Anti-Cancer Drugs 1994, **5**, pp. 115–130

Review paper

Hypothalamic hormones: from neuroendocrinology to cancer therapy

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The discovery of hypothalamic hormones, especially LH-RH and somatostatin has led to practical clinical use of their analogs in the field of cancer treatment. Bombesin/GRP antagonists can be also considered for the development of new methods for treatment of various tumors. The understanding of functions of these peptide hormones and the availability of their synthetic analogs should permit the clinicians to treat a variety of cancers more successfully than in the past.

Key words: Antitumor activity, bombesin/gastrin releasing peptide, corticotropin-releasing factor, cytotoxic peptide analogs, luteinizing hormone-releasing hormone, somatostatin.

Introduction

The discovery of hypothalamic hormones achieved during the last few decades[1] and the ready availability of their synthetic analogs permits clinicians to diagnose and treat a variety of endocrine and reproductive diseases and conditions much better than in the past. No less important is the development of new hormonal methods for the therapy of various cancers based on analogs of various hypothalamic hormones, which has been proceeding for the past 15 years.[2,3]

I have been asked to give my account of the events dealing with some of my contributions to these fields. It is my hope that the material covered will be a source of learning, and a stimulus for future experimental and clinical research.

Delivered in Montreal, Quebec, Canada, on 5 November 1993, as part of the Foundations of Medical Science lecture series and the Heinz Lehmann lecture series, celebrating the 50th anniversary of the Department of Psychiatry of McGill University.

Corticotropin-releasing factor (CRF)

I was attracted into the hypothalamic endocrine field in the mid 1950s while still an undergraduate student at McGill University in Montreal. I was given the opportunity to work with Murray Saffran in the laboratory of experimental therapeutics of the Allan Memorial Institute of Psychiatry headed by Dr RA Cleghorn. The work at this laboratory was devoted to ACTH and adrenal cortical steroids.[4] That period marked the beginning of my interest in the relationship between brain function and endocrine activity, and it was there that my involvement in the hypothalamic field began.

A decisive stimulus was provided by the brilliant hypothesis of Harris and others[5,6] concerning the hypothalamic control of secretion of the anterior pituitary gland. Harris postulated that neurohumoral substances might originate in the median eminence of the tuber cinereum, penetrate to the anterior lobe by way of the hypophysial portal blood supply and thus regulate pituitary secretion.[5,6] There were other theories too at that time, but we reached the conclusion that the hypothalamic theory explained most of the experimental facts. However, it was obvious that despite a strong circumstantial case favoring this hypothesis, it would remain speculative until direct evidence for the existence of hypothalamic chemotransmitters controlling release of pituitary hormones could be demonstrated.

In the beginning it was not possible to isolate these substances because of lack of specific methods for the detection of their activity. We sought to provide evidence for this theory by demonstrating an unequivocal direct effect of hypothalamic substances on the pituitary. We were encouraged by the success of the *in vitro* assay system for ACTH based on incubation of rat adrenal glands.[4] Working with

the isolated adrenals of the rat, we have developed a method for the assay of ACTH based on the stimulation of the formation of corticoids by the addition of ACTH to the tissue. It is possible to divide the adrenals into quarters and then assign two flasks to each dose in an eight-flask assay. The adrenal corticoids released during the incubation were extracted with methylene chloride and measured in a spectrophotometer. This test enables us to measure ACTH activity quickly and easily.[4]

Our success with the isolated adrenal glands encouraged us to attempt the utilization of the isolated rat anterior pituitary gland in a test for the putative CRF in the hypothalamus (Figure 1).[7] The pituitary of a rat was cut in two halves. One half of the anterior lobe of the pituitary served as the control tissue to indicate the level of secretion of ACTH by the isolated tissue. The other half was exposed to the materials under test. Then, the response of the adrenal was used to measure the ACTH liberated by each half of the pituitary. We found that hypothalamic or posterior pituitary tissue added along with either adrenaline or noradrenaline significantly increased the rate of liberation of ACTH.[7] We chose to explore then the known hormones of the posterior pituitary for activity in this system as well as posterior pituitary and hypothalamic extracts. We found that hypothalamic or neurohypophysial extracts added to anterior pituitary tissue caused a clear increase in ACTH release.[8,9] We 'knew' then that the existence of a substance which stimulated the release of ACTH had been demonstrated experimentally for the first time. We named it CRF.[8,9] Vasopressin had some activity, but when it was further purified, it lost its CRF activity.[8,9] The activity in the original vasopressin was found in one of the impurities separated from the vasopressin spot by paper chromatography.[8,9] Using protopituitrin, a posterior lobe preparation as starting material, we separated a small amount of CRF by consecutive chromatography in four different solvent systems.[9] The purified CRF was active in our test system in low doses and did not require noradrenaline for activity.[9] We obtained evidence that CRF was a polypeptide,[9] but despite 7 years of effort, 2 years with M Saffran in Montreal and 5 years with R Guillemin in Houston, we were unable to isolate enough material for the determination of its structure.

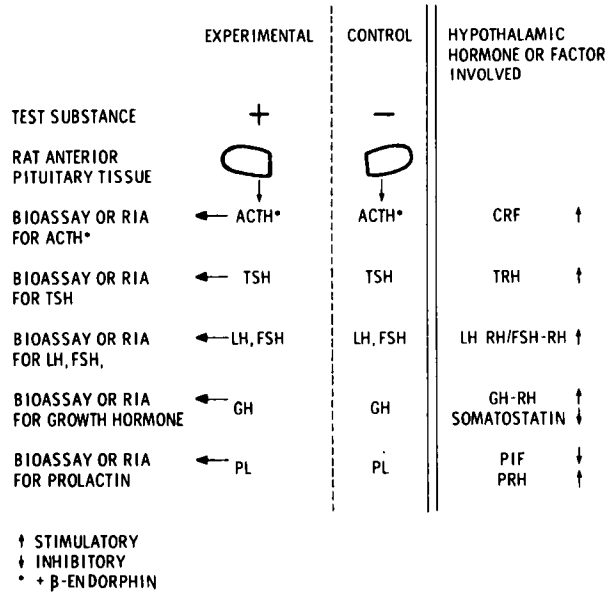

Figure 1. A diagrammatic representation of the *in vitro* test system for the detection of hypothalamic hormones and factors controlling the release of anterior pituitary hormones. (From © Les Prix Nobel en 1977, p. 202, with permission of the Nobel Foundation.)

In that period, techniques such as high performance liquid chromatography (HPLC) and microdetermination of the structure of peptides on a microscale were simply not available and the structure of CRF was not elucidated until some 25 years later, when Vale and collaborators[10] reported the isolation, characterization and synthesis of ovine CRF. At that time, we were already deeply involved in our work on cancer, but we isolated and determined the amino acid sequence of porcine CRF.[11] Since the term 'factor' was meant to apply to substances whose activity cannot be ascribed to a specific chemical structure, the name corticotropin releasing hormone (CRH) should now be used. The nomenclature followed by most neuroendocrinologists and clinicians employs the name releasing hormone (RH) for those hypothalamic substances which have had their structures determined and which have been shown to be likely physiological regulators of secretion of respective anterior pituitary hormones.[1,12]

During the intervening time, I started work on other hypothalamic hormones.[12] The *in vitro* pituitary incubation system, developed for CRF proved to be useful for demonstrating the existence of hypothalamic hormones regulating the secretion of TSH, LH, FSH and GH (Figure 1).[1,12]

In 1962, after I moved to VA Hospital and Tulane University, New Orleans, I made arrangements for procurement of the hundreds of thousands of hypothalami of pigs, necessary for purification of useful quantities of hypothalamic hormones and I was able to obtain about a million pig hypothalami. This enabled us to undertake a large-scale effort aimed at the purification of amounts of material adequate for

chemical characterization. We systematically investigated the purified fractions for the presence of materials, which would control the release of TSH, LH, FSH and GH, since the discovery of CRF opened the way to their demonstration.[12]

Thyrotropin-releasing hormone (TRH)

Having demonstrated the presence of TRH in pig, beef and human hypothalami,[1,12,13] we undertook its purification with the help of CY Bowers and TW Redding.[14] In 1966, we isolated TRH from 100 000 pig hypothalami and showed that TRH had three amino acids, i.e. glutamic acid, histidine and proline, which established for the first time that it was a peptide.[14] In 1969, we solved the structure of porcine TRH and synthesized it.[15] The structural work of Burgus, Guillemin and collaborators on ovine TRH paralleled that of our group and they elucidated the structure of ovine TRH a few weeks after us.[16]

We carried out the clinical studies using TRH and showed that it stimulated TSH release in humans.[17] After synthetic TRH became available, it was established that TRH is very useful diagnostically for the evaluation of the pituitary–thyroid function.[1,18]

Luteinizing hormone-releasing hormone (LH-RH)

After identification of TRH, we increased our efforts on LH and FSH releasing hormone. My interest became intensified further by the idea that the discovery of LH-RH might open up new possibilities for the inhibition and stimulation of fertility at the level of the brain. Evidence for the existence of LH-RH and FSH-RH in hypothalamic extracts was provided in the early 1960s by SM (Don) McCann,[19] my group[12] and others.[20] This started a race for the isolation and structure of LH-RH between several groups.

It was initially thought that LH-RH and FSH-RH activities were due to two separate substances.[12] We purified some LH-RH from porcine hypothalami and in view of its relative purity and absence of toxicity, it was decided to test it in humans. These studies carried out in 1968 and 1969 in Mexico unequivocally established that highly purified LH-RH released LH and FSH in men and women under a variety of conditions.[21,22] Realizing that LH-RH might be useful clinically, we intensified our efforts to establish its structure. The efforts of my group resulted in the first isolation of LH-RH from pig hypothalami, elucidation of its amino acid se-

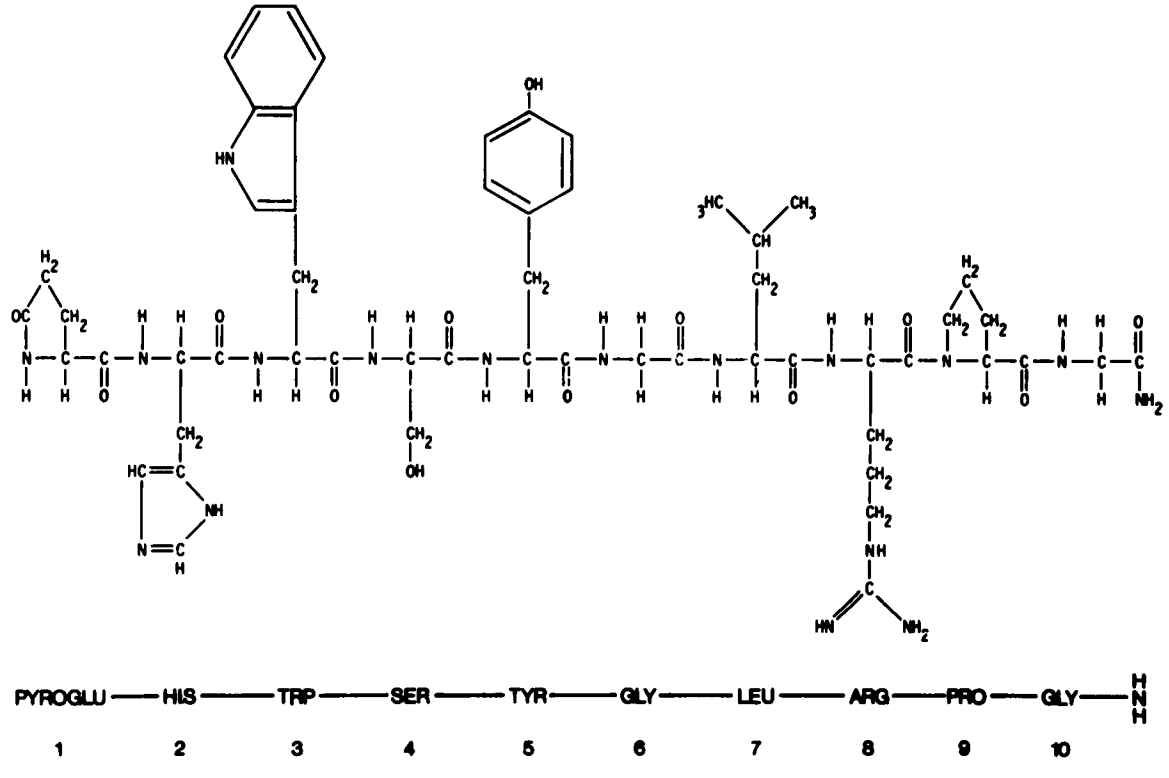

Figure 2. Molecular structure of LH-RH/FSH-RH.

quence and its synthesis.[23–29] The structure of LH-RH/FSH-RH, also called gonadotropin releasing hormone (Gn-RH), is shown in Figure 2. Synthetic LH-RH stimulated the release of LH and FSH *in vivo* in rats and *in vitro*.[28,29] Clinical studies demonstrated that in human beings, synthetic LH-RH also raised plasma LH and FSH levels.[29,30]

Because both natural and synthetic LH-RH released FSH as well as LH, we proposed that one hypothalamic hormone could be responsible for this dual effect.[28,29] This concept is now supported by much evidence. Intense activity in the synthesis of LH-RH all over the world followed our announcement of its structure. The interest in possible medical applications of LH-RH stimulated various groups including ours to synthesize some 3000 LH-RH analogs.[1–3,31–33] The aims were to develop analogs with prolonged biological activity, so that they would be more useful therapeutically than LH-RH itself, and to obtain antagonistic analogs which could form the basis of new birth control methods.[31]

However, at that time we could not imagine the impact and the variety of applications, including major uses in oncology, that LH-RH analogs would eventually have.[1–3]

Synthetic LH-RH is used as a diagnostic agent for evaluating hypothalamic–pituitary gonadotrophin function.[1,18,34] LH-RH permits the assessment of the disorders of the hypothalamic–pituitary axis both in men and women.[1,18,31] Synthetic LH-RH itself is also used for induction of ovulation. During the past few years, several investigators have used automatic portable pumps designed to deliver pulses of synthetic LH-RH, and have demonstrated the effectiveness and safety of this mode of ovulation induction in patients with hypothalamic amenorrhea.[34]

Several LH-RH analogs substituted in positions 6, 10 or both are much more active than LH-RH and also possess prolonged activity.[2,3,32–38] Of these, the most important are [D-Trp^6]LH-RH (Decapeptyl, Triptorelin),[1–3,31,34] [D-Leu^6, Pro^9 NHEt]LH-RH (Leuprolide, Lupron),[35] [D-Ser(Bu^t)^6, Pro^9-NHEt]LH-RH (Buserelin),[36] [D-Ser(Bu^t)^6, Aza-Gly^{10}]LH-RH (Zoladex, Goserelin),[37] and [D-Nal(2)^6]LH-RH (Nafarelin),[38] which are 50–100 times more potent than LH-RH. Various investigators have determined that large doses of superactive agonistic analogs of LH-RH cause paradoxical antifertility effects in animals and human beings.[39–41] The phenomena of down-regulation of pituitary receptors for LH-RH, desensitization of pituitary gonadotropes and inhibition of sex steroid levels by LH-RH agonists are being used for treatment of precocious puberty, endometriosis, leiomyomas, benign prostate hyperplasia and other conditions.[1–3,33,34] Chronic administration of LH-RH agonists is being utilized to induce the regression of hormone-dependent malignant neoplasms, especially prostate and breast cancer, ovarian cancer, and, more recently, endometrial carcinoma.[1–3,34,42] LH-RH agonists are also being used in *in vitro* fertilization and embryo transfer (IVF-ET), gamete intra-fallopian transfer (GIFT) and polycystic ovarian disease.[34,43,44]

Potent inhibitory analogs of LH-RH, which block ovulation in laboratory animals, have been synthesized.[45–47] Several antagonists of LH-RH, such as SB-75 (Cetrorelix), have been tested in men and women, and shown to be active enough for practical use.[48–50] It is probable that the LH-RH antagonists will be useful for treatment of precocious puberty and endometriosis, and for management of breast, ovarian and prostate carcinoma.[2,3,42,51] The approach based on analogs of LH-RH has been proved feasible for the development of new methods of birth control, but the exact clinical regimens are still lacking.[34] Thus, the discovery of LH-RH has led to many practical clinical uses and analogs of LH-RH have various important applications such as the treatment of hormone sensitive tumors. Oncological uses of the agonistic and antagonistic analogs of LH-RH, in specific malignancies are reviewed below.

Somatostatin

The presence of somatostatin in hypothalamic extracts was first established by Krulich and McCann.[52] In 1973, Brazeau *et al.* isolated from sheep hypothalami a tetradecapeptide, which inhibited the release of growth hormone (GH) *in vitro* and *in vivo*, and which they named somatostatin.[53] They also established its structure.[53] Subsequently, we isolated and determined the structure of porcine somatostatin, the structure of which was identical to ovine.[54] We also isolated a larger form of somatostatin from pig hypothalami with amino-terminal extension, that is somatostatin 28.[55] Somatostatin 14 and 28 also suppress the secretion of glucagon and insulin and decrease the release and action of gastrin and other GI hormones.[3,18,54–58] Somatostatin is present in discrete cells of the pancreas, gastric mucosa, duodenum and other tissues, and may play an important role in the regulation not only of the pituitary, but also of the endocrine pancreas and gastrointestinal tract.[3,18,56,57] Soma-

tostatin appears to be an endogenous growth inhibitor.[57,59] Somatostatin was synthesized by several groups, including ours.[53–55]

Somatostatin itself is of little therapeutic value because it has multiple actions and a short biological half-life. However, our group and others produced superactive analogs of somatostatin with prolonged and more selective activity.[2,3,57,58,60–62]

Epilogue and reflections on the work on neuroendocrinology

Following the isolation, structural elucidation and synthesis of TRH, LH-RH and somatostatin, the hypothalamus suddenly rocketed to the top of the endocrine popularity list. The domination of various US and foreign endocrine meetings by papers on TRH, LH-RH and somatostatin had been remarkable. I have had the satisfaction that my work in the hypothalamus was honored by top US and Canadian awards, and a share of the Nobel Prize in Physiology and Medicine for 1977.

The Nobel Prize made it possible for me to meet with top medical leaders in various countries and to become acquainted with international health problems, among them cancer. In one of my lectures in Manilla, the Philippines, I presented our work on the production of antisera to LH-RH, which were made for a variety of immunological and immunocytochemical studies.[63] Male rabbits that were actively immunized with LH-RH developed a marked testicular atrophy associated with aspermatogenesis.[63] Since breast cancer as well as prostate cancer are well known to be sex-steroid dependent, I was encouraged to produce liters of LH-RH antisera in horses for treatment of women with breast cancer in the Philippines.

However, our approach to treating cancer was based on synthetic peptide chemistry, the making of synthetic super analogs of LH-RH and somatostatin, inasmuch as in the preceding years, we became profoundly influenced by the enormous activity of these analogs.[1–3,31,45,57,58,61] Since previously I also collaborated in clinical trials on various endocrine tumors,[3,18,57,64] I thought that it would be scientifically foolish not to take advantage of the high activity of both agonistic and antagonistic analogs of hypothalamic hormones, and after 1978 I became more and more interested in their therapeutic applications in the cancer field.

Oncological applications of analogs of hypothalamic hormones

I will review now some of our experimental and clinical studies on endocrine-dependent or hormone-sensitive cancers. Originally only breast cancer and prostate cancer were thought to be hormonally dependent.[3] Now the list of hormone-sensitive malignant neoplasms includes exocrine ductal pancreatic cancer, epithelial ovarian cancer, endometrial carcinoma, gastric cancer, colon cancer, tumors of the bone and cartilage such as osteo and chondro-sarcomas, small cell lung-carcinoma (SCLC), which represents about 25% of all lung cancers, and other tumors.[3] Even brain tumors may be hormone sensitive. For those cancers which are endocrine-dependent, various exogenous hormones can be used to nullify the effects of endogenous hormones or growth factors which are responsible for the growth of these malignancies.[2,3,57]

Hormonal therapy, in contrast to radiation and chemotherapy, is relatively free of side effects.[3] Moreover, the therapy proposed by us is based on the peptide analogs of hypothalamic and other hormones, and peptides as a class of compounds, have fewer side effects than steroids, synthetic estrogens like DES, antiestrogens or antiandrogens.[3]

Prostate cancer

Introduction. Carcinoma of the prostate is the most common malignancy in the American male and the second leading cause of death from cancer among adult men.[65] About 70% of human prostate cancers are testosterone-dependent. The treatment of advanced (stage C or D) prostate cancer is usually based upon androgen dependence of the tumor.[2,3,66,67] Endocrine therapy for carcinoma of the prostate includes orchiectomy and the administration of estrogen or antiandrogens.[2,3,67] However, surgical castration is associated with a psychological impact while estrogens have cardiovascular, hepatic and mammotropic side effects. A new, radically different endocrine therapy without apparent toxicity is based on the use of agonistic analogs of LH-RH.[2,3,66] The first clinical study with LH-RH agonists in men with prostate cancer was carried out by Tolis *et al.*[68] in 1980–81 and was based on experimental studies performed in my laboratory.[69] This and other clinical trials documented a fall in testosterone levels, and marked subjective and objective improvement in patients with stage C or D

prostate carcinoma after treatment with agonistic analogs, [D-Trp-6]LH-RH (Decapeptyl), Buserelin, Leuprolide and Zoladex.[70–75]

Development of long-acting delivery systems. Initially, superagonists of LH-RH were given daily by the subcutaneous or intranasal route.[68,70–75] Subsequently, we developed a long-acting delivery system for [D-Trp6]LH-RH in microcapsules of a biocompatible, biodegradable polymer, poly (DL-lactide-co-glycolide), designed to release a controlled dose of the peptide over a 30-day period.[43,45,76,77]

Normally the microcapsules contain 2–6% of analog and 94–98% polymer, but now up to 20% of peptide analog can be incorporated into microcapsules or microgranules.[43] Microcapsules of peptide analogs that can be injected once a month make the treatment of patients not only with malignancies, but also other conditions, more convenient.[2,3] The first trial with microcapsules of [D-Trp6]LH-RH in men with prostate cancer carried out in England showed 87% objective response in the microcapsule treated group versus 81% for total orchidectomy.[78] Other agonists of LH-RH are also used now in the form of depot preparations.

Present status of therapy with LH-RH agonists. Clinical results accumulated so far indicate that long-term therapy with agonists of LH-RH is the preferred alternative to surgical castration or therapy with estrogens in men with advanced prostate cancer.[68,70–80] In a recent survey, LH-RH analogs were chosen by more than 70% of patients as primary treatment.[66] However, LH-RH agonists alone[2,3] or in combination with antiandrogen[81,82] will not prevent an eventual relapse.[2,3] Continued investigation of combinations of LH-RH agonists with somatostatin analogs, bombesin antagonists or the use of LH-RH antagonists and LH-RH analogs carrying cytotoxic radicals may lead to an improvement in treatment of prostate cancer and an increase in the survival rate.[66]

Growth factors and prostate cancer. Various growth factors may be responsible for the relapse. IGF polypeptides (somatomedins) and various growth factors including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF-α) and others appear to be involved in the proliferation of both normal and neoplastic cells or phenotypic transformation of cells.[83–87] Human prostate cancers have receptors for EGF and IGF.[88,89] Somatostatin and its analogs reduce the levels of growth hormone and IGF-I.[57] Analogs of somatostatin such as RC-160 induce dephosphorylation of EGF receptors[90] and antagonists of bombesin also reduce the levels of EGF receptors on tumors.[66] Thus, somatostatin analogs and bombesin antagonists might inhibit the growth of prostate cancers by reducing the secretion of endogenous growth factors or levels of their receptors.[66] Experimental results and early clinical findings support this view.

LH-RH antagonists. LH-RH antagonists represent another class of compounds that may be useful for treatment of sex-hormone-dependent cancers. While repeated chronic administration of LH-RH agonists is required to induce an inhibition of LH and FSH release and reduction in the levels of sex steroids, similar effects can be obtained after the first administration of LH-RH antagonists.[2,3,34,45] Antagonistic analogs of LH-RH were originally developed for contraception. Modern antagonists possess modifications in positions 1, 2, 3, 6, 10 and others.[45] These antagonists act on the same receptor sites as LH-RH and cause an immediate inhibition of the release of gonadotropins and sex steroids. Early antagonists had side effects.[2,3]

In order to eliminate the undesirable edematogenic effect of the LH-RH antagonists containing basic D-amino acids at position 6, new analogs with D-ureidoalkyl amino acids such as D-Cit and D-Hci at position 6 were synthesized in our laboratory, and evaluated *in vitro* and *in vivo* in several systems in rats,[2,3,45] subhuman primates and, subsequently, clinically.[2,3,51]

The advantage of the antagonists is based on the fact that they inhibit LH and FSH and thus sex steroids from the start of administration and thus reduce the time of the onset of therapeutic effect. The use of antagonists would also prevent the temporary clinical 'flare-up' of the disease which can occur with the agonists.[2,3,66]

When we treated nude mice bearing xenografts of human prostate adenocarcinoma PC-82 with the agonist [D-Trp6]LH-RH or the antagonist SB-75 we were able to demonstrate that in mice which received microgranules of antagonist SB-75 there was a greater decrease in tumor weight and volume than that produced by the agonist.[91] Serum levels of testosterone were decreased by 90% in mice given the LH-RH agonist and by 94% in response to the antagonist SB-75.[91] Serum levels of prostate-specific antigen (PSA) were significantly lower in mice treated with LH-RH analogs, the antagonist SB-75 causing a greater reduction.[91]

Clinical studies with antagonist SB-75 in patients with prostate cancer and BPH demonstrated a marked clinical improvement parallel to lowering of serum testosterone levels to castration values.[51] LH-RH antagonists are already being used for the treatment of patients with advanced prostate cancer and benign prostate hyperplasia (BPH).[51]

Bombesin/gastrin releasing peptide (GRP) antagonists. Another class of antitumor compounds could consist of antagonists of bombesin/GRP. Bombesin contains 14 amino acids and was first found in the skin of the frog *Bombina bombina*[92] and in the stomach and brain.[93] Gastrin releasing peptide, which has 27 amino acids, is the mammalian equivalent of bombesin,[94] and is found in the stomach and gut.[93] Both bombesin and GRP are also found in the hypothalamus.[93,95,96] Since bombesin and GRP are produced by various cancers, such as small cell lung carcinoma[97,98] and breast[99] and pancreatic cancer,[100] and could act as autocrine growth factors, the development of hormone therapy based on bombesin antagonists should be considered.

We have synthesized more than 200 bombesin/GRP receptor antagonists with different modifications at positions 6, 7, 13 and 14, and a pseudopeptide bond at positions 13–14.[101] These antagonists inhibit the binding of labeled GRP(14–27) and Tyr^4-bombesin to the receptors, and are also active *in vivo*.[101,102]

In nude mice with transplanted hormone-dependent human prostate cancer PC-82, bombesin antagonist RC-3095 and the combination of [D-Trp^6]LH-RH and RC-160 caused a greater inhibition of tumor growth than [D-Trp^6]LH-RH or RC-160 alone.[103] Similarly, in nude mice bearing xenografts of the androgen-independent human prostate cancer cell lines PC-3 or DU-145, tumor volumes and weights were significantly reduced by somatostatin analog RC-160 and bombesin RC-3095.[104,105] In all three human prostate cancer models, administration of RC-160 or RC-3095 produced a significant down-regulation of EGF receptors.[103–105] Our results suggest that somatostatin analog RC-160 and bombesin/GRP antagonist RC-3095 can inhibit the growth of androgen-independent prostate cancer when the therapy is started at an early stage of tumor development.[66,103–105]

LH-RH analogs carrying cytotoxic radicals. Additional new classes of antitumor drugs are being developed by us based on LH-RH analogs containing various cytotoxic radicals such as melphalan and platinum complexes related to cisplatin.[2,3,106–108] LH-RH agonists and antagonists carrying various cytotoxic radicals were designed as targeted chemotherapeutic agents intended for treatment of cancers that contain receptors for LH-RH, such as prostate cancer, breast cancer, and ovarian and endometrial cancer.[2,3,106–108]

Such analogs could exert the effect of agonists or antagonists and, at the same time, act as chemotherapeutic agents targeted to the tumor cells by their peptide portion for which binding sites are present on the cell membrane. Because the antitumor action may be exerted to a greater degree locally or at least at more selective sites that have the cell membrane receptors, the peripheral toxicity would be reduced. The availability of cytotoxic compounds linked to hormonal peptides that can be targeted to certain cancers possessing receptors for these peptides, and therefore more selective for killing cancer cells, could be of significant practical therapeutic importance.[2,3]

In an attempt to produce better cytotoxic analogs, chemotherapeutic–antineoplastic radicals, including alkylating nitrogen mustard derivative of D-phenylanine (D-melphalan), reactive cyclopropane, anthraquinone derivatives (2-hydroxymethyl) anthraquinone and anticancer antibiotic adriamycin (doxorubicin) and an antimetabolite (methotrexate), were coupled to suitably modified agonists and antagonists of LH-RH which function as carriers.[108] The effects of hybrid cytotoxic LH-RH analogs produced by linking anthraquinone or methotrexate to carrier LH-RH agonist [D-Lys^6]LH-RH were evaluated in rats bearing Dunning R-3327H prostate adenocarcinoma.[109] The cytotoxic LH-RH analogs caused a somewhat greater tumor growth inhibition than the carrier peptide, while anthraquinone or methotrexate alone, administered in equimolar doses, were ineffective.[109]

Perspectives for improvement in the treatment of prostate cancer. Our experimental findings suggest that therapeutic response in men with prostate cancer could be further improved by: (i) combination of a once a month sustained delivery system of LH-RH agonists with somatostatin analogs; (ii) Modern LH-RH antagonists like SB-75, particularly in combination with somatostatin analogs; (iii) cytotoxic LH-RH analogs (LH-RH agonists or antagonists linked to cytotoxic radicals), and (iv) bombesin/GRP antagonists in combination with LH-RH analogs. Some of these approaches are being tried clinically in an attempt to delay or prevent the relapse.[66,110]

Breast cancer

Breast cancer is the most common malignancy among American women and is responsible for about 46 000 deaths annually.[65] About 30% of breast cancers in women are estrogen dependent.[2,42] Various experimental and clinical studies suggest that analogs of LH-RH are useful for treatment of estrogen-dependent breast cancer.[2,42,111–113] The mechanism of the main effect of LH-RH analogs is based on estrogen deprivation, but some direct antitumor effects of LH-RH analogs on mammary carcinomas are also possible,[2,42] since several groups, including ours, found LH-RH receptors in human breast cancers.[114–116] In 260 of 500 breast cancer samples (52%), two classes of [D-Trp^6]LH-RH membrane receptor sites were also detected, one class showing high affinity and low capacity, and the second class low affinity and high capacity.[116] SS-14 receptor was found in 36% of patients[116] and is linked with good prognosis.[117] In clinical trials in France, we have used sustained release formulations of [D-Trp^6]LH-RH for hormonal treatment of women with breast cancer.[2,34,42,118]

In one study, 23 patients with advanced breast carcinoma were treated with the microcapsules of [D-Trp^6]LH-RH.[118] Eight patients were pre-menopausal and 15 post-menopausal; five of eight pre-menopausal patients were estrogen receptor positive (ER$^+$) and three of them responded. Three of 15 post-menopausal patients also responded; two of them were ER$^+$. These results indicate that the treatment with [D- Trp^6]LH-RH is more efficacious in ER$^+$ patients, in accord with the results of other studies. The responses recorded in post-menopausal patients suggest that [D-Trp^6]LH-RH may have a direct antitumoral action.[2,118] Other studies, including a large trial by Kaufman *et al.*,[112] support the view that LH-RH agonists are efficacious for the treatment of pre-menopausal women with estrogen-dependent breast cancers.[112] The women with breast cancer who do not respond to [D-Trp^6]LH-RH therapy could be treated with somatostatin analogs such as RC-160.[2,57] Somatostatin analogs might inhibit breast cancers by reducing release of GH and interfering with the action, signal transmission or secretion of endogenous growth factors.[57] Our experimental results[99,119–121] suggest that the combination of LH-RH agonists or antagonists with somatostatin analogs or bombesin antagonists could result in an increase in the therapeutic response in patients with breast cancer.

In female BDF$_1$ mice inoculated with MXT estrogen-independent mouse mammary carcinoma, the combination of SB-75 or [D-Trp^6]LH-RH with somatostatin analog RC-160 caused a greater reduction of tumor volume or tumor weights than single analogs.[119] The finding that LH-RH agonists and antagonists and somatostatin analogs inhibit the growth of estrogen independent mammary tumors and that combinations are more effective than single analogs might be of practical importance in human breast cancer therapy.[119]

The growth of estrogen-independent MXT breast cancers in mice was also powerfully inhibited by bombesin/GRP antagonist RC-3095, in contrast to surgical ovariectomy, which had no effect.[120] The inhibitory effect of the bombesin antagonist on growth of MXT breast cancers appeared to be linked with a major decrease in EGF receptor levels in tumors.[120] Bombesin receptor antagonists like RC-3095 might be useful for therapy of ER$^-$ breast cancer, alone or in combination with other agents.

Cytotoxic LH-RH analogs were likewise tested in mice bearing MXT estrogen- independent mammary tumors.[122] Cytotoxic LH-RH analogs T-98 and T-121/B produced a significant inhibition of tumor growth. Tumor volumes and tumor weight were significantly reduced.[122] In nude mice bearing MCF-7 MIII human breast cancer, cytotoxic analog AJ-04 also inhibited tumor growth (Pinski, Yano and Schally, in preparation).

Endometrial carcinoma

Endometrial carcinoma is the fourth most common cancer in American women.[42] Surgery or radiotherapy is successful in 75% of patients, but new methods are needed for advanced or relapsed cases.[42] Specific LH-RH binding sites are present in samples of surgically removed endometrial cancers.[123] Emons *et al.*[124] evaluated whether these binding sites can mediate direct effects of LH-RH analogs on the proliferation of HEC-1A and Ishikawa human endometrial cancer cell lines. Effects on cell proliferation were studied using the LHRH agonist [D-Trp^6]LH-RH or the LH-RH antagonist SB-75 (Cetrorelix). In both cell lines two binding sites for [D-Trp^6]LH-RH, one with high affinity and low capacity, and the other with low affinity and high capacity cells were found.[124] [D-Trp^6]LH-RH and SB-75 significantly inhibited the proliferation of both cell lines.[124] These data indicated that both agonistic and antagonistic analogs of LH-RH can directly inhibit proliferation of human endometrial

carcinoma. Clinical trials with [D-Trp^6]LH-RH are in progress in women with endometrial carcinoma.

Epithelial ovarian cancer

Epithelial ovarian cancer is the fourth most frequent cause of cancer related deaths in women.[42,65] Surgery and chemotherapy are not very effective.[42] Ovarian cancer may be dependent on LH and FSH, and suppression of the secretion of gonadotropins produced by LH-RH analogs appears to inhibit the growth of ovarian tumors.[42] Specific binding sites for LH-RH and its analogs have been characterized in samples of surgically removed human ovarian carcinomas.[125] Emons *et al.*[126] evaluated whether these binding sites are also present in EFO-21, EFO-27, human ovarian cancer cell lines and if they mediated direct LH-RH-analog effects on their proliferation. Proliferation effects were ascertained by incubating the cells in the presence of the LH-RH agonist [D-Trp^6]LH-RH or the LH-RH-antagonist SB-75 (Cetrorelix).[126] In both cell lines a high affinity and low capacity binding site for [D-Trp^6]LH-RH, and a low affinity and high capacity binding site were found.[126] At 10^{-9} M concentrations the LH-RH agonist significantly reduced proliferation in both cell lines:[126] comparable inhibitory effects were found with the LH-RH antagonist SB-75 on the proliferation of EFO-21 cells. Thus, the LH-RH binding sites in these cancer cells might mediate direct antiproliferative effects of LH-RH-analogs.[126]

Response to [D-Trp^6]LH-RH (Decapeptyl) microcapsules in ovarian carcinoma. We treated 41 patients with advanced ovarian carcinoma (FIGO Stage III and IV), who had relapsed following conventional treatment, with long acting microcapsules preparation of [D-Trp^6]LH-RH once a month.[127,128] Five patients showed stabilization (SD) on [D-Trp^6]LH-RH where tumor size remained the same. Six patients had clinical or radiological evidence of partial remission where the tumor size decreased by more than 50%.[128] Thus, 11 patients showed remission or stabilization of the disease. Clinical benefit of therapy with microcapsules of [D-Trp^6]LH-RH in about 26% of patients is encouraging.[128] Treatment with [D-Trp^6]LH-RH offers an important non-toxic alternative in patients who do not tolerate chemotherapy or who have progressive disease following chemotherapy.[127,128]

Multi-center clinical trials with [D-Trp^6]LH-RH in women with ovarian cancer are in progress in West Germany, Scandinavia and Israel.[42] Preliminary experimental results suggest that LH-RH antagonist SB-75 inhibits growth of human epithelial ovarian cancers better than agonist [D-Trp^6]LH-RH and therefore may be more efficacious clinically (Yano, Pinski and Schally, in preparation).

Carcinoma of the exocrine pancreas

Ductal exocrine carcinoma of the pancreas has a poor prognosis.[2,34,57,129] Our experimental studies and those of others are consistent with the view that the exocrine pancreatic carcinomas are sensitive to GI hormones, sex steroids and growth factors.[2,34,57,129–131] Various experimental and clinical findings suggest that it might be possible to develop a new hormonal therapy for exocrine cancer of the pancreas based on somatostatin analogs or bombesin/GRP antagonists in combination with LH-RH analogs.[2,34,57,99,132–139] Somatostatin analogs suppress the secretion and/or action of gastrin, secretin and cholecystokinin (CCK), which produce hyperplasia and hypertrophy of the exocrine pancreas and probably influence the growth of the malignant cells of the pancreas.[57,130] Somatostatin analogs might also inhibit the growth of pancreatic cancer by suppressing the action or secretion of growth factors, which are thought to be involved in neoplastic processes.[57,90,138] Various observations suggest that bombesin acts as a growth factor and stimulates proliferation of pancreatic cancers through specific receptors for bombesin/GRP present on the tumors.[99,134,135] Bombesin/GRP antagonist RC-3095 appears to inhibit the growth of pancreatic cancers by blocking the interaction of bombesin with its receptors.[134,135] Sex steroids may also play a role in the growth of the cancerous pancreas[131] and their secretion could be inhibited by LH-RH analogs.[136,137]

Various experimental studies were carried out to explore this view in Syrian Golden hamsters with ductal pancreatic cancers induced with *N*-nitrosobis (2-oxopropyl)amine (BOP)[132–136] or in nude mice bearing xenografts of human pancreatic cancers.[100] In a recent study, ductal pancreatic cancers were induced with carcinogen BOP in golden hamsters.[133] The animals were then treated for two months with 5-fluorouracil (5-FU), agonist [D-Trp^6]LH-RH, antagonist SB-75 and somatostatin analog (RC-160), and with some combinations thereof. In the first experiment, the treatment with [D-Trp^6]LH-RH plus 5-FU resulted in 52% inhibition of tumors. In the second experiment, both LH-RH antagonist SB-75 and somatostatin analog RC-160 caused a significant

inhibition of tumors, and their combination had the strongest tumor inhibitory effect.[133]

Phase I trials with RC-160 were conducted by Dr Graeme Poston in England in patients with advanced pancreatic cancer.[138,139] RC-160 was tried clinically as a single drug in patients with inoperable pancreatic cancer. Preliminary results indicate clinical improvement and stabilization in some patients.[138,139] However, RC-160 alone even in large doses may not be sufficient to produce effective palliation in most patients and bombesin antagonists or various combinations of peptides should be tried.

In female Syrian golden hamsters with nitrosamine-induced pancreatic cancers, bombesin antagonist RC-3095 exerted a dose-dependent inhibitory effect on the growth of pancreatic cancers.[134,135] Chronic administration of RC-3095 also inhibited the growth of CFPAC-1 human pancreatic cancer in nude mice.[100] Bombesin antagonist RC-3095 could be considered for the development of new approaches for the treatment of human pancreatic cancers.

Colon cancer

Advanced disseminated colon cancer is difficult to treat.[2,34,57,140] Sex steroids, GI hormones and growth factors appear to be involved in the tumorigenesis of the colon.[2,34,57] An approach similar to that on pancreatic cancer and based on hormonal manipulations such as the combined use of analogs of somatostatin, antagonists of bombesin/GRP and LH-RH analogs could also be envisioned for colorectal cancer.[2,34,57]

In nude mice bearing xenografts of HT-29 human colon cancer, somatostatin analog RC-160 significantly reduced tumor growth.[141] Bombesin/GRP antagonists had the greatest and consistently significant inhibitory effect on tumor growth. Bombesin/GRP antagonists and somatostatin analogs could be considered for the development of new hormonal therapies for colon cancer.[141]

Gastric cancer

Patients with unresectable stomach cancer have a poor prognosis and new therapeutic approaches are needed.[142] In nude mice bearing xenografts of the gastrin-responsive human gastric adenocarcinoma MKN45 cell line, somatostatin analog RC-160 reduced tumor volumes and weights.[143] Bombesin/GRP antagonist RC-3095 had the greatest inhibitory effect on tumor growth. Receptors for EGF were down-regulated in MKN 45 tumor cells after treatment with RC-3095 or RC-160.[143] Bombesin antagonists also inhibit *in vitro* and *in vivo* growth of Hs746T human gastric cancer.[144] These data demonstrate that the growth of human gastric cancer in nude mice can be inhibited by somatostatin analogs and by bombesin antagonist, and these approaches could be tried in patients suffering from this malignancy.[143,144]

Lung cancers

Lung cancer is the leading cause of cancer related deaths.[65,145] SCLC accounts for 20–25% of all cases of lung cancer.[145] Recent evidence indicates that SCLC may be hormone dependent.[97,98] SCLC produces peptides such as bombesin or GRP which act as autocrine growth factors.[97,98] Consequently, the development of hormonal therapy based on bombesin antagonists should be considered.[2,57] Non-SCLC (which include squamous or epidermoid, adenocarcinoma and large cell carcinoma) are dependent of growth factors like EGF and IGF-I.[146,147] We have demonstrated an inhibition of growth of NCI H-69 SCLC cells implanted into nude mice by bombesin/GRP antagonists and somatostatin analog RC-160.[148] We have also investigated the effects of SB-75, RC-160 and bombesin antagonist RC-3095 on growth of NCI H-157 human non-SCLC cells implanted into nude mice.[148] SB-75, RC-3095 and castration had no effect, but RC-160 caused a major inhibition of proliferation of non-SCLC.[148] These results are in accord with those of other investigators.[149]

Brain tumors

There are more than 12 000 new cases of primary brain tumors every year and 10 000 deaths attributed to brain tumors in USA.[65,150] Benign brain tumors like meningiomas can be treated by surgery, but new therapeutic modalities are needed for malignant tumors. Brain tumors have receptors for EGF, IGF-I and somatostatin.[57,151] Recently, we have demonstrated inhibitory effect of somatostatin analog RC-160 and bombesin/GRP antagonist RC-3095 on proliferation of various human glioblastomas (astrocytomas grade 3, malignant) transplanted into nude mice. Bombesin/GRP antagonist RC-3095 and somatostatin analogs inhibited the growth of these

malignant gliomas (Pinski and Schally, in preparation). Somatostatin analogs penetrate the blood–brain barrier[152] and along with bombesin antagonists can be considered for the development of new methods for treatment of brain tumors.

Conclusions

Inhibition of the pituitary–gonadal axis forms the basis for oncological applications of LH-RH agonists and antagonists, but direct effects on tumors may also play a role. Various endocrine-dependent or hormone-sensitive tumors can be treated with LH-RH analogs and the use of sustained delivery systems based on microcapsules or microgranules makes the treatment more practical and efficacious. A successful utilization of various agonistic analogs of LH-RH for treatment of androgen-dependent prostate cancer has been documented in thousands of patients and of antagonist SB-75 in dozens of patients. Agonists and antagonists of LH-RH might be also beneficial for treatment of breast cancer in pre-menopausal and post-menopausal women. The development and clinical testing of new LH-RH antagonist SB-75 is continuing. The advantage of the LH-RH antagonists is based on the fact that they inhibit LH and FSH and thus sex steroids from the start of the administration. The use of antagonists would prevent the temporary clinical 'flare-up' of the disease which can occur with the agonists. Work is in progress on the application of LH-RH analogs for treatment of ovarian and endometrial cancer. Methods based on the analogs of LH-RH or somatostatin might supplement or in some cases replace conventional procedures for the treatment of hormone-sensitive cancers. New hormonal therapy for pancreatic cancer, colon cancer, gastric cancer, lung cancer (SCLC or non-SCLC), brain tumors, and chondrosarcomas and osteosarcomas could be based on somatostatin analogs and/or bombesin/GRP antagonists.

It is gratifying to see that the discovery of hypothalamic hormones, especially LH-RH and somatostatin, has led to practical clinical use of their analogs in the field of cancer treatment. During the past 15 years, it has been a challenge to work in the field of cancer exploring the use of analogs of LH-RH and somatostatin, and now bombesin/GRP. I hope that I can continue to contribute to this field.

It was my good fortune to have arrived on the scientific scene at such a crucial time and to have helped in the discoveries of hypothalamic hormones, and more recently in the applications of their analogs in the field of cancer. The understanding of functions of hypothalamic hormones and the availability of their synthetic analogs should permit clinicians to diagnose and treat a variety of diseases including cancer much more successfully than in the past.

Acknowledgments

Basic studies done by us since 1962 and quoted here have been supported by the Veterans Affairs Department and the National Institutes of Health. I wish to thank many collaborators, scientists and clinicians cited in the references for their valuable contributions. Modern research is based on team-work and without their help, few of the advances described herein would have been possible. A special mention is due to Dr Murray Saffran for joint entry into the CRF field some 30 years ago. The support of my former superiors, especially Dr RA Cleghorn during my years in Canada and Dr Joe Meyer and EH (Manny) Bresler (Veterans Affairs Department in the period 1961–1978) is also gratefully acknowledged.

References

1. Schally AV. Aspects of hypothalamic regulation of the pituitary gland. *Science* 1978; **202**: 18–28.
2. Schally AV, Comaru-Schally AM, Hollander V. Hypothalamic and other peptide hormones. In: Hollander JR, Frei E, Bast RC, *et al.* eds. *Cancer medicine*, 3rd edn. Philadelphia, PA: Lea & Febiger 1993: 827–40.
3. Schally AV, Radulovic S, Comaru-Schally AM. Experimental and clinical studies in hormone dependent cancers. In: Mazzaferri E, Samaan N, eds. *Endocrine tumors*. Boston: Blackwell 1993: 49–73.
4. Saffran M, Schally AV. *In vitro* bioassay of corticotropin: Modification and statistical treatment. *Endocrinology* 1955; **56**: 523–32.
5. Green JD, Harris GW. The neurovascular link between the neurohypophysis and adenohypophysis. *J Endocrinol* 1947; **5**: 136–46.
6. Harris GW. *Neural control of the pituitary gland*. London: E Arnold 1955.
7. Saffran M, Schally AV. The release of corticotrophin by anterior pituitary tissue *in vitro*. *Can J Biochem Physiol* 1955; **33**: 408–15.
8. Saffran M, Schally AV, Benfey BG. Stimulation of the release of corticotropin from adenohypophysis by a neurohypophysial factor. *Endocrinology* 1955; **57**: 439–44.
9. Schally AV, Saffran M, Zimmerman B. A corticotrophin releasing factor: partial purification and amino acid composition. *Biochem J* 1958; **70**: 97–103.

10. Vale V, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 1981; **213**: 1394–7.
11. Patthy M, Horvath J, Mason-Garcia M, *et al.* Isolation and amino acid sequence of corticotropin-releasing factor from pig hypothalami. *Proc Natl Acad Sci USA* 1985; **82**: 8762–6.
12. Schally AV, Arimura A, Bowers CY, *et al.* Hypothalamic neurohormones regulating anterior pituitary function. *Recent Prog Hormone Res* 1968; **24**: 497–590.
13. Bowers CY, Redding TW, Schally AV. Effect of thyrotropin releasing factor (TRF) of ovine, bovine, porcine and human origin on thyrotropin release *in vitro* and *in vivo*. *Endocrinology* 1965; **77**: 609–16.
14. Schally AV, Bowers CY, Redding TW, *et al.* Isolation of thyrotropin releasing factor (TRF) from porcine hypothalamus. *Biochem Biophys Res Commun* 1966; **25**: 165–9.
15. Boler J, Enzmann F, Folkers K, *et al.* The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidine-proline amide. *Biochem Biophys Res Commun* 1969; **37**: 705–10.
16. Burgus R, Dunn TF, Desiderio D, *et al.* Characterisation of ovine hypothalamic hypophysiotropic TSH-releasing factor. *Nature* 1970; **226**: 321–5.
17. Anderson MS, Bowers CY, Kastin A, *et al.* Synthetic thyrotropin releasing hormone (TRH): a potent stimulator of thyrotropin secretion in man. *New Engl J Med* 1971; **285**: 1279–83.
18. Hall R, Gomez-Pan A. The hypothalamic regulatory hormones and their clinical applications. *Adv Clin Chem* 1976; **18**: 173–212.
19. McCann SM, Taleisnik S, Friedman HM. LH-releasing activity in hypothalamic extracts. *Proc Soc Exp Biol Med* 1960; **104**: 432–34.
20. Mittler JC, Meites J. *In vitro* stimulation of pituitary FSH release by Hypothalamic extract. *Proc Soc Exp Biol Med* 1964; **117**: 309–13.
21. Kastin AJ, Schally AV, Gual C, *et al.* Stimulation of LH release in men and women by hypothalamic LH-releasing hormone purified from porcine hypothalami. *J Clin Endocrinol Metab* 1969; **29**: 1046–50.
22. Kastin AJ, Schally AV, Gual C, *et al.* Administration of LH-releasing hormone to selected human subjects. *Am J Obstet Gynecol* 1970; **108**: 177–82.
23. Schally AV, Arimura A, Baba Y, *et al.* Isolation and properties of the FSH and LH-releasing hormone. *Biochem Biophys Res Commun* 1971; **43**: 393–9.
24. Schally AV, Nair RMG, Redding TW, *et al.* Isolation of the LH and FSH-releasing hormone from porcine hypothalami. *J Biol Chem* 1971; **246**: 7230–6.
25. Matsuo H, Baba Y, Nair RMG, *et al.* Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. *Biochem Biophys Res Commun* 1971; **43**: 1334–9.
26. Baba Y, Matsuo H, Schally AV. Structure of porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analysis. *Biochem Biophys Res Commun* 1971; **44**: 459–63.
27. Matsuo H, Arimura A, Nair RMG, *et al.* Synthesis of the porcine LH- and FSH-releasing hormone by the solid-phase method. *Biochem Biophys Res Commun* 1971; **45**: 822–7.
28. Schally AV, Arimura A, Kastin AJ, *et al.* The gonadotropin-releasing hormone: one polypeptide regulates the secretion of luteinizing and follicle stimulating hormones. *Science* 1971; **173**: 1036–8.
29. Schally AV, Kastin AJ, Arimura A. Hypothalamic FSH and LH-regulating hormone: structure, physiology and clinical studies. *Fertil Steril* 1971; **22**: 703–21.
30. Kastin AJ, Schally AV, Gual C, *et al.* Release of LH and FSH after administration of synthetic LH-releasing hormone. *J Clin Endocrinol Metab* 1972; **34**: 753–6.
31. Schally AV, Kastin AJ, Coy DH. LH-Releasing hormone and its analogues: recent basic and clinical investigations. *Int J Fertil* 1976; **21**: 1–30.
32. Karten MJ, Rivier JE. Gonadotropin-releasing hormone analog design. Structure-function studies toward the development of agonists and antagonists: rationale and perspective. *Endocr Rev* 1986; **7**: 44–66.
33. Vickery BH. Comparison of the potential for therapeutic utilities with gonadotrophin-releasing hormone agonists and antagonists. *Endocrine Rev* 1986; **7**: 115–24.
34. Schally AV. LH-RH analogues in medicine. Keynote address, *XIV World Congress of Fertility and Sterility*, Caracas, Venezuela, November 22–27, 1992. In Rodriguez Armas O, Baumgartner W, Burgos L, ed. *Progress in research practice*. Parthenon Publishing, Carnforth, UK 1994.
35. Fujino M, Yamazaki I, Kobayashi S, *et al.* Some analogs of luteinizing hormone-releasing hormone (LH-RH) having intense ovulation-inducing activity. *Biochem Biophys Res Commun* 1974; **57**: 1248–58.
36. Konig W, Sandow J, Geiger R. Structure–function relationships of LH-RH/FSH-RH. In: Walter R, Meienhofer J, eds. *Peptides: chemistry, structure and biology. Proceedings of the Fourth American Peptide Symposium*. Ann Arbor, MI: Ann Arbor Science 1975; 883–88.
37. Dutta AS, Furr BJA, Giles MB, *et al.* Potent agonist and antagonist analogues of luliberin containing an azaglycine residue in position 10. *Biochem Biophys Res Commun* 1978; **81**: 382–90.
38. Nestor Jr JJ, Ho TL, Simpson RA, *et al.* Synthesis and biological activity of some very hydrophobic superagonist analogues of luteinizing hormone-releasing hormone. *J Med Chem* 1982; **25**: 795–801.
39. Sandow J, von Rechenberg W, Jerzabek G, *et al.* Pituitary gonadotropins inhibition by a highly active analog of luteinizing hormone-releasing hormone. *Fertil Steril* 1978; **30**: 205–9.
40. Johnson B, Gendrich RL, White WF. Delay of puberty and inhibition of reproductive processes in the rat by a gonadotropin-releasing hormone agonist analog. *Fertil Steril* 1976; **27**: 853–60.
41. Corbin A, Beattie CW, Tracy J, *et al.* The anti-reproductive pharmacology of LHRH and antagonistic analogs. *Int J Fertil* 1978; **23**: 81–92.
42. Emons G, Schally AV. The use of LH-RH agonists and antagonists in gynecological cancers. *Human Reproduction* 1994; **9**(3).
43. Zorn JR, Boyer P, Guichard A. Never on a Sunday: programming for IVF-ET and GIFT. *Lancet* 1987; **1**: 385–6.
44. Ayalon D, Ben-David M, Wohl R, *et al.* Induction of ovulation with [D-Trp^6]LHRH combined with purified

FSH in patients with polycystic ovarian disease. *Gynecol Endocrinol* 1988; **2**: 319–30.
45. Bajusz S, Csernus VJ, Janaky T, *et al.* New antagonists of LHRH: II. Inhibition and potentiation of LHRH by closely related analogues. *Int J Peptide Prot Res* 1988; **32**: 425–35.
46. Ljungqvist A, Feng DM, Hook W, *et al.* Antide and related antagonists of luteinizing hormone release with long action and oral activity. *Proc Natl Acad Sci USA* 1988; **85**: 8236–40.
47. Rivier JE, Porter J, Rivier CL, *et al.* New effective gonadotropin releasing hormone antagonists with minimal potency for histamine release *in vitro*. *J Med Chem* 1986; **29**: 1846–51.
48. Gonzalez-Barcena D, Vadillo-Buenfil M, Garcia-Procel E, *et al.* Inhibition of LH and FSH release in hypergonadotropic patients with new potent antagonists free of anaphylactoid reactions. *Abstract Endocrine Society Meeting*, Seattle, WA 1989: 1712.
49. Gonzalez-Barcena D, Vadillo-Buenfil M, Guerra-Arguero L, *et al.* Potent antagonistic analog of LH-RH (SB-75) inhibits LH, FSH, and testosterone levels in human beings. *Abstract Endocrine Society Meeting*, Atlanta, GA 1990: 1318.
50. Pavlou SN, Wakefield G, Schlechter NL, *et al.* Mode of suppression of pituitary and gonadal function after acute or prolonged administration of a luteinizing hormone-releasing hormone antagonist in normal men. *J Clin Endoc Metab* 1989; **68**: 446–54.
51. Gonzalez-Barcena D, Vadillo-Buenfil M, Gomez-Orta F, *et al.* Response to the antagonistic analog of LH-RH (SB-75) in patients with benign prostatic hyperplasia (BPH) and prostatic cancer. *The Prostate* 1994; **24**: 84–92.
52. Krulich I, Dhariwal APS, McCann SM. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary *in vitro*. *Endocrinology* 1968; **83**: 783–90.
53. Brazeau P, Vale W, Burgus R, *et al.* A hypothalamic polypeptide that inhibits the secretion of pituitary growth hormone. *Science* 1973; **179**: 77–9.
54. Schally AV, Dupont A, Arimura A, *et al.* Isolation and structure of growth hormone-release inhibiting hormone (somatostatin) from porcine hypothalami. *Biochemistry* 1976; **15**: 509–14.
55. Schally AV, Huang WY, Chang RCC, *et al.* Isolation and structure of pro-somatostatin: A putative somatostatin precursor from pig hypothalamus. *Proc Natl Acad Sci USA* 1980; **77**: 4489–93.
56. Reichlin S. Somatostatin. *N Engl J Med* 1983; **309**: 1495–501, 1556–63.
57. Schally AV. Oncological application of somatostatin analogues. *Cancer Res* 1988; **48**: 6977–85.
58. Konturek SJ, Bilski J, Jaworek J, *et al.* Comparison of somatostatin and its highly potent hexa- and octapeptide analogs on exocrine and endocrine pancreatic secretion. *Proc Soc Exp Biol Med* 1988; **187**: 241–9.
59. Mascardo RN, Sherline P. Somatostatin inhibits rapid centrosomal separation and cell proliferation induced by epidermal growth factor. *Endocrinology* 1982; **111**: 1394–6.
60. Bauer W, Briner U, Boepfner W, *et al.* SMS-201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci* 1982; **31**: 1133–40.
61. Cai RZ, Szoke B, Lu R, *et al.* Synthesis and biological activity of highly potent octapeptide analogs of somatostatin. *Proc Natl Acad Sci USA* 1986; **83**: 1896–900.
62. Veber DF, Saperstein R, Nutt RF, *et al.* A superactive cyclic hexapeptide analog of somatostatin. *Life Sci* 1984; **34**: 1371–78.
63. Arimura A, Sato H, Kumasaka T, *et al.* Production of antiserum to LH-RH associated with marked atrophy of the gonads in rabbits: characterization of the antibody and development of a radioimmunoassay for LH-RH. *Endocrinology* 1973; **93**: 192–1103.
64. Hall R, Besser GM, Schally AV, *et al.* Action of growth hormone-release inhibitory hormone in healthy men and in acromegaly. *Lancet* 1973; **ii**: 581–4.
65. Boring CC, Squires TS, Tong T. Cancer statistics. *Ca-A Cancer J Clinicians* 1992; **42**: 19–38.
66. Schally AV, Comaru-Schally AM, Gonzalez-Barcena D. Present status of agonistic and antagonistic analogs of LH-RH in the treatment of advanced prostate cancer. *Biomed Pharmacother* 1992; **46**: 465–71.
67. Walsh PC. Physiological basis for hormonal therapy in carcinoma of the prostate. *Urol Clin N Am* 1975; **2**: 125–40.
68. Tolis G, Ackman D, Stellos A, *et al.* Tumor growth inhibition in patients with prostatic carcinoma treated with luteinizing hormone-releasing agonists. *Proc Natl Acad Sci USA* 1982; **79**: 1658–62.
69. Redding TW, Schally AV. Inhibition of prostate tumor growth in two rat models by chronic administration of D-Trp-6-LH-RH. *Proc Natl Acad Sci USA* 1981; **78**: 6509.
70. Warner B, Worgul TJ, Drago J, *et al.* Effect of high dose D-Leucine^6-gonadotropin-releasing hormone proethylamide on the hypothalamic-pituitary testicular axis in patients with prostatic cancer. *J Clin Invest* 1983; **17**: 1842–53.
71. Waxman JH, Wass JAH, Hendry WF, *et al.* Treatment with gonadotrophin releasing hormone analog in advanced prostatic cancer. *Br Med J* 1983; **286**: 1309–12.
72. Walker KJ, Nicholson RI, Turkes AO, *et al.* Therapeutic potential of the LHRH agonist, ICI 118630, in the treatment of advanced prostatic carcinoma. *Lancet* 1983; **ii**: 413–5.
73. Gonzalez-Barcena D, Perez-Sanchez P, Ureta-Sanchez S, *et al.* Treatment of advanced prostatic carcinoma with D-Trp-6-LH-RH. *Prostate* 1985; **7**: 21–30.
74. Mathe G, Schally AV, Comaru-Schally AM, *et al.* A phase II trial with D-Trp-6-LH-RH in prostatic carcinoma; A comparison with other hormonal agents. *Prostate* 1986; **9**: 327–42.
75. The Leuprolide Study Group. Leuprolide versus diethylstilbestrol for metastatic prostate cancer. *N Engl J Med* 1984; **311**: 1281–6.
76. Redding TW, Schally AV, Tice TR, *et al.* Long-acting delivery systems for peptides: inhibition of rat prostate tumors by controlled release of (D-Trp-6) luteinizing hormone-releasing hormone from injectable microcapsules. *Proc Natl Acad Sci USA* 1984; **81**: 5845–8.
77. Csernus VJ, Szende B, Schally AV. Release of peptides from sustained delivery systems (microcapsules and microparticles) *in vivo*: a histological and immunohistological study. *Int J Peptide Prot Res* 1990; **35**: 557–65.
78. Parmar H, Lightman SL, Allen L, *et al.* Randomised controlled study of orchidectomy vs long-acting D-Trp-6-LH-RH microcapsules in advanced prostatic carcinoma. *Lancet* 1985; **ii**: 1202–5.

79. Peeling WB. Phase III studies to compare goserelin (Zoladex) with orchiectomy and with diethylstilbestrol in treatment of prostatic carcinoma. *Urology* 1989; **33**(suppl): 45–52.
80. Sharifi R, Soloway M. Leuprolide Study Group: clinical study of leuprolide depot formulation in the treatment of advanced prostate cancer. *J Urol* 1990; **143**: 68–72.
81. Labrie F, Belanger A, Dupont A, *et al.* Combined treatment with LHRH agonist and pure antiandrogen in advanced carcinoma of prostate. *Lancet* 1984; **ii**: 1090.
82. Crawford ED, Eisenberger MA, McLeod DG, *et al.* A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. *N Engl J Med* 1989; **321**: 419–24.
83. D'Ercole AJ, Stiles AD, Underwood LE. Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. *Proc Natl Acad Sci USA* 1984; **81**: 935–9.
84. Stoscheck CM, King LE, Jr. Role of epidermal growth factor in carcinogenesis. *Cancer Res* 1986; **46**: 1030–7.
85. Heldin CH, Westermark B. Growth factors: mechanism of action and relation to oncogenes. *Cell* 1984; **37**: 9–20.
86. Lippman ME, Dickson RB, Kasid A, *et al.* Autocrine and Paracrine growth regulation of human breast cancer. *J Steroid Biochem* 1986; **24**: 147–54.
87. Goustin AS, Leof EB, Shipley GS, *et al.* Growth factors and cancer. *Cancer Res* 1986; **46**: 1015–29.
88. Connolly JM, Rose DP. Secretion of epidermal growth factor and related polypeptides by the DU 145 human prostate cancer cell line. *Prostate* 1989; **15**: 177–86.
89. Hofer DR, Sherwood ER, Bromberg WD, *et al.* Autonomous growth of androgen-independent human prostatic carcinoma cells: role of transforming growth factor α. *Cancer Res* 1991; **51**: 2780–5.
90. Liebow C, Reilly C, Serrano M, *et al.* Somatostatin analogues inhibit growth of pancreatic cancer by stimulating tyrosine phosphatase. *Proc Natl Acad Sci USA* 1989; **86**: 2003–7.
91. Redding TW, Schally AV, Radulovic S, *et al.* Sustained release formulations of luteinizing hormone-releasing hormone antagonist SB-75 inhibit proliferation and enhance apoptotic cell death of human prostate carcinoma (PC-82) in male nude mice. *Cancer Res* 1992; **52**: 2538–44.
92. Erspamer VG, Falconieri E, Inselvini M. Some pharmacological actions of alytesin and bombesin. *J Pharm Pharmacol* 1970; **22**: 275–6.
93. Sunday ME, Kaplan LM, Motoyama E, *et al.* Biology of disease, gastrin-releasing peptide (mammalian bombesin) gene expression in health and disease. *Lab Invest* 1988; **59**: 5–24.
94. McDonald TH, Jornvall H, Nilsson G, *et al.* Characterization of a gastrin releasing peptide from porcine nonantral gastric tissue. *Biochem Biophys Res Commun* 1979; **90**: 227–33.
95. Moody TW, Thoa NB, O'Donohue TL, *et al.* Bombesin-like peptides in rat brain: Localization in synaptosomes and release from hypothalamic slices. *Life Sci* 1980; **26**: 1707–12.
96. Moody TW, O'Donohue TL, Jacobowitz DM. Biochemical localization and characterization of bombesin-like peptides in discrete regions of rat brain. *Peptides* 1981; **2**: 75–9.
97. Cuttitta F, Carney DN, Mulshine J, *et al.* Bombesin-like peptides can function as autocrine growth factors in human small cell lung cancer. *Nature* 1985; **316**: 823–6.
98. Moody TW, Russel DK, O'Donohue TL, *et al.* Bombesin-like peptides in small cell lung cancer: biochemical characterization and secretion from a cell line. *Life Sci* 1983; **32**: 487–93.
99. Yano T, Pinski J, Groot K, *et al.* Stimulation by bombesin and inhibition by bombesin/GRP antagonist RC-3095 of growth of human breast cancer cell lines. *Cancer Res* 1992; **52**: 4545–7.
100. Qin Y, Ertl T, Cai RZ, *et al.* Inhibitory effect of bombesin receptor antagonist RC-3095 on the growth of human pancreatic cancer cells *in vivo* and *in vitro*. *Cancer Res* 1994; **54**: 1035–41.
101. Radulovic S, Cai RZ, Serfozo P, *et al.* Biological effects and receptor binding affinities of new pseudononapeptide bombesin/GRP receptor antagonists with N-terminal D-Trp or D-Tpi. *Int J Peptide Protein Res* 1991; **38**: 593–600.
102. Cai RZ, Radulovic S, Pinski J, *et al.* Pseudononapeptide bombesin antagonists containing C-terminal Trp or Tpi. *Peptides* 1992; **13**: 267–71.
103. Milovanovic SR, Radulovic S, Groot K, *et al.* Inhibition of growth of PC-82 human prostate cancer line xenografts in nude mice by bombesin antagonist RC-3095 or combination of agonist [D-Trp^6]-luteinizing hormone-releasing hormone and somatostatin analog RC-160. *The Prostate* 1992; **20**: 269–80.
104. Pinski J, Schally AV, Halmos G, *et al.* Effect of somatostatin analog RC-160 and bombesin/gastrin releasing peptide antagonist RC-3095 on growth of PC-3 human prostate cancer xenografts in nude mice. *Int J Cancer* 1993; **55**: 962–7.
105. Pinski J, Halmos G, Schally AV. Somatostatin analog RC-160 and bombesin/gastrin releasing peptide antagonist RC-3095 inhibit the growth of androgen-independent DU-145 human prostate cancer line in nude mice. *Cancer Lett* 1993; **71**: 189–96.
106. Bajusz S, Janaky T, Csernus VJ, *et al.* Highly potent metallopeptide analogues of luteinizing hormone-releasing hormone. *Proc Natl Acad Sci USA* 1989; **86**: 6313–7.
107. Bajusz S, Janaky T, Csernus VJ, *et al.* Highly potent analogues of luteinizing hormone-releasing hormone containing D-phenylalanine nitrogen mustard in position 6. *Proc Natl Acad Sci USA* 1989; **86**: 6318–22.
108. Janaky T, Juhasz A, Bajusz S, *et al.* Analogues of luteinizing hormone-releasing hormone containing cytotoxic groups. *Proc Natl Acad Sci USA* 1992; **89**: 972–6.
109. Pinski J, Schally AV, Yano T, *et al.* Inhibition of growth of experimental prostate cancer in rats by LH-RH analogs linked to cytotoxic radicals. *The Prostate* 1993; **23**: 165–78.
110. Gonzalez-Barcena D, Vadillo-Buenfil M, Gomez-Orta F, *et al.* Treatment of patients with advanced prostatic cancer with LH-RH antagonist SB-75 and of the relapse by the combination of agonist D-Trp-6-LH-RH and somatostatin analog RC-160. *Abstract Endocrine Society Meeting*, Las Vegas, NV 1993: 389.
111. Williams MR, Walker KJ, Turkes A, *et al.* The use of an LH-RH agonist (ICI 118630, Zoladex) in advanced premenopausal breast cancer. *Br J Cancer* 1986; **53**: 629–36.
112. Kaufmann M, Jonat W, Kleeburg U, *et al.* The German

zoladex trial group: Goserelin, a depot gonadotropin releasing hormone agonist in the treatment of premenopausal patients with metastatic breast cancer. *J Clin Oncol* 1989; **7**: 1113–9.
113. Santen RJ, Manni A, Harvey H, *et al.* Endocrine treatment of breast cancer in women. *Endoc Rev* 1990; **11**: 221–65.
114. Miller WR, Scott WN, Morris R, *et al.* Growth of human breast cancer cells inhibited by a luteinizing hormone-releasing hormone agonist. *Nature* 1985; **313**: 231–3.
115. Eidne KA, Flanagan CA, Harris NS, *et al.* Gonadotropin-releasing hormone (GnRH)-binding sites in human breast cancer cell lines and inhibitory effects of GnRH antagonists. *J Clin Endocrinol Metab* 1987; **64**: 425–32.
116. Fekete M, Wittliff JL, Schally AV. Characteristics and distribution of receptors for [D-Trp^6]-luteinizing hormone-releasing hormone, somatostatin, epidermal growth factor, and sex steroids in 500 biopsy samples of human breast cancer. *J Clin Lab Anal* 1989; **3**: 137–47.
117. Foekens JA, Portengen H, van Putten WLJ, *et al.* Prognostic value of receptors for insulin-like growth factor 1, Somatostatin, and epidermal growth factor in human breast cancer. *Cancer Res* 1989; **49**: 7002–9.
118. Mathe G, Mauvernay R, Schally A, *et al.* D-Trp-6-LH-RH, Sustained Release Formulation, a hormonal treatment efficient in prostate cancer, breast cancer and genital dysplasias. *Abstract 2nd International Conference of the Metastasis Research Society*, Heidelberg, Germany, September 26–29, 1988.
119. Szepeshazi K, Milovanovic S, Lapis K, *et al.* Growth Inhibition of Estrogen Independent MXT mouse mammary carcinomas in mice treated with an agonist or antagonist of LH-RH, an analog of somatostatin, or a combination. *Breast Cancer Res Treat* 1992; **21**: 181–92.
120. Szepeshazi K, Schally AV, Halmos G, *et al.* Growth inhibition of estrogen-dependent and estrogen-independent MXT mammary cancers in mice by the bombesin and gastrin-releasing peptide antagonist RC-3095. *J Natl Cancer Inst* 1992; **84**: 1915–22.
121. Yano T, Pinski J, Szepeshazi K, *et al.* Inhibitory effect of bombesin/gastrin releasing peptide (GRP) antagonist RC-3095 and LH-RH antagonist SB-75 on growth of MCF-7 MIII human breast cancer xenografts in athymic nude mice. *Cancer* 1994; **73**: 1229–38.
122. Szepeshazi K, Schally AV, Juhasz A, *et al.* Effect of LH-RH analogs containing cytotoxic radicals on growth of estrogen independent MXT mouse mammary carcinoma *in vivo*. *Anti-Cancer Drugs* 1992; **3**: 109–16.
123. Srkalovic G, Wittliff JL, Schally AV. Detection and partial characterization of receptors for [D-Trp-^{6}]-luteinizing hormone-releasing hormone and epidermal growth factor in human endometrial carcinoma. *Cancer Res* 1990; **50**: 1841–6.
124. Emons G, Schroder B, Ortmann O, *et al.* High affinity binding and direct antiproliferative effects of LH-RH agonists and antagonists in human endometrial cancer cell lines. *J Clin Endocrinol Metab* 1993; **77**: 1458–64.
125. Emons G, Pahwa GS, Brack C, *et al.* Gonadotropin releasing hormone binding sites in human epithelial ovarian carcinomata. *Eur J Cancer Clin Oncol* 1989; **25**: 215–21.
126. Emons G, Ortmann O, Becker M, *et al.* High affinity binding and direct antiproliferative effects of LHRH analogues in human ovarian cancer cell lines. *Cancer Res* 1993; **53**: 5439–46.
127. Parmar H, Phillips RH, Rustin G, *et al.* Response to [D-Trp^6]LHRH (Decapeptyl) microcapsules in advanced ovarian cancer. *Br Med J* 1988; **296**: 1229.
128. Parmar H, Phillips RH, Rustin G, *et al.* Therapy of advanced ovarian cancer with D-Trp-6-LH-RH (decapeptyl) microcapsules. *Biomed Pharmacother* 1988; **42**: 531–38.
129. Theve NO, Pousette A, Carlstrom K. Adenocarcinoma of the pancreas—a hormone sensitive tumor? A preliminary report on Nolvadex treatment. *Clin Oncol* 1983; **6**: 193–197.
130. Poston GJ, Gillespie J, Guillou PJ. The biology of pancreatic cancer. *Gut* 1991; **32**: 800–12.
131. Greenway BA. Carcinoma of the exocrine pancreas: a sex hormone responsive tumor? *Br J Surg* 1987; **74**: 441–2.
132. Szende B, Srkalovic G, Schally AV, *et al.* Inhibitory effects of analogs of luteinizing hormone-releasing hormone and somatostatin on pancreatic cancers in hamsters. *Cancer* 1990; **65**: 2279–90.
133. Szepeshazi K, Lapis K, Schally AV. Effect of combination treatment with analogs of luteinizing hormone releasing hormone (LH-RH) or somatostatin and 5-fluorouracil on pancreatic cancer in hamsters. *Int J Cancer* 1991; **49**: 260–6.
134. Szepeshazi K, Cai RZ, Radulovic S, *et al.* Inhibitory effect of bombesin/GRP antagonist RC-3095 and high dose of somatostatin analog RC-160 on nitrosamine-induced pancreatic cancers in hamsters. *Cancer Res* 1991; **51**: 5980–6.
135. Szepeshazi K, Schally AV, Groot K, *et al.* Effect of bombesin, gastrin-releasing peptide (GRP)(14-27) and bombesin/GRP antagonist RC-3095 on growth of nitrosamine-induced pancreatic cancers in hamsters. *Int J Cancer* 1993; **54**: 282–9.
136. Szende B, Srkalovic G, Groot K, *et al.* Regression of nitrosamine-induced pancreatic cancers in hamsters treated with LH-RH antagonists or agonists. *Cancer Res* 1990; **50**: 3716–21.
137. Gonzalez-Barcena D, Ibarra-Olmos MA, Garcia-Carrasco F, *et al.* Influence of D-Trp-6-LH-RH on the survival time in patients with advanced pancreatic cancer. *Biomed Pharmacother* 1989; **43**: 313–7.
138. Poston GJ, Schally AV. Somatostatin analogs and pancreatic cancer. *Int J Pancreatol* 1993; **14**: 64–6.
139. Poston GJ, Davies N, Schally AV, *et al.* Phase one B study of somatostatin analogue RC-160 in the treatment of patients with advanced exocrine pancreatic cancer. *Abstract Eurpean Pancreatic Club*, Basel, Switzerland, October 15–17. *Digestion* 1990; **46**: 170.
140. Fleisher DE, Goldberg SB, Browning TH, *et al.* Detection and surveillance of colorectal cancer. *J Am Med Ass* 1989; **261**: 580–5.
141. Radulovic S, Miller G, Schally AV. Inhibition of growth of HT-29 human colon cancer xenografts in nude mice by treatment with agonists or antagonists of LH-RH. *Cancer Res* 1991; **51**: 6006–9.
142. Schein PS. Locally advanced and metastatic gastric carcinoma. *Adv Oncol* 1987; **3**: 15–22.
143. Pinski J, Halmos G, Yano T, *et al.* Inhibition of growth of MKN45G human gastric adenocarcinoma xenografts in nude mice by treatment with bombesin/gastrin re-

leasing peptide antagonist (RC-3095), and somatostatin analogue RC-160. *Int J Cancer* 1994; in press.
144. Qin Y, Halmos G, Cai RZ, *et al.* Bombesin antagonists inhibit *in vitro* and *in vivo* growth of human gastric cancer and binding of bombesin to its receptors. *J Cancer Res Clin Oncol* 1994; in press.
145. Minna JD. Neoplasms of the lung. In: Braunwald E, Isselbacher KJ, Petersdorf RG, eds. *Harrison's principles of internal medicine*, 11th edn. New York: McGraw Hill 1987: 1115–23.
146. Veale D, Kerr N, Gibson GJ, *et al.* Characterization of epidermal growth factor receptor in primary human non-small cell lung cancer. *Cancer Res* 1989; **49**: 1313–7.
147. Macauly VM, Teale JD, Everard MJ, *et al.* Somatomedian-C/insulin-like growth factor-I is a mitogen for human small cell lung cancer. *Br J Cancer* 1988; **57**: 91–3.
148. Pinski J, Halmos G, O'Byrne K, *et al.* Inhibitory effect of bombesin/GRP antagonists and somatostatin analogs on the growth of S.C.L.C. and Non-S.C.L.C. xenografts in nude mice. *Br J Cancer* 1994; in press.
149. Mahmoud S, Staley J, Taylor J, *et al.* [Psi^{13,14}] bombesin analogues inhibit growth of small cell lung cancer *in vitro* and *in vivo*. *Cancer Res* 1991; **51**: 1798–802.
150. Salcman M, Kaplan RS. Intracranial tumors in adults. In: Moossa AR, Robson MC, Schimpff, SC, eds. *Comprehensive textbook of oncology*. Baltimore, MD Williams and Wilkins, 1986: 617–29.
151. Gammeltoft A, Ballotti R, Kowalski A, *et al.* Expression of two types of receptors for insulin-like growth factors in human malignant glioma. *Cancer Res* 1988; **48**: 1233–7.
152. Banks WA, Kastin AJ, Radulovic S, *et al.* Selective uptake of the somatostatin analog RC-160 across the blood–brain tumor barrier of mice with KHT sarcomas. *Anti-Cancer Drugs* 1992; **3**: 519–23.

(Received 14 December 1993; accepted 6 January 1994)