that the newly established cell lines were stimulated by pentagastrin at passage 2, but long-established cell lines did not respond to pentagastrin. Similarly, the colon adenocarcinoma DHD/K12 was inhibited by RC-160 when it grew as xenografts in BD IX rats, but was not responsive to RC-160 and somatostatin-14 in vitro (53). These findings, which indicate that tumor cell response to hormones could be decreased or lost during in vitro cultures, could explain the different extent of tumor growth inhibition obtained with RC-160 in vivo and in vitro in our study.

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Somatostatin Analogue and Bombesin/Gastrin-releasing Peptide Antagonist RC-3095 Inhibit the Growth of Human Glioblastomas in Vitro and in Vivo

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