Therapeutic Potential of Growth Factors and Their Antagonists

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This article describes studies with four peptides, epidermal growth factor (EGF), transforming growth factor alpha (TGF alpha), gastrin-releasing peptide/bombesin (GRP), and gastrin. The mitogenic and anti-secretory activities of EGF/TGF alpha appear to be mediated by a single class of high-affinity membrane receptors but may involve different signal transducing mechanisms. Biological activity of EGF resides in the N-terminal 42 amino acid fragment with the C-terminal undecapeptide determining binding affinity. A parenteral depot formulation of an EGF-related peptide or a small molecule agonist of the EGF receptor could have utility in treating various ulcerative disorders of the gut. Although antagonism of EGF (and thus TGF alpha) receptors and/or transducing mechanisms is frequently cited as a potential therapeutic approach to hyperproliferative diseases, blocking the action of TGF alpha, GRP, or gastrin with neutralizing antibodies or receptor antagonists did not influence the growth of a wide range of solid tumors in nude mice. These findings suggest that, unless tumor growth displays absolute dependency on one particular mitogen, antagonism of a specific growth factor is unlikely to have great effect in cancer therapy.

BACKGROUND

Antagonism of receptors or enzymes has underpinned the development of the modern pharmaceutical industry, as evidenced by the success of drugs such as beta-blockers, H2 antagonists, and cyclo-oxygenase inhibitors. Although many chronic diseases, such as hypertension, peptic ulceration, and arthritis, are effectively controlled by currently available agents, there has been almost no progress in the drug treatment of solid tumors for the past 30 years [1]. Currently available cytotoxic drugs have little or no influence on tumor growth or life expectancy and cause severe side effects. Indeed, such is the severity of the side effects caused by anti-proliferative therapy that recently introduced hemopoetic growth factors and anti-emetic drugs have themselves created multi-million dollar markets.

The one exception to this dismal picture is the success of drugs to combat hormone-dependent disease, pioneered by the development of anti-estrogens for treating breast cancer [2]. The possibility of identifying other solid tumors dependent on a particular mitogenic stimulus has provided an impetus to develop inhibitors of tumor growth factors. Blocking the action of these mitogens by preventing their production, by neutralization with antibodies, or by antagonists acting on cell surface receptors or enzymes involved in signal transduction is consistent with the time-

Abbreviations: EGF: epidermal growth factor GI: gastrointestinal GRP: gastrin-releasing peptide iv: intravenous SCLC: small-cell lung cancer TGF alpha: transforming growth factor alpha

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Mitogenic activity was assayed by measuring bromodeoxyuridine (Brdu) or tritiated thymidine incorporation into DNA and the effects confirmed by an increase in cell number [unpublished data].

honored approach to drug design in other therapeutic areas. This strategy has the best chance of success when the response in dependent on a single factor, as in an autocrine growth loop.

This paper describes studies directed at three growth-promoting peptides: the epidermal growth factor/transforming growth factor (EGF/TGF) alpha family, gastrin-releasing peptide (GRP/bombesin), and gastrin itself (G-17). All these peptides are capable of stimulating proliferation of gastrointestinal tumor cell lines, as evidenced by an increase in the rate of thymidine incorporation into DNA (Table 1). EGF interacts with the same receptor as the more widely distributed mitogen TGF alpha and is capable of stimulating proliferation in a wide range of cell types [3]. GRP is produced by human small-cell lung cancer (SCLC), stimulates proliferation of SCLC cells, and has been proposed as an autocrine growth factor in this disease [4]. In addition to inhibiting the action of GRP directly, a GRP receptor antagonist could interfere with the action of G-17 by inhibiting its release. G-17 is well established as a growth factor for gastric fundic mucosa [5]. In the rat, prolonged elevation in plasma levels associated with continuous acid suppression causes carcinoid tumors in the stomach [6].

EPIDERMAL GROWTH FACTOR

EGF (human homologue urogastrone) is a 53 amino acid peptide containing three intramolecular disulphide bonds (Fig. 1). The major sites of production are platelets,
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FIG. 2. EGF was injected (iv) at the arrow into anesthetized rats at 5.0 μg/kg (left-hand panel) or Heidenhain pouch dogs at 0.1 μg/kg (right-hand panel); controls received saline. The percentage of inhibition of histamine-stimulated secretion was computed from acid output, measured as the product of volume and H⁺ concentration. Values are mean (SEM) of four to six animals per group [unpublished data].

Together with salivary and Brunner's glands from where it is secreted into the gut [7], EGF interacts with the same receptor and activates the same intracellular signaling pathway as TGF alpha, with which it shares about 40 percent sequence homology [8]. In addition to cells of epithelial origin, EGF is a potent mitogen for a large number of other cell types, including fibroblasts and endothelial cells. As a result, EGF enhances angiogenesis and wound healing as well as stimulating proliferation throughout the gastrointestinal tract [3,7,9]. Stimulation of vascular development can be readily demonstrated by application of EGF to the surface of the chick yolk sac, a preparation used widely to assay angiogenic factors [10]. EGF is also a potent inhibitor of gastric acid secretion in humans, laboratory animals, and in vitro [11,12,13]. The anti-secretory profiles following intravenous (iv) bolus injection of EGF in the rat and dog are shown in Fig. 2.

EGF stimulates mitogenesis via interaction with a 170 kDa transmembrane receptor which displays intrinsic tyrosine kinase activity [14]. The N-terminal, 621 amino acid extracellular domain contains cysteine-rich regions, which presumably equate with the ligand binding site. There is a short membrane spanning region and a cytoplasmic domain which is autophosphorylated in response to EGF binding and receptor dimerization [15]. Clustering of receptors, internalization, and their subsequent recycling to the cytoplasmic membrane provide a mechanism for regulating sensitivity of the cells to exogenous EGF. Less is known about the signal transduction mechanism leading to inhibition of gastric acid secretion. In isolated parietal cells, EGF has been shown to inhibit histamine-stimulated cAMP accumulation and decrease phosphorylation of the substrates for cAMP-dependent protein kinase [16,17]. The fact that anti-secretory responses are blocked by pertussis toxin implies that EGF exerts an anti-secretory effect via the inhibitory (G_i) GTP-binding protein.

Structure-activity studies were undertaken to determine whether existence of receptor subtypes could account for the diverse (mitogenic and anti-secretory)
biological actions of EGF. Progressive enzymic degradation of the C terminus was used to produce a series of peptides of between 53 and 42 amino acids [18]. Truncation of EGF was accompanied by a parallel reduction in its potency as a stimulant of mitogenesis and an inhibitor of acid secretion. All the peptides behaved as full agonists or antagonists. Disruption of tertiary structure by treatment with mercaptoethanol followed by cysteine carboxyamidomethylation to prevent reformation of disulphide bonds abolished the biological activity of EGF.

The above findings are consistent with a single class of receptors for EGF. The data would also indicate that the C-terminal undecapeptide determines affinity of the structurally constrained, 42 amino acid N-terminal domain. Marked differences were, however, found with respect to the duration of EGF exposure required to evoke the two biological responses. Thus sequestration of EGF with an antibody up to ten hours after addition of the ligand inhibited mitogenesis, whereas anti-secretory activity could only be attenuated if the neutralizing antibody was administered prior to EGF [19]. This latter finding demonstrates very rapid transduction of the anti-secretory signal. It is even conceivable that a monomeric ligand-receptor complex can inhibit secretion by the parietal cell, as opposed to the receptor dimerization thought necessary to elicit a mitogenic response.

EGF has the ideal spectrum of biological activity to act as a mediator of mucosal protection in the upper gastrointestinal tract. Thus rapid inhibition of acid secretion would serve to limit damage caused by H+ back-diffusion immediately following injury, while stimulation of proliferation and wound healing would repair damage and restore mucosal cell mass over the longer term. A proposed protective role for endogenous EGF is not inconsistent with the lack of biological activity of luminal EGF, since “absorption” can be induced by superficial damage. For example, EGF inhibited gastric acid secretion in the rat when instilled into segments of small intestine in the presence of molar NaCl but not when the peptide was administered in 0.15 M NaCl [20]. This profile of activity has led us to consider that luminal EGF may serve a “housekeeper” function with respect to gastrointestinal mucosal integrity.

Appearance of EGF-secreting cell lineages in both the gastric and intestinal mucosa of man has recently been described in association with the presence of ulceration [21]. While short-term induction of EGF would serve a reparative function, continuous over-expression of EGF-related growth factors could predispose to tumor formation. Increased production of both TGF alpha peptide and EGF receptor have been reported in various human tumors, including gastric cancer [22]. In order to define the role of TGF alpha in an experimental model of breast cancer, MCF7 tumors were grown in athymic mice and the animals treated with a TGF alpha antiserum prepared by a method similar to that employed to generate EGF antiserum [18]. Despite clear evidence for suppression of TGF alpha activity, tumor growth was not inhibited [Gregory H, Garner A: unpublished observations]. Because of the negative result obtained in this tumor growth study and in similar in vivo studies with GRP and gastrin-sensitive tumors described below, we have been extremely careful to validate each stage of the experiments. Thus the cell culture was shown to respond to TGF alpha in vitro and the antiserum shown to block this mitogenic effect. Furthermore, in vivo activity and duration of action of the antiserum was established in a separate series of experiments from a dosing regime which completely abolished the anti-secretory effect of TGF alpha in mice.
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Fig. 3. The GRP/bombesin receptor antagonist ICI 216,140 at 2 mg/kg subcutaneously inhibited bombesin-stimulated pancreatic secretion (left-hand panel) and caused a dose-related inhibition of bombesin-stimulated amylase output measured after three hours. Values are mean (SEM) of five animals per group.

GASTRIN-RELEASING PEPTIDE

GRP, first isolated from porcine intestine, shares a common C-terminal amino acid sequence with the frog skin peptide bombesin [23]. This heptapeptide region is responsible for agonist activity, including contraction of smooth muscle and stimulation of pancreatic secretion, gastrin release, and cellular proliferation. GRP has been proposed to act as an autocrine growth factor in human small-cell lung cancer (SCLC), based on the presence of GRP-like immunoreactivity in tumor biopsies, existence of GRP receptors on SCLC cells, the ability of GRP to stimulate growth of SCLC clones, and, seemingly most convincing, the ability of a GRP monoclonal antibody to inhibit growth of SCLC tumors in nude mice [24]. These findings led a number of pharmaceutical companies to search for GRP receptor antagonists as potential therapeutic agents for SCLC.

Assays for displacement of $^{125}\text{I}$-GRP binding and inhibition of GRP-mediated $^3\text{H}$-thymidine incorporation were developed in 3T3 fibroblasts and used to screen synthetic peptides [25]. Active compounds were evaluated in vivo for their ability to inhibit GRP-stimulated pancreatic secretion in rats in order to select potent, long-acting analogs for screening against human SCLC xenografts in nude mice. Systematic synthesis of truncated and side-chain deletion analogs of the GRP (18–27) sequence and screening of substance P antagonists led to identification of specific GRP antagonists with in vitro activity in the nM range. Further structural modifications led to the identification of analogs which inhibited GRP-stimulated amylase output and displayed a prolonged duration of action in vivo [26]. One such compound, ICI 216,140, with the structure \((\text{CH}_3)\text{CHCO-His-Trp-Ala-Val-D-Ala-His-Leu-NHCH}_3\), was studied in detail. This compound was a very effective antagonist of GRP in vivo (Fig. 3). It failed, however, to inhibit the growth of a range of SCLC tumors in animals.
While ICI 216,140 did not display activity against tumors in which GRP was allegedly an autocrine growth factor, it is proving to be a useful tool in terms of defining the biological actions of GRP [27,28]. For example, we have investigated gastro-pancreatic reflexes in the anesthetized turkey. In this model, distention of the proventriculus with a solution of peptone produces an increase in pancreatic electrolyte and protein secretion. This reflex response was inhibited by a GRP antagonist but not by antagonists of acetylcholine, CCK, or gastrin. Thus protein-rich solutions in the avian stomach appear to cause the release of GRP, which then acts directly on the pancreas to stimulate secretion. This finding may indicate a wider role for GRP in mediating the gastric phase of digestion.

GASTRIN

Gastrin (G-17) is well established as a stimulant of fundic mucosal growth [29]. Although exogenous G-17 is capable of stimulating proliferation of various intestinal cells in vitro, there is little evidence to suggest this hormone plays any significant role in regulating mucosal proliferation elsewhere in the gut. In the stomach, hypergastrinemia in response to G-17 or to hypochlorhydria induced by surgical or pharmacological means have each been reported to cause hyperplasia of antral G cells and fundic ECL cells [30,31]. The latter topic has been a focus of attention as a consequence of the finding that long-term administration of omeprazole and other potent inhibitors of acid secretion induces carcinoid tumors in rats [32]. Furthermore, procedures which elevate G-17 have been reported to stimulate the growth of gastrointestinal (GI) tumor cell lines and to promote formation of chemically induced tumors in animals [33,34]. There are also reports that postprandial levels of G-17 are elevated in patients with colon cancer [35]; however, any link between hypergastrinemia and GI cancer in man remains circumstantial.

More convincing evidence for a causal role of G-17 in tumorigenesis would be provided by a demonstration of tumor regression after blocking the action of endogenous G-17 with receptor antagonists or by neutralization with antibodies. Since these tools are now available, we have investigated their influence on growth of a range of GI tumors in vivo and in vitro. Four transformed GI cell lines were used; MKN45 (human gastric), AR42J (rat pancreatic), MC26 (mouse colonic), and C523 (human colonic). Presence of high-affinity G-17 binding sites on these cells and/or stimulation of proliferation by 0.1–1 nM G-17 were used to indicate the presence of functional receptors. For in vivo studies, the GI cell lines were grown as xenografts in athymic mice and the effects of gastrin antagonism determined. Three different strategies for inhibiting the action of gastrin were used: the synthetic receptor antagonist L365260 [36], a monoclonal CURE Gas-93 (kindly provided by Dr. John Walsh), and an antiserum raised in sheep against gastrin 2–17 linked to keyhole limpet hemocyanin [37]. Prior to investigating anti-tumor activity, all treatment regimes were shown to inhibit gastrin-stimulated acid secretion in mice. With the exception of a statistically significant inhibitory effect of the antiserum against growth of AR42J and C523, none of the treatments influenced tumor growth, as summarized in Table 2. Although these findings are largely negative, G-17 is but one of numerous circulating mitogens capable of stimulating growth of experimental tumors. As proved to be the case with TGF alpha and GRP, it seems equally unlikely that inhibiting G-17 will have a major effect in the therapy of cancer.
which seek gastric phase distention P.B. Particular EGFindization nists in largely roles and continue neutralizing therapy. Accelerated estimated similar therapeutic GRP/bombesin stimulate basolateral membrane ulcerative diseases. Of cellular activity rapid studies and active activity as (anti-secretory) proliferation, of EGF receptors. In the gastrointestinal tract, EGF receptors are restricted to the basolateral membrane of mucosal cells, which probably accounts for the lack of oral activity of EGF and TGF alpha. A parenteral slow-release formulation or an orally active small molecule mimetic could, however, have utility in treating various ulcerative diseases.

Antagonizing the action of EGF/TGF alpha has also been proposed to have therapeutic potential, in this case as a treatment for solid tumors. Thus both peptides stimulate proliferation of a wide range of cell lines and enhance tumor growth in vivo. Similar findings have been made with a number of other peptides, including GRP/bombesin and G-17. Growth of various transplanted tumors in nude mice was, however, unaffected by antagonism of these peptides, suggesting that strategies which seek to block the action of single mitogens will have little effect in cancer therapy.

The literature contains many accounts of studies in which tumor growth has been accelerated by administration of a particular mitogen and the response then inhibited by co-administration of the corresponding antagonist. Such experiments are largely self-fulfilling, and provide little to support the use of growth factor antagonists in anything other than “autocrine” disease. Nevertheless, development of neutralizing antibodies and receptor antagonists to these regulatory peptides will continue to enable important findings to be made concerning their physiological roles and possible therapeutic uses. Thus stimulation of gastric secretion by neutralization of circulating EGF suggests the parietal cell is subject to tonal inhibition by EGF in vivo. Similarly, inhibition of pancreatic secretion in response to gastric distention by a GRP antagonist suggests that GRP may have a role in mediating the gastric phase of digestion.

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TABLE 2
Effect of Gastrin Antagonism on Tumor Growth in Nude Mice

| Treatment      | MKN45 | AR42J | C523 | MC26 |
|----------------|-------|-------|------|------|
| L365 260       | Inactive | Inactive | Inactive | Inactive |
| G(2–17)antisera| Inactive | Active | Active | Inactive |
| CURE Gas-93    | Inactive | Inactive | Inactive | Inactive |

Growth of four GI tumor lines in groups of seven to ten nude mice was assessed after 18–36 days of treatment with a gastrin receptor antagonist (L365260) or two different immunoneutralization strategies [37].

SUMMARY AND CONCLUSIONS

EGF interacts with the same receptor and shares the same profile of biological activity as TGF alpha, including inhibition of gastric acid secretion, stimulation of cellular proliferation, and enhancement of wound repair. Evidence from binding studies and biological activity of a series of truncated EGF peptides suggests both the rapid (anti-secretory) and prolonged (trophic) actions are mediated via a single class of receptors. In the gastrointestinal tract, EGF receptors are restricted to the basolateral membrane of mucosal cells, which probably accounts for the lack of oral activity of EGF and TGF alpha. A parenteral slow-release formulation or an orally active small molecule mimetic could, however, have utility in treating various ulcerative diseases.

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