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Antitumor Effects of Analogs of Hypothalamic Hormones in Endocrine-Dependent Cancers (41797)

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The isolation, determination of structure, and synthesis of hypothalamic peptide hormones and the subsequent synthesis of their analogs have opened radically new approaches to diagnosis and treatment of some endocrine and nonendocrine disorders (1–10). Clinical applications of the hypothalamic hormones TRH, LH-RH, somatostatin CRF, and GH-RF which have been proven or which are being tried include: determination of pituitary reserve of thyrotropin (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), ACTH, and GH, differentiation between hypothalamic and pituitary lesion, stimulation of fertility, birth control, and treatment of precocious puberty, cryptorchidism, endometriosis, diabetes mellitus, acromegaly, acute pancreatitis, and peptic ulcers (1–10). The use of analogs of hypothalamic hormones for treatment of hormone-sensitive tumors is very recent, but most promising. It was initially viewed as a sideline of the application of hypothalamic peptides, but now appears to be of growing and major clinical value. Ongoing or planned investigations of the application of analogs of hypothalamic hormones in the field of neoplasms, benign as well as malignant, include treatment of prostate and breast cancer, chondrosarcomas and osteosarcomas, pancreatic cancer, pituitary tumors, ovarian cancer and neoplasms of the female genital tract, and other hormone-dependent tumors (11–43). In this review we shall summarize results of experimental and clinical investigations on the effects of hypothalamic hormones on various endocrine-dependent tumors, with emphasis on the treatment of prostate cancer. We shall also briefly mention some new approaches which, in all likelihood, will be tried in the near future.

1. Prostate Cancer. Carcinoma of the prostate is the second most common tumor in men and the second leading cause of cancer-related deaths among adult American men over 55 years of age (44–46). Pioneering work by Huggins and collaborators established that carcinoma of the prostate is frequently hormone dependent (47, 48). Androgen-dependent prostate cancer constitutes about 70% of all cases of prostate neoplasms (44–46). Prolactin also appears to be a promoter of prostate growth and could be involved in prostate cancer as a cofactor (49–52). The treatment of prostate cancer is usually based upon androgen dependence of the tumor (44–48, 53, 54). Endocrine therapy for adenocarcinoma of the prostate includes orchiectomy, hypophysectomy, adrenalectomy, administration of estrogens and antiandrogens (44–48, 53, 54). Elimination or inhibition of testicular androgen secretion can induce a high degree of initial response (44–46). However, surgical castration is associated with a psychological impact and estrogens have serious cardiovascular, hepatic, and mammotropic side effects, and the mortality from cardiovascular disease is significantly increased in patients receiving estrogen therapy (21, 55).

A radically different endocrine therapy, without apparent toxicity, is based on the use of highly potent, long-acting, agonistic analogs of luteinizing hormone-releasing hormone (LH-RH).

Experimental studies with LH-RH agonists in rat models of prostate cancer. LH-RH agonists characterized by a D-amino acid in position 6 were originally developed to treat infertility (1–5, 11–14). D-Trp-6-LH-RH and D-Ser(Bu^t)^{6}des-Gly-NH_2^{10}-LH-RH ethylamide (HOE 766) are superactive long-acting LH-RH agonists, about 100 times more potent than LH-RH (56). D-Leu-6-LH-RH ethylamide (Leuprolide) is about 50 times more active than LH-RH (1–5). The properties of D-

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Ser(Bu^t)^{6}AZ-Gly^{10}-LH-RH (I.C.I. 118630) are similar to those of Leuprolide (57). Although an acute injection of superactive agonists of LH-RH induces a marked and prolonged release of LH and FSH, paradoxically, chronic administration produces dramatic inhibitory effects through a process of "down regulation" of the pituitary gonadotrophs and gonads and desensitization of receptors (1–5, 16, 21, 58–61). In male animals this is manifested by a fall in the weights of testes, seminal vesicles, and prostate, a decrease in plasma testosterone levels, and a reduction in testicular LH/hCG receptors (1–5, 61–64). In men, a persistent suppression of Leydig cell function, manifested by a fall in serum testosterone, dihydrotestosterone, and estradiol levels, has been observed after chronic administration of D-Trp-6-LH-RH or D-Ser(Bu^t)^{6}des-Gly-NH_2^{10}-LH-RH ethylamide (HOE 766) (1–5, 21, 22, 65–69).

The finding that prolonged treatment with agonistic analogs of LH-RH can result in testicular inhibition and chemical castration prompted us to use this method in an attempt to induce the regression of prostate tumors in rats. We found that chronic administration of D-Trp-6-LH-RH can inhibit prostate tumor growth in two different rat models; Segaloff 11095 squamous cell carcinoma and Dunning R-3327H adenocarcinoma (11). Administration of D-Trp-6-LH-RH for 14–21 days significantly inhibited growth of the chemically induced squamous cell carcinoma 11095 in Fisher 344 male rats. Treatment of male Copenhagen F-1 rats bearing the Dunning R-3327H prostate adenocarcinoma with D-Trp-6-LH-RH for 42 days reduced the percentage increase in tumor volume to one-third, and decreased actual tumor weight by 58% as compared to untreated controls (11). Tumor cell doubling time was more than four times longer in rats receiving D-Trp-6-LH-RH than in controls. Serum LH and FSH levels were significantly decreased in rats receiving this analog. In both Fisher 344 and Copenhagen F-1 rats, serum prolactin and testosterone levels were significantly reduced after treatment with D-Trp-6-LH-RH, whereas progesterone levels were increased. This study (11) demonstrated for the first time the potential clinical efficacy of D-Trp-6-LH-RH in the treatment of prostate carcinoma and other hormone-sensitive tumors in man.

In a subsequent study chronic administration of D-Trp-6-LH-RH for 21 days to rats bearing Dunning R-3327H prostate tumor induced a highly significant reduction (10-fold) in tumor weight as compared with controls (12). Tumor volume of control animals showed a 215% increase, while final tumor volume of the D-Trp-6-LH-RH-treated group was smaller by nearly 18% than initial volume. Long-term administration of D-Trp-6-LH-RH significantly reduced LH and prolactin levels but did not affect GH values. D-Trp-6-LH-RH greatly suppressed serum testosterone values, the levels being diminished by 93% (12). These two studies (11, 12) demonstrated that chronic administration of the agonist D-Trp-6-LH-RH inhibits the growth of Dunning prostate adenocarcinoma R-3327H. The hormone dependency of the Dunning tumor is well documented. Histological and biochemical similarities of this tumor to human prostatic adenocarcinoma have made it an acceptable model for the study of human prostatic cancer. The principal mechanism of remission of this tumor is probably linked to the suppression of testosterone and prolactin levels. The most effective means of suppressing tumor growth in the Dunning rat model is hypophysectomy, alone or in combination with orchidectomy. The suppressive effect of LH-RH agonists on gonadotropins, prolactin, and testosterone can mimic, in part, the effect of hypophysectomy and orchiectomy. Prolactin has been shown to stimulate prostate growth and may also enhance the response of the prostate to androgen (49–52). Inhibition of both testosterone and prolactin by chronic administration of LH-RH agonists would thus be expected to suppress tumor growth more effectively than a deficiency of androgen alone. It is also possible that D-Trp-6-LH-RH could have some direct inhibitory effect on prostate tumors. LH-RH receptors have been found in the rat testes and ovaries (64). We have been unable to find LH-RH receptors in normal rat prostate tissue, but we observed binding of D-Trp-6-LH-RH to plasma membranes from Dunning R3327H prostate tumors (70). These findings appear to support the view that LH-RH analogs, in addition to their main effect which is exerted on the pituitary, and a possible action on the gonads, might also act directly on prostatic tumors.

Clinical trials with LH-RH agonists in men

with prostate cancer. The demonstration that D-Trp-6-LH-RH inhibits the growth of prostate tumors in rats (11) led to clinical trials. The first successful palliative treatment of advanced prostatic carcinoma by agonistic analogs of LH-RH was demonstrated in a collaborative trial carried out at the Royal Victoria Hospital in Montreal (23). Ten patients with prostatic carcinoma, 6 with stage C and 4 with stage D disease, were treated for 6 weeks to 12 months with agonistic analogs of LH-RH. Estrogen therapy had been tried unsuccessfully in 1 patient and was contraindicated for the remaining 9 patients because of a history of previous myocardial infarction or other thromboembolic episodes. D-Trp-6-LH-RH was given subcutaneously once daily in a dose of 100 μg, and [D-Ser(Bu^t)^6]des-Gly-NH_2^{10}-LH-RH ethylamide (HOE 766) was given subcutaneously, 50 μg once daily, or intranasally, 500 μg twice daily. In all patients, mean plasma testosterone levels showed a 75% fall by the third week of treatment and remained at castration values thereafter. This was followed by a decrease or normalization of serum acid phosphatase levels by the second month of treatment and a 47% or more decrease in serum alkaline phosphatase by the 10th week of treatment in all but one patient, whose tumor was androgen-insensitive. In patients with stage C disease presenting with urinary obstruction, there was a noticeable clinical improvement. In two such patients, a decrease in the size of the prostate was confirmed by ultrasonography. In patients with stage D disease manifested by diffuse bone metastases, there was relief of bone pain, and in one patient treated for more than 12 months improvement was documented by radioisotope bone imaging. The only side effects consisted of a decrease in libido and climacteric-like vasomotor phenomena. This trial demonstrated for the first time that superactive agonistic LH-RH analogs are efficacious therapeutic agents in patients with androgen-sensitive prostatic adenocarcinoma (23). Treatment based on LH-RH agonists might avoid the psychological impact of castration or even an unnecessary castration, as well as cardiovascular, hepatic, and mammotropic side effects of estrogen.

These findings have been confirmed and extended by other clinical trials in Europe and North America (21, 22, 24–32, 41–43). The Abbott group has reported therapeutic trials at 13 US centers with the agonist D-Leu-6-des-Gly-NH_2^{10}-LH-RH ethylamide (leuprolide) (24). Parenteral administration of Leuprolide in doses of 1–20 mg for 19 or more weeks to 118 patients with stage D prostatic carcinoma resulted in objective responses, such as a fall in acid phosphatase levels in 60–70% of patients. There was a transient flare-up of the disease in about 10% of patients, but after 4 weeks androgen values were at castrate levels. The therapy appeared to be effective for prostatic carcinoma, with fewer side effects than those caused by estrogen administration (24). Borgmann *et al.* (22) treated nine patients with advanced carcinoma of the prostate with HOE 766 and found a fall in testosterone levels after 3 weeks of therapy, and regressive changes in some tumors after 3–6 months of treatment similar to those seen after surgical castration. In a subsequent study the same group used HOE 766 analog for 7 to 19 months in 21 patients with stage C prostate cancer (29). Seventeen patients showed good therapeutic responses with cytological signs of tumor regression. Tolis *et al.* (27, 28) studied 22 patients with advanced prostatic carcinoma and compared the effects of orchiectomy on Group I versus the effect of chronic administration of analog HOE 766 (Group II). Both therapies resulted in a decrease in prostatic size as estimated by transabdominal ultrasonography, and by the sixth month there was no statistical difference in prostatic size between the two groups. A follow-up of 24 months showed a persistent suppression of prostatic size and improvement in urinary obstruction in nearly all the patients treated with the LH-RH analog (27, 28). Bone scanning of patients with stage D_2 disease revealed an improvement in the number and/or intensity of bone lesions in some patients. Labrie *et al.* (25, 26) used a combination of HOE 766 with an antiandrogen RU-23908 for treatment of patients with stage C and D_2 prostate carcinoma and reported subjective and objective improvement, including a decrease in bone pain and a fall in acid phosphatase. It was stated that the combined treatment with the LH-RH analog and antiandrogen is more effective than the analog alone (25, 26).

A significant, sometimes dramatic decrease in bone pain in patients with stage D meta-

static prostate carcinoma after treatment with LH-RH agonists is a frequent and consistent finding (23–32). A decrease in the number and intensity of bone lesions has also been reported (23–32), as well as an improvement in appetite (30) and an increase in body weight (30). Mathé *et al.* (31), used D-Trp-6-LH-RH to treat 21 patients with stage D prostatic carcinoma and 3 at stage C. After 3 months of therapy, 84% of patients showed a normalization or decrease of tumor mass (measured by ultrasonography), and an improvement in bone metastases (evaluated by bone scanning) was observed in 43% of the cases. Prostatic acid phosphatase levels decreased in 75% of the cases. Nineteen out of 21 patients showed a total disappearance or improvement in bone pain (31). Aside from hot flashes and a decrease in potency no side effects were observed. Four independent studies in England, three of which used I.C.I. analog 118630 (41–43), and one HOE766 (32), also support the view that LH-RH agonists may be the treatment of choice in adenocarcinoma of the prostate.

Thus, data are accumulating which suggest that long-term therapy with agonists of LH-RH might be the preferred alternative to surgical castration or treatment with estrogens. The regression of prostatic tumors and metastatic disease manifested clinically, radiologically, biochemically, and cytologically after treatment with LH-RH agonists is most encouraging (22–32, 41–43). However, more time is needed to assess the duration of response to LH-RH agonists and the effect on survival rate.

Future treatment of prostate carcinoma should be made more convenient by the development of injectable microcapsules with D-Trp-6-LH-RH for once a month administration (12–14). These microcapsules, prepared from biodegradable biocompatible polymer, poly(D,L-lactide-co-glycolide) (pLGA) system, are designed for a constant, controlled release of this analog over a 30-day period. Our findings indicate that a single intramuscular administration of microcapsules with D-Trp-6-LH-RH can suppress the growth of Dunning R 3327H prostate tumor in Copenhagen–Fisher rats for at least 30 days. Preliminary results indicate that these microcapsules lead to a greater reduction of tumor volume and weight than twice daily injection of the analog. The suppression of testosterone levels also seemed to be better with microcapsules than with twice daily injections of the analog. The use of microcapsules would make therapy with D-Trp-6-LH-RH more practical and convenient and would also better ensure patient compliance. This approach should increase the utility of LH-RH analogs for treatment of prostate carcinoma and other hormone-sensitive neoplasms. Preliminary findings indicate that microencapsulated D-Trp-6-LH-RH is efficacious in human beings.

It is also likely that treatment with LH-RH agonists can be combined with chemotherapy. Thus, simultaneous administration of one of the cytotoxic drugs, probably cyclophosphamide, and D-Trp-6-LH-RH or other LH-RH agonists, early in the diagnosis of metastatic prostate cancer could inhibit the proliferation of androgen-independent cell clones and therefore enhance the therapeutic response. Such a combination of chemotherapy and hormonal approach has already been tried with other agents by Murphy *et al.* (71).

An increase in the therapeutic response could also result from the combination of LH-RH agonists with some peptides which inhibit prolactin secretion. This hypothesis is based on the evidence that prolactin may be a cofactor in the development or growth of prostate tumors (49–52). Thus the reduction in prolactin levels produced by the administration of a suitable somatostatin analog, or a peptide with prolactin inhibiting factor (PIF) activity, combined with the decrease in testosterone values which results from chronic treatment with LH-RH agonists may lead to a greater inhibition of prostate tumors than that which can be obtained with LH-RH agonists alone. The administration of somatostatin analogs would also decrease GH levels and this might also contribute to an additional inhibition of tumor growth.

Experimental studies with LH-RH antagonists on prostate tumors in rats. While repeated administration of LH-RH agonists is required to induce a paradoxical inhibition of LH and FSH release and a reduction in the levels of sex steroids, similar effects can be obtained with a single administration of LH-RH antagonists (1–5, 10, 72–78). Antagonistic analogs of LH-RH were developed for contraception (1–5, 10, 75, 76) and possess modifications in positions 2, 3, and 6, and in more recent compounds other changes, especially

in positions 1 and 10. These analogs act on the same receptor sites as LH-RH and inhibit the release of gonadotropins. A series of potent LH-RH antagonists has been synthesized and tested in animals and human beings (1–5, 10, 72–78). In women and subhuman primates these antagonists disrupt the normal events of the menstrual cycle, block ovulation, and inhibit the rise in LH and FSH induced by oophorectomy (1–5, 10, 75–78). In male rats administration of LH-RH antagonists has been shown to decrease gonadotropin and testosterone levels and reduce the weights of testes and accessory sex organs (12, 15, 72, 73). An early antagonistic analog blocked the LH and FSH responses to LH-RH in men for at least 24 hr (1–5, 10, 74).

Successful suppression of Segaloff and Dunning prostate tumors by chronic administration of the agonist D-Trp-6-LH-RH (11) prompted us to investigate whether inhibitory analogs would also have an effect. So far three antagonistic analogs have been used in our studies on rat models of prostate tumors: [*N*-Ac-D-*p*-F-Phe^{1},*p*-Cl-D-Phe^{2},D-Trp^{3,6},D-Ala-10]LH-RH, [*N*-Ac-D-*p*-Cl-Phe^{1,2},D-Trp^{3},D-Phe^{6},D-Ala^{10}]LH-RH, and [*N*-Ac-D-*p*-Cl-Phe^{1,2},D-Trp^{3},D-Arg^{6},D-Ala^{10}]LH-RH (12, 15). First we investigated the effects of *N*-Ac-D-*p*-F-Phe-1,*p*-Cl-D-Phe-2,D-Trp-3,6,D-Ala-10-LH-RH on the growth of the chemically induced Segaloff 11095 squamous cell carcinoma in Fisher 344 male rats (15). Administration of this antagonist for 21 days significantly reduced tumor weight as compared with controls, and markedly decreased serum LH, FSH, and testosterone levels. When male Copenhagen F-1 rats bearing the Dunning R-3327H prostate adenocarcinoma were treated with this antagonist or with *N*-Ac-*p*-Cl-D-Phe-1,2,D-Trp-3,D-Phe-6,D-Ala-10-LH-RH, the percentage increase in tumor volume was decreased to half or less, and actual tumor volume was diminished 34 to 96% compared to controls (15). Tumor weights were decreased 30 to 89%. Tumor doubling time was three- to four-fold longer in rats receiving inhibitory analogs than in controls (15). Serum LH, FSH, and testosterone levels in Copenhagen F-1 rats bearing Dunning tumors were significantly decreased after treatment with the inhibitory analogs, but progesterone levels were increased (15). In the second study (12) we used a more modern antagonist, *N*-Ac-D-*p*-Cl-Phe-1,2,D-Trp-3,D-Arg-6,D-Ala-10-LH-RH (Org 30276), which is considerably more potent in blocking ovulation than analogs used previously, and active when administered orally to rats (10, 76). In addition, this antagonist has more desirable solubility properties, being soluble in water and saline (10, 76). Chronic administration of *N*-Ac-*p*-Cl-D-Phe-1,2,D-Trp-3,D-Arg-6,D-Ala-10-LH-RH (Org 30276) significantly inhibited growth of the Dunning prostate tumor. Mean tumor weight in the antagonist-treated group at autopsy was only 0.38 ± 0.05 g as compared with 8.55 ± 4.6 g for controls. Tumor volumes increased by only 13.7% during the 3-week treatment with the antagonist, in contrast to a 215% increase in the control group (12). Administration of this antagonist also greatly suppressed serum testosterone values, the levels being diminished by 97% (12). The antagonist also significantly reduced LH and prolactin levels. The inhibition of animal models of prostate carcinomas by antagonistic LH-RH analogs is probably mainly due to the suppression of plasma levels of testosterone and prolactin (12, 15) similar to that achieved by chronic administration of LH-RH agonists. The binding of a powerful antagonist, Ac-D-*p*-Cl-Phe-1,2,D-Trp-3,D-Lys-6,D-Ala-10-LH-RH, to plasma membranes from Dunning R-3327H prostate tumors indicates that LH-RH antagonists might also act directly on prostatic tumors (70).

These experimental studies, which showed an inhibition of growth of prostate tumors in rats after administration of three different LH-RH antagonists (12, 15), suggest that this class of compounds should also be considered for the therapy of prostate carcinoma. The use of antagonistic analogs of LH-RH for the treatment of prostate cancer would avoid the transient stimulation of release of gonadotropins and testosterone which occurs initially in response to LH-RH agonists, thus preventing the temporary clinical flare-up of the disease (12, 14, 24, 25). However, LH-RH antagonists have been tried so far only in acute studies in human beings aimed at contraception (1–5, 10), and clinical data are not yet available on the effects of chronic administration. Large scale synthesis and recently completed toxicological investigations will permit a clinical evaluation of *N*-Ac-D-*p*-Cl-Phe-1,2,D-Trp-3,D-Arg-6,D-Ala-10-LH-RH in the treatment of

prostate cancer, and a comparison of its therapeutic efficacy with D-Trp-6-LH-RH and other agonists. It is also possible that treatment with LH-RH antagonists can be combined with chemotherapy, or with substances inhibiting prolactin secretion.

Future experimental studies in animal models of prostate cancer and eventual clinical trials will be made more convenient by the development of long-acting pharmaceutical dosage forms.

Benign prostate hyperplasia. It is clear that a similar approach based on administration of agonists or antagonists of LH-RH should be tried for the treatment of benign prostatic hyperplasia. Accumulation of dihydrotestosterone (DHT) within the prostate could cause this hyperplasia in both dogs and men (79, 80). Plasma DHT levels can be markedly reduced by chronic administration of LH-RH agonists (23, 27, 65). Recently Vickery *et al.* showed that implantation of pellets of D-Trp^6-LH-RH ethylamide decreased prostate size in geriatric dogs (81).

In view of the very encouraging results with agonistic analogs of LH-RH in the treatment of prostatic carcinoma in men (21–32, 41–43), similar approaches could be tried in prostate hyperplasia. The aim of such trials would be to evaluate the efficacy of agonists and antagonists of LH-RH in decreasing the size of the hypertrophied prostate gland, thus avoiding the need for surgery.

2. Breast Cancer. Breast carcinoma is the most common type of tumor in women over 40 years of age and a leading cause of deaths from malignancies among females in the U.S. and other countries. It is well established that breast cancer can be hormone-dependent, as evidenced by regression following endocrine manipulations, or hormone-independent (82–92). Endocrine manipulations, employed for the treatment of metastatic breast carcinoma include oophorectomy, hypophysectomy, adrenalectomy, and treatment with androgens, progestins, aminoglutethimide, glucocorticoids, and antiestrogens such as tamoxifen. Tumor regression occurs in approximately 30–40% of all breast cancer patients following endocrine ablation or hormonal therapy (84), thus reinforcing the view that a certain proportion of these tumors are hormone dependent.

The presence of estrogen and prolactin receptors in mammary tumors, including human breast cancer cells, also supports the concept that they can be estrogen or prolactin dependent (83, 85–92). In cases where the estrogen receptor test is positive, endocrine therapy can be applied (87–91). The status of receptors for estrogen and progesterone may predict the response to endocrine therapy, since there is a correlation between the levels of these receptors and the likelihood of a favorable response (89–91). Various studies also support the hypothesis that prolactin may play a role in the growth of breast neoplasms, not only in rodents but also in humans (84–86, 92–94). In addition, it has been suggested that growth hormone and somatomedins may be involved in the growth of human breast cancer (95).

Several hormone dependent mammary tumors developed in the rat and mouse are widely used as models of human breast cancer (86, 96–98). The growth of a large proportion of mammary tumors induced in the rat by 7,12-dimethylbenz(a)anthracene (DMBA) is markedly influenced by the endocrine status of the animal (94). This tumor has been reported to be prolactin as well as estrogen dependent. Estrogen-, progesterone-, glucocorticoid-, prolactin-, and more recently androgen-binding components have been found in this experimental mammary tumor. The finding of specific hormone-binding receptors, combined with the effect of hormone therapy on the growth and development of the DMBA-induced mammary tumor in rats, indicate that this neoplastic tissue represents a suitable model of human hormone-dependent breast cancer (82). In addition, there are several other good animal models of mammary cancer, among them MT/W9A tumors in rats and MXT tumors in mice.

The MT/W9A mammary tumor, a subline developed from the mammotropin-dependent MT/W9 mammary tumor, has been characterized as estrogen dependent, and although it does not require extraneous prolactin, physiological levels of both prolactin and estrogen are still necessary for growth (96). Another good mammary tumor model is the mouse MXT tumor. This tumor has been regarded as an improvement over the DMBA tumor model since histologically, it is a papillary

ductal carcinoma closely resembling human carcinomas, which are also of ductal origin. Originally, this tumor arose in urethan-treated BD2F-1 female mice carrying a pituitary isograft under the renal capsule. This tumor has been characterized as estrogen-dependent and estrogen-receptor positive (99).

Inhibition of animal models of mammary tumors by agonistic and antagonistic analogs of LH-RH. A new endocrine therapy for breast carcinoma could be based on the use of potent agonistic or antagonistic analogs of LH-RH (1–5, 10, 16, 17).

Chronic administration of superactive agonistic analogs of LH-RH induces paradoxical inhibitory effects on pituitary gonadotrophs and gonads in female animals analogous to those reported in males and discussed above (1–5, 16). Among the effects reported in female rats are: delay in vaginal opening, reduction in ovarian and uterine weight, interference with mating and implantation, termination of gestation, and a fall in ovarian receptors for LH/hCG (1–5, 57, 58, 60). These antifertility effects, which occur in response to treatment with LH-RH agonists, have been observed in females of other species, including primate and human (60,100), but not in mice (101). Various groups have demonstrated a regression in the growth of DMBA-induced mammary carcinoma in rats after administration of superactive LH-RH agonists D-Leu-6-LH-RH-ethylamide and I.C.I. 118630 (33–37). We have used D-Trp-6-LH-RH in rat and mouse models of endocrine-dependent mammary tumors (17). Tumor weights in BDF-1 mice bearing the MXT mammary adenocarcinoma were significantly decreased after administration of D-Trp-6-LH-RH for 21 days (17). Final tumor volume was also reduced by 47%. Treatment with D-Trp-6-LH-RH lowered plasma progesterone, but not to ovariectomy levels. Since mice are reputedly refractory to inhibitory effects of LH-RH agonists (101), it is possible that D-Trp-6-LH-RH induced mammary tumor regression by some other mechanism.

In Wistar–Furth rats bearing the MT/W9A mammary adenocarcinoma, treatment with D-Trp-6-LH-RH for 28 days significantly decreased tumor weights by more than 50% and tumor volume by 67% (17). The percentage change in tumor volume (−51%), based on individual responses, was also highly significant when compared to controls. Administration of D-Trp^6-LH-RH significantly decreased progesterone levels by 74% and estradiol by 42%. Serum LH did not fall appreciably, but prolactin levels were suppressed by 70% as compared to control rats (17). A small number of rats were ovariectomized after transplantation of the tumor to test for estrogen dependency of the carcinoma. After 28 days tumor weights were decreased by more than 90%, and in some rats the tumor had disappeared.

The estrogen-dependent MT/W9A mammary tumor requires physiological levels of prolactin for its growth. Progesterone may also have a primarily stimulatory role. Regression of this tumor may be due mainly to the suppressive effects of D-Trp-6-LH-RH on estrogen and progesterone levels (17), and is in agreement with results obtained with other agonists of LH-RH in the DMBA rat model (33–37). A direct action of the agonist D-Trp-6-LH-RH on tumor tissue itself also cannot be ruled out. However, it has not been determined whether LH-RH-like receptors exist in mammary tumor tissue. The regression of mammary tumors in rats and mice after chronic administration of agonistic analogs of LH-RH suggests that they could be tried for treatment of breast carcinoma in premenopausal women.

Two powerful antagonistic analogs of LH-RH, *N*-Ac-D-*p*-Cl-Phe-1,2,Phe-3,D-Arg-6,D-Ala-10-LH-RH and *N*-Ac-D-*p*-Cl-Phe-1,2,D-Trp-3,D-Arg-6,D-Ala-10-LH-RH (Antagonist I), were also tested by us in rat and mouse models of endocrine-dependent mammary tumors (17). Administration of Ac-D-*p*-Cl-Phe-1,2,Phe-3,D-Arg-6,D-Ala-10-LH-RH for 3 weeks significantly reduced the weight and volume of estrogen-dependent MXT mammary tumors in BDF1 mice. Ovarian weights and plasma levels of progesterone were also diminished after treatment with this antagonist.

N-Ac-D-*p*-Cl-Phe-1,2,D-Trp-3,D-Arg-6,D-Ala-10-LH-RH, administered for 4 weeks to Wistar–Furth rats, reduced the weight and volume of MT/W9A mammary tumor by 58 and 42%, respectively (17). The percentage change in tumor volume (−40%) was similarly highly significant after treatment with the an-

tagonist. LH, estrogen, and progesterone levels were also greatly reduced in rats treated with the antagonist. Suppression of tumor growth by antagonists is most likely linked with inhibition of the levels of sex steroids, but some direct action of the antagonists on mammary tumors cannot be excluded. The modern antagonist *N*-Ac-D-*p*-Cl-Phe-1,2,D-Trp-3,D-Arg-6,D-Ala-10-LH-RH, used in our study, is considerably more potent in blocking ovulation than analogs used previously, and active when administered orally to rats (10, 76).

The use of antagonistic analogs of LH-RH for the treatment of breast cancer would avoid the transient stimulation of the release of gonadotropins and estrogens which occurs initially in response to LH-RH agonists, thus preventing the temporary clinical flare-up of the disease. However, LH-RH antagonists have been tried so far only in acute studies in human beings aimed at contraception (1–5, 10, 77), and clinical data are not yet available on the effects of chronic administration. Large scale synthesis and toxicological investigations will permit a clinical evaluation of *N*-Ac-D-*p*-Cl-Phe-1,2,D-Trp-3,D-Arg-6,D-Ala-10-LH-RH in the treatment of breast cancer, and a comparison of its therapeutic efficacy with D-Trp-6-LH-RH and other agonists. Future experimental studies in animals will be made more convenient by the development of injectable microcapsules with D-Trp-6-LH-RH and with *N*-Ac-D-*p*-Cl-Phe-1,2,D-Trp-3,D-Arg-6,D-Ala-10-LH-RH, designed for a constant, controlled release of these analogs over a 30-day period (10).

Clinical trials with LH-RH analogs in women with breast cancer. The responses of postmenopausal women with metastatic breast cancer to therapy with LH-RH agonists have been disappointing (14, 38, 39). Nevertheless, occasional responses have been recorded. Recently Mathé *et al.* reported 70% regression of tumor mass and disappearance of the axillary lymph node metastases in a postmenopausal patient treated with D-Trp-6-LH-RH for 3 months (cited in (14)). This patient had a diagnosis of bilateral breast cancer with pulmonary metastases and was previously treated with local irradiation and tamoxifen, but had a relapse (14). On the basis of our experimental work (17), we forecast that premenopausal patients with breast cancer should respond better to the LH-RH analogs then postmenopausal (13, 14). Recent preliminary results are in agreement with this view. Klijn and de Jong (39) reported that two of four premenopausal women with metastatic breast cancer improved after 7 weeks of treatment with LH-RH agonist HOE 766 (Buserelin). In another study, in which the Leuprolide analog of LH-RH was used, some clinical improvement in about 40% of premenopausal women with breast cancer was also detected (38).

It is likely that antagonistic analogs of LH-RH may prove to be superior to agonists in inhibiting mammary tumors, and thus lead to a better regression of breast cancer in premenopausal and perhaps even postmenopausal patients. For instance, autoradiograms of polyacrylamide gel electrophoresis of proteins from MT/W9A mammary tumors indicate a greater suppression of phosphorylation in regressing tumors of rats treated with the LH-RH Antagonist I than with the agonist D-Trp-6-LH-RH (102). The inhibition of phosphorylation in our experiments is in good agreement with previous observations by Cho-Chung and Redler (103) on phosphorylation of proteins after ovariectomy-induced tumor regression in the DMBA model.

The use of microcapsules would make the treatment of breast cancer with LH-RH agonists or antagonists more practical and convenient, and would also better ensure patient compliance. It is also possible that treatment with LH-RH agonists or antagonists can be combined with chemotherapy or with tamoxifen. Thus, simultaneous administration of one of the cytotoxic drugs and antagonists or agonists of LH-RH, early in the diagnosis of breast cancer could enhance the therapeutic response. Similar considerations could apply to tamoxifen.

Inhibition of prolactin release by peptides with prolactin release-inhibiting factor (PIF) activity may be beneficial for the treatment of mammary carcinoma, since prolactin may be a cofactor in the development or growth of mammary tumors (84–86, 92–94, 104). It has also been reported recently that physiological concentrations of prolactin and pharmacological quantities of GH can promote the growth of human breast tumor cells in culture (93). Thus reduction in prolactin levels, induced by administration of a suitable so-

matostatin analog or a peptide with PIF activity, combined with the decrease in estrogen values which results from chronic treatment with LH-RH agonist or antagonist, may lead to a greater inhibition of mammary tumors than that which can be obtained with LH-RH analog alone. Peptides which inhibit prolactin secretion could be tried as adjuncts together with agonistic or antagonistic analogs of LH-RH in the treatment of breast cancer. The administration of somatostatin analogs would also decrease the levels of GH and this might also contribute to an additional inhibition of tumor growth. Development of a new method of treatment for breast cancer in women based on agonistic or antagonistic analogs of LH-RH might reduce the need for mastectomies and/or increase the survival rate of some patients with hormone-sensitive tumors. At any rate, this method might supplement more conventional procedures for the treatment of hormone-dependent breast cancer.

3. Pituitary Tumors. *Introduction.* Pituitary tumors are common lesions which are presently treated by neurosurgery (transsphenoidal or transfrontal hypophysectomy or selective microadenomectomy) (105, 106), radiotherapy, or dopamine agonists such as 2-bromo-α-ergocriptine (CB 154; Bromocriptine), Lisuride, and Pergolide. Some tumors are not completely resectable by neurosurgery and do not always respond to radiation therapy.

Hormones secreted by functional pituitary tumors most commonly include prolactin, growth hormone, and ACTH; the overproduction of LH, FSH, and TSH is rare. Some pituitary tumors can be nonfunctional. Bromocriptine is particularly effective for functional tumors, prolactinomas characterized by amenorrhea–galactorrhea, and to a lesser extent for tumors associated with acromegaly. Treatment with bromocriptine frequently fails to normalize the hGH level in acromegaly (107, 108). Occasionally, plasma levels of prolactin and growth hormone rise during treatment despite increases in the dosage of bromocriptine.

There are various experimental animal models of pituitary tumors (40, 110–113). Transplantable pituitary tumors originated by J. Furth provide well-established models (110, 111). Prolactin- and GH-secreting pituitary tumors produced in rats by administration of estrogens or irradiation are estrogen dependent. MtT-F4 transplantable pituitary tumor secretes ACTH, prolactin, and GH (112). Transplantable GH_3 pituitary tumor, a clonal subline of MtT/W5 tumor produces both GH and prolactin (113). The growth of prolactin and ACTH-secreting pituitary tumors 7315a in rats is estrogen dependent and can be inhibited by the antiestrogen tamoxifen but not by bromocriptine (40). There are also MtT/W10 pituitary tumors (steroid independent) in rats and MtT prolactin-producing tumors in mice.

Various basic studies in animals have demonstrated that somatostatin-14 (S-S-14), its analogs, and somatostatin-28 (S-S-28), decrease the release of GH, TSH, prolactin, and other hormones (4, 5, 114). Another approach for inhibiting pituitary tumors could be based on the use of potent agonistic or antagonistic analogs of LH-RH (13, 14, 20, 40). Lambert *et al.* (40) have shown recently that chronic administration of an LH-RH agonist (I.C.I. 118,630) inhibited the growth of estrogen-dependent pituitary tumor 7315a in rats. The results of our studies (20) are in agreement with their findings.

We have investigated the effects of D-Trp-6-LH-RH (agonistic analog), *N*-Ac-D-*p*-Cl-Phe-1,2,D-Trp-3,D-Phe-6,D-Ala-10-LH-RH (antagonistic analog) and D-5-methoxy-Trp-8-somatostatin (somatostatin analog) on the growth of the prolactin (PRL)- and ACTH-secreting pituitary tumor 7315a in female Buffalo rats (20). Chronic administration of D-Trp-6-LH-RH starting 18 days after inoculation with the tumor, inhibited the growth of transplantable prolactin-secreting pituitary tumor (20). Tumor weight and volume were also reduced when this analog was administered 3 days after inoculation. The antagonistic LH-RH analog injected for 14 to 24 days also significantly inhibited pituitary tumor growth. Chronic administration of the somatostatin analog likewise decreased tumor weights in comparison with controls. The inhibition of pituitary tumor growth by LH-RH agonist, antagonist and somatostatin analog was accompanied by a decrease in serum prolactin levels. It was concluded that LH-RH agonist, antagonist, and somatostatin analog can inhibit the growth of estrogen-dependent PRL/ACTH-secreting pituitary tumor in rats (20).

In rats bearing pituitary tumor GH_3, daily administration of D-Trp-6-LH-RH for 7 weeks produced more than 90% decrease in tumor weight and volume when compared to controls (115). Pituitary and serum PRL concentrations, and serum GH levels were greatly reduced in these animals. It is possible that these findings (13, 20, 40, 115) could be of some clinical significance.

Several clinical studies in normal subjects have clearly shown that S-S-14, S-S-28, and analogs of somatostatin-14 can depress the release of growth hormone. Under certain conditions (hypothyroids, Nelson's syndrome) the secretion of TSH, prolactin, and ACTH were also inhibited (4–6, 116, 117). It has also been clearly documented that GH levels in acromegalic patients are markedly reduced during infusion of somatostatin-14, somatostatin-28, or analogs of somatostatin (4–6, 116–119). These results appear to indicate that somatostatin analogs affect pituitary adenoma cells and thus might be useful for the treatment of acromegaly. Moreover, some acromegalic patients, in spite of adenomectomy and bromocriptine treatment, still have elevated plasma hGH (120). During infusion of somatostatin analogs in these patients GH levels fell (120). Thus certain long-acting selective somatostatin analogs could inhibit both prolactin and growth hormone release and induce the suppression of some pituitary tumors. It cannot be excluded that a therapy based on analogs of LH-RH alone or combined with somatostatin analogs could be of value in the management of patients with pituitary tumors who have failed to respond to surgery, irradiation, and bromocriptine treatment.

4. Ovarian Cancer and Neoplasms of the Female Genital Tract. Ovarian cancer is the fourth leading cause of cancer deaths among American women (121) and kills more women than cervical and endometrial cancer combined. Ovarian cancer is not a single entity, but encompasses numerous cell types (121). The majority of ovarian cancers are of epithelial origin. The primary treatment of ovarian cancer is surgical removal (121), but chemotherapy is more commonly used today in the management of disseminated ovarian carcinoma because of unsatisfactory results following operation alone (121). Most common functioning ovarian neoplasms are referred to as granulosa–theca cell tumors (121, 122). These tumors produce estrogens, progesterone, and androgens (122) and account for 15–20% of all solid ovarian neoplasias. Castrated rodents with intrasplenic ovarian transplants have elevated levels of gonadotropins, and this results in a high rate of granulosa cell tumors of thecal cell origin in the transplanted ovaries (104, 123, 124). Animal models of granulosa cell tumors are dependent on hypophyseal gonadotropins for stimulation of hormone secretion, and concentrate ^{125}I-labeled HCG (125–127). A similar dependency on gonadotropins may occur in certain human ovarian tumors (125–127). In epithelial ovarian cancer, gonadotropins are not directly stimulatory to the ovarian epithelial cells, but these cells replicate after each ovulation to cover the exposed surface of the ovary (104). This hormone excess hypothesis, supported by epidemiological findings, indicates that interruption of the normal cyclic gonadotropin stimulation of the ovary, e.g., by pregnancy or oral contraceptives, would be protective and would decrease the risk of ovarian cancer (104). No animal model for epithelial ovarian tumors exists. However, human ovarian tumor tissue transplanted into the nude mouse or immune-deficient nude rat provides models to study the effect of hormones on the growth of this tumor (126, 127).

The most common neoplasm of the female genital tract is the uterine myoma (128). It is apparently hormone dependent and continues to grow to a varying degree during reproductive years, but regresses after menopause (128). Hysterectomy is the usual method of treatment, particularly in older women. Carcinoma of the uterine body is generally endometrial adenocarcinoma (128) and it occurs predominantly in the older age group. The important role estrogens play in the etiology of endometrial cancer has long been recognized (104). Early studies of women with endometrial cancer demonstrated evidence of high estrogen levels. The importance of estrogen is also suggested by studies in animals in which administration of estrogens has produced endometrial cancer (129). Epidemiological observations in postmenopausal women support this estrogen excess hypothesis. Postmenopausal estrogen replacement therapy might increase the risk of endometrial cancer. Numerous

comparative studies have confirmed this opinion that estrogen plays some role in the development of these tumors (130, 131). Progesterone and other progestins are therapeutically useful in treating endometrial carcinoma partially because of their antiestrogenic effect (104). There are several animal models of hormone-dependent uterine sarcomas. Uterine sarcomas have been produced in GR mice by the long-term administration of a combination of progesterone and estrone. These tumors retain their hormone dependency as tested by retransplantation (132). Other hormone-responsive mesenchymal tumors such as the Noble rat uterine leiomyoma are available (133). Human endometrial adenocarcinoma transplanted into nude mice retains its estrogen dependency.

In view of the evidence that gonadotropins and estrogens might increase the incidence of cancers of the ovary and endometrium, respectively, in animals and humans (104, 123–134), the effects of chronic administration of agonists and antagonists of LH-RH are being investigated in murine models and in animals bearing transplanted human tumors in order to determine if an inhibition of tumor growth can be obtained. The basis for this inhibition has been discussed in detail in the section on breast cancer. Suppression of the secretion of gonadotropins and a reduction in estrogens levels produced by chronic administration of LH-RH agonists and antagonists could on theoretical grounds inhibit the growth of some of these neoplasms.

This information from tests in animals should be useful in view of projected clinical use of agonistic and antagonistic analogs of LH-RH for the treatment of ovarian cancer, uterine myoma, and endometrial adenocarcinoma.

5. Chondrosarcomas and Osteosarcomas or Tumors of Cartilage and Bone. Sarcomas can be classified by the tissue of origin as either soft tissue or bone and cartilage tumors (135). The most common tumors of bone and cartilage of nonhematopoietic origin are osteogenic sarcomas (osteosarcomas), chondrosarcomas, chondromas, Ewing sarcomas, fibrosarcomas, and other sarcomas (135, 136). The usual treatment for sarcomas includes chemotherapy, radiotherapy, and radical surgical ablation, but survival rates are low (135–137). Moreover, chondrosarcomas are resistant to chemotherapy (135). The incidence curve of osteosarcoma peaks in adolescence and again in old age (104). Epidemiological findings suggest that the peak of osteosarcoma in adolescence is associated with the pattern of skeletal growth (104). In addition to growth hormone, thyroid hormones, androgens, and estrogens are also involved in skeletal growth. Johnson (138) proposed that the incidence of osteosarcomas is a function of the amount of cellular activity in the bones, and may be influenced by growth hormone. Osteosarcomas in adolescents occur most frequently in the epiphyses of long bones, sites of maximal bone growth (139). In older people, osteosarcomas often occur in conjunction with Paget's disease and excessive bone turnover (104, 139).

It is also recognized that hormonal factors play an important role in the growth and differentiation of normal cartilage tissue (18). Some murine sarcoma models, for instance C3H/HeJ osteosarcoma (137), and Swarm chondrosarcomas (140, 141), are similar to human tumors. Hormonal requirements of transplantable rat osteosarcoma have been well defined (142). Several groups have established that, as in the case of normal cartilage, chondrosarcoma chondrocytes are also hormone dependent (143–147). Swarm rat chondrosarcoma is a malignant, transplantable, hormone-responsive tumor whose growth is dependent upon GH or GH-induced serum factors (somatomedins), glucocorticoids, and insulin (143, 144). The Swarm chondrosarcoma is a cartilagenous tumor which spontaneously arose in a female Sprague–Dawley rat and was originally classified as an osteochondrosarcoma (140). Histochemically this tumor resembled a human osteogenic sarcoma, but in subsequent passage the bone components were lost and the cartilage-like consistency was retained (140, 141). Salomon *et al.* (143) reported that the growth of this chondrosarcoma was greatly reduced in adrenalectomized or hypophysectomized rats. Since cortisone administration restores the growth of this tumor in adrenalectomized rats, but ACTH or growth hormone are only partially effective in supporting tumor growth in hypophysectomized rats, chondrosarcoma appears to be dependent upon hormones from the adrenal and pituitary glands (143).

McCumbee *et al.* (144–146) demonstrated that GH and cortisone act synergistically to stimulate growth of this chondrosarcoma in hypophysectomized rats, and also showed that chondrosarcomas respond, *in vitro,* to GH-dependent serum growth factors (somatomedins) and insulin by an increase in amino acid transport and macromolecule synthesis (145). Recent biochemical data indicate that somatomedins stimulate both RNA and protein synthesis in chondrosarcoma tissue (147).

Inhibitory effect of analogs of hypothalamic hormones on growth of chondrosarcomas and osteosarcomas. Various basic studies in animals have demonstrated that somatostatin-14 (S-S-14), its analogs, and somatostatin-28 (S-S-28), decrease the release of GH, TSH, insulin, and other hormones (4, 5, 114). Several clinical studies in normal and acromegalic patients have clearly shown that somatostatin 14 and 28 and analogs of somatostatin 14 can depress the release of growth hormone, insulin, and under certain conditions also the secretion of prolactin and ACTH (6, 116). The growth hormone dependence of rat chondrosarcomas prompted us to test experimentally the hypothesis that somatostatin analogs, by inhibiting the GH secretion, could have potential therapeutic implications in treatment of this neoplasm (18). D-Trp-6-LH-RH was also tried since agonists of LH-RH inhibit pituitary gonadotropin and sex hormone levels (1–4) and induce regression of prostate and mammary tumors (1–3, 11, 17). Various analogs of S-S-14 and S-S-28 were injected sc starting 3 days after transplanting Swarm chondrosarcoma into male Sprague–Dawley rats. Administration for 30 days of pNH_2-Phe^4-S-S or Val-Gly-Tyr-Val-Ile-Leu-Gly-S-S-28 significantly decreased tumor weights by 38% as compared to controls (18). Treatment of tumor rats with D-5F-Trp-8-S-S-14 for 14 to 21 days also significantly decreased tumor weights by 32–46% and tumor volume by 50–60% vs controls. S-S-28 given for 22 days decreased tumor weight by 38% and volume by 50% as compared to controls. D-5-Methoxy-Trp-8-S-S-14 decreased tumor volume by 27% as compared to controls (18). In three different experiments, D-Trp-6-LH-RH administered alone or with somatostatin analogs also significantly reduced the weight or volume or both of chondrosarcomas (18). GH and prolactin levels were significantly decreased in rats treated with D-5F-Trp-8-S-S or with D-Trp-6-LH-RH. There appeared to be a greater suppression of serum GH and prolactin levels when D-5F-Trp-8-S-S was administered together with D-Trp-6-LH-RH (18). The inhibitory effect of analogs of somatostatin-14 and somatostatin-28 on the growth of chondrosarcomas can most likely be explained by suppression of GH levels. It is also possible that some somatostatin analogs act directly on tumor tissue. D-Trp-6-LH-RH appears to have exerted its action by inhibiting the GH and prolactin level and/or the adrenal. Two experiments were also carried out in mice with Dunn osteosarcomas. In the first experiment, L-5F-Trp-8-D-Cys-14-S-S and D-Trp-6-LH-RH prolonged the survival rate by 86 and 29%, respectively (14). In the second experiment, the prolongation of survival of mice with Dunn osteosarcomas was 37% in the case of D-Trp-6-LH-RH and 73% for L-5Br-Trp-8-D-Cys-14-S-S (14). It is possible that growth of other sarcomas (148, 149) could be inhibited by these analogs. Growth inhibition of the Swarm chondrosarcomas and increase in survival rate in Dunn osteosarcomas by administration of analogs of somatostatin and LH-RH is encouraging (18) and suggests that they might lead to a new endocrine therapy for chondrosarcomas, osteosarcomas and related hormone-dependent neoplasias. Future experimental work and eventual treatment of patients should be made more convenient by the development of injectable microcapsules with D-Trp-6-LH-RH and somatostatin analogs for once a month administration. In patients with osteogenic malignancies where conventional therapy has failed, treatment with analogs of somatostatin alone or combined with D-Trp-6-LH-RH could perhaps be of value.

6. Pancreatic Tumors. Carcinoma is the most common neoplasm of the pancreas, causing over 20,000, or 5% of all cancer deaths per year in the U.S. Malignant exocrine tumors arise most frequently from the ducts and thus about 70–75% of carcinomas are in the head of the pancreas (150–152). About 25–30% of tumors arise in the body or tail of the pancreas (151, 152). Histologically pancreatic cancer is most commonly of ductal or acinar type, the former constituting about 80–90% of cases. Carcinoma of the head of the pancreas

has a poor prognosis and the 5-year survival rate is only 2 to 5%. The resectability rate is about 15 to 20%. Radiotherapy and chemotherapy are usually ineffective (150, 152). Tumors of the endocrine pancreas (islet-cell tumors), may be benign or malignant and they may be functioning or nonfunctioning (153). There is a variety of pancreatic endocrine (or neurocrine) tumors (153, 154). At least five different endocrine cell types have been recognized in the pancreas which elaborate polypeptides and amines (serotonin), and an occasional overproduction of some hormones by tumors, causing syndromes, would not be unexpected. These tumors are thus normally classified according to their secretory products, although some secrete more than one product. Entopic endocrine tumors include insulinomas, glucagonomas, somatostatinomas, and pancreatic polypeptide (PP) islet cell tumors (PPomas, PP apudomas) (154). Carcinoid tumors of the pancreas can elaborate various polypeptides of the APUD system in addition to serotonin or another amine (154). Among the chief ectopic endocrine tumors of the pancreas are gastrinomas associated with Zollinger–Ellison syndrome and vasoactive intestinal polypeptide (VIP)-producing tumors (VIPomas) which result in Verner–Morrison watery diarrhea syndrome. Other ectopic tumors of the pancreas can produce ACTH and parathyroid hormone or multiple hormones. The treatment for Zollinger–Ellison syndrome is usually gastrectomy (154) or administration of receptor blockers such as cimetidine. Complete surgical excision of other resectable endocrine tumors of the pancreas is the treatment of choice, but in many patients such treatment is not possible or may ultimately fail (154). Various drugs and specific chemotherapeutic agents such as streptozotocin, 5-fluorouracil, adriamycin, or irradiation are also used for the management of endocrine pancreatic tumors (153), but the overall results have been disappointing. Various experimental and clinical findings suggest that it might be possible to develop a new hormonal therapy for some malignant tumors of the pancreas based on somatostatin analogs. Thus, studies carried out during the past 10 years in several species, including humans, have shown that somatostatin and its analogs exert inhibitory actions on the endocrine and exocrine pancreas as well as on the stomach and gut (4–6). These actions include inhibition of the release of insulin and glucagon from the pancreas and suppression of the secretion and/or action of gastrin, secretin, cholecystokinin (CCK), and VIP (4, 154, 155). Gastrin, CCK, and secretin promote the growth of the exocrine pancreas and increase DNA, RNA and protein content, in addition to stimulating gastric and pancreatic (enzyme and water plus bicarbonate) secretions, respectively (156). The role these gastrointestinal hormones play in the growth of pancreatic cancer is not clearly established, but it is likely that they influence the growth of malignant cells of the pancreas as well (156, 157).

Several *in vitro* studies have shown that CCK, secretin, and gastrin can stimulate the growth of pancreatic adenocarcinoma cells, duodenal cells, gastric mucosa cells, and rat stomach cancer cells in tissue culture (157–161). Townsend *et al.* have shown that caerulein, which is structurally closely related to CCK, given in high doses together with secretin, stimulated the *in vivo* growth of hamster pancreatic ductal adenocarcinoma (157). Clinical studies have demonstrated that infusion of somatostatin inhibited the secretion of insulin in patients with insulinomas and of glucagon in a case of glucagonoma (162, 163, 4–6). The secretion of ectopic endocrine tumors of the pancreas can also be inhibited by somatostatin. Suppression by somatostatin of gastrin secreted from a pancreatic tumor in Zollinger–Ellison syndrome was accompanied by a marked fall in gastric acid secretion (164). Similarly, inhibition of vasoactive intestinal polypeptide secretion from a pancreatic tumor in the Verner–Morrison syndrome by infusion of somatostatin has been reported (165, 166). Although these studies have demonstrated a temporary suppression of endocrine pancreatic tumor activity by infusion of somatostatin or its analogs, the substances used were not selective enough in their actions and have not been tried chronically in patients with carcinoma of the pancreas. Development of selective superactive analogs of somatostatin and controlled delivery systems for once a month administration may be required for this approach.

Sex steroids may also play a role in the growth of the normal and cancerous pancreas

(167–171). The incidence of carcinoma of the pancreas is greater in the male, indicating that this neoplasm could be in part androgen dependent. The presence of specific membrane or intracellular hormone receptors for estrogen and androgen in pancreatic cell fractions indicates that sex hormones could exert some influence on neoplastic cell processes (167–171). All these findings suggest that pancreatic adenocarcinoma may also be sensitive to sex hormones.

Recent development of animal models of pancreatic cancer has permitted experimental studies on this topic. Longenecker *et al.* (172, 173) described the generation of acinar cancer of pancreas in Wistar–Lewis rats by administration of azaserine. Pour *et al.* (174) reported the development of ductal pancreatic neoplasms in Syrian hamsters after single administration of carcinogen *N*-Nitroso-2-methoxy-2,6-dimethyl-morpholine (MeNDMM). A ductal pancreatic adenocarcinoma model has also been developed by Scarpelli *et al.* (175) by chronic administration of *N*-nitrobis (2-oxopropyl) amine to inbred Syrian golden hamsters. Other transplantable models of pancreatic adenocarcinoma have also been reported in the Syrian hamster (WDPaCa and PD PaCa tumors) (176). Pancreatic carcinomas induced in rats (172, 173) and in hamsters (174, 175) provide models of pancreatic acinar and ductal tumors with certain similarities to human tumors.

Using animal models of pancreatic cancer with acinar and ductal phenotypic characteristics, we investigated the effect of analogs of hypothalamic hormones on the growth of pancreatic tumors (19). In Wistar–Lewis rats bearing the acinar pancreatic tumors DNCP-322, chronic administration of L-5Br-Trp-8-S-S, a powerful analog of somatostatin-14, significantly decreased tumor weights and volume. Somatostatin-28 and the cyclic hexapeptide of somatostatin, cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe) (177) failed to influence the growth of this tumor (19). The agonistic analog D-Trp-6-LH-RH also significantly decreased tumor weight and volume and significantly reduced testes and ventral prostate weights and testosterone levels. In Syrian hamsters bearing ductal form of pancreatic cancer, chronic administration of L-5-Br-Trp-8-S-S for 21–30 days diminished tumor weights and volume (19). The percentage change in tumor volume was significantly decreased when compared to control animals. In one experiment cyclic hexapeptide of somatostatin (177) also inhibited growth of this tumor. D-Trp-6-LH-RH, given twice daily or injected in the form of controlled-release microcapsules, significantly decreased tumor weight and volume, reduced the weights of the testes, and suppressed serum levels of testosterone (19). Hamsters castrated 4 days after transplantation of the pancreatic tumor also showed a significant decrease in tumor weight and volume. This suggests that pancreatic cancers may at least in part be sex-hormone sensitive (19). D-Trp-6-LH-RH may decrease the growth of pancreatic carcinomas by eliminating the stimulatory effect of androgens. Somatostatin analogs reduce the growth of pancreatic ductal and acinar cancers, probably by inhibiting the release and/or stimulatory action of gastrointestinal hormones on tumor cells (155, 178). A combined administration of a suitable somatostatin analog with LH-RH agonist may lead to a greater inhibition of cancers of the pancreas than that which can be obtained with somatostatin analogs alone. The inhibition of the growth of ductal and acinar pancreatic tumors by D-Trp-6-LH-RH or analogs of somatostatin obtained in our laboratory appears to be the first attempt at endocrine management of these tumors. The observations that chronic administration of analogs of somatostatin and D-Trp-6-LH-RH inhibit the growth of the rat and hamster pancreatic cancers could be of clinical significance. Inhibition of animal models of pancreatic tumors in rats and hamsters in response to chronic administration of somatostatin analogs and D-Trp-6-LH-RH suggests that these compounds should be considered for the development of a new hormonal therapy for cancer of the pancreas (19).

7. Hypothalamic Antimitogenic (Cell-Growth-Inhibiting) Factor. Cell growth is regulated by a multiplicity of stimulatory, inhibitory, and synergistic hormones and growth factors (179, 180). Several polypeptides present in various tissues may regulate cell growth rate, but the mechanisms of this regulation are incompletely understood (181, 182). Fibroblast growth factor (FGF), a basic polypeptide isolated from brain and pituitary, is a potent mitogen for mesoderm-derived cells,

while epidermal growth factor (EGF), an acidic polypeptide isolated from mouse submaxillary glands, stimulates the proliferation of ectoderm-derived cells (181, 182). A peptide isolated from bovine placenta was reported to inhibit thymidine incorporation in DNA and tumor growth to a greater extent in tumors than in normal cells (183). Continuing our investigations on the effects of hypothalamic substances on cancer, we have examined the influence of hypothalamic fractions on cell proliferation. We have demonstrated that a peptide present in acetic acid extracts of porcine hypothalami can inhibit the growth of cells in several normal and neoplastic cell lines (184). This peptide inhibited [^{3}H]thymidine incorporation into cellular DNA as well as the incorporation of amino acids into proteins and of [^{3}H]uridine into RNA (184). A reduction in 3T6 fibroblast cell numbers was also observed (184). An antimitogenic factor of natural origin might find a possible application in the treatment of some neoplasms, as it could provide an alternative to chemotherapeutic agents, most of which have deleterious side effects. Further work might show, whether in addition to the recently developed treatment of endocrine dependent tumors with synthetic analogs of hypothalamic hormones, another approach based on this naturally occurring hypothalamic substance could prove feasible. This substance might possibly be effective against endocrine-dependent as well as hormone-insensitive cancers.

8. Potential Use of Analogs of LH-RH for Protection against Gonadal Damage during Chemotherapy. Gonadal dysfunction with sterility in patients who received chemotherapy for cancer is a significant problem (185–196). Many chemotherapeutic agents have been shown to cause variable degrees of gonadal dysfunction in both sexes and in all age groups (185). Among the commonly used antineoplastic drugs, alkylating agents, especially chlorambucil and cyclophosphamide have been most frequently associated with the development of infertility (186). Although lasting clinical remissions have been obtained in many patients with Hodgkin's disease, acute lymphoblastic leukemia, choriocarcinoma, testicular cancer, and the nephrotic syndrome, cytotoxic chemotherapy has resulted in gonadal dysfunction (186, 187). The severity of the dysfunction depends on the total drug dose and the age at time of therapy. In general, cytotoxic agents produce infertility in men while they produce premature menopause in women. Men develop azoospermia and compensated Leydig-cell function; women sustain ovarian damage causing impaired fertility in the short term, and early menopause later (186, 187, 193). It has been estimated, for example, that about 80% of males treated with combined chemotherapy for Hodgkin's disease become azoospermic or severely oligospermic, and that approximately 50% of females become amenorrheic (186, 187). The damage to the germinal epithelium may be greater than that to Leydig cells (186). There may be differences in the sensitivity of the prepubertal, pubertal, and adult testis to alkylating agent chemotherapy (185, 186). Dividing cells are known to be more sensitive to alkylating agents than resting cells. The prepubertal testis may be more resistant to the effects of alkylating agents than is the adult gonad (186). Prepubertal boys and boys in early puberty treated with cyclophosphamide for nephrosis appear to have normal FSH and LH levels after therapy, as well as normal levels of serum testosterone. This effect seems to be also dependent on the dosage and duration of therapy. (185, 194–196). In one study cyclophosphamide used for chemotherapy in a dose of 50–100 mg daily in adult males for 4 months induced azoospermia (190). In another study with cyclophosphamide in children with nephrosis, results showed two distinct patterns. Four males who received 2–4 mg/kg/day for 49 to 60 days had normal semen, while four patients who received 2–5 mg/kg/day for 89 to 489 days showed azoospermia (196). Although it still has not been established whether temporary suppression of spermatogenesis and ovarian function during chemotherapy would have any protective effect against the development of testicular and ovarian failure, LH-RH analogs are being tried in animals in an attempt to test this hypothesis. Extensive experimental work reviewed in Sections 1 and 2 indicates that LH-RH agonists and antagonists effectively but reversibly inhibit the pituitary–gonadal function and fertility in animals (1–3, 58–61, 66, 72, 73, 100, 101). Chronic administration of superactive agonists of LH-RH produces dramatic inhib-

itory effects through a process of "down regulation" of the pituitary gonadotrophs and gonads (1–3, 58–61, 101). In male animals this is manifested by a fall in the weights of testes, seminal vesicles and prostate, inhibition of spermatogenesis, and a decrease in plasma LH, FSH, and testosterone levels (1–5, 59, 61–64). In men a persistent suppression of Leydig-cell function, manifested by a fall in serum testosterone, dihydrotestosterone, and estradiol levels and inhibition of spermatogenesis have been observed after chronic administration of D-Trp-6-LH-RH or D-Ser(Bu^t)^{6}des-Gly-NH$_2^{10}$-LH-RH ethylamide (1–5, 22, 66, 69). Among the effects reported in female rats are: delay in vaginal opening, reduction in ovarian and uterine weight, interference with mating and implantation, and termination of gestation (1–5, 16, 58, 60). These antifertility effects of LH-RH agonists, have been observed in females of other species, including primate and human (100). The inhibition of reproductive functions in animals and human beings of both sexes by agonists of LH-RH is reversible and fertility is restored after the administration is stopped (1–5, 16, 60, 66, 100). The recovery of spermatogenesis in men occurs 10 to 14 weeks after cessation of treatment (66) and in women normal cycles resume rapidly (100). In male rats administration of LH-RH antagonists has been shown to decrease testosterone production and reduce the weights of testes and accessory sex organs and inhibit spermatogenesis (1–5, 10, 72, 73). In male rats (72), fertility is restored 4–8 weeks after the last injection. In women and subhuman primates LH-RH antagonists disrupt the normal events of the menstrual cycle, block ovulation and inhibit the rise in LH and FSH induced by oophorectomy (1–5, 10, 77). The inhibitory effect is nullified one to two cycles after the last injection (10). The working hypothesis is that administration of LH-RH agonists or antagonists for a few weeks before the therapy with antineoplastic agents is started, during the chemotherapy, and for about 2 weeks after it, would suppress ovarian or testicular function and maintain inactive gonads. Consequently chemotherapy might not damage the testes and ovaries temporarily suppressed by LH-RH analogs to the extent it does in the case of the unsuppressed gonads. The use of microcapsules would make the eventual treatment with LH-RH agonists or antagonists practical and convenient. Pretreatment with antagonistic and agonistic analogs of LH-RH of patients about to undergo chemotherapy with alkylating agents could decrease testicular and ovarian damage frequently caused by these drugs.

Recently Glode *et al.* (197) reported that temporary inhibition of the pituitary–gonadal axis by administration of [D-Leu-6]-LH-RH ethylamide induced protection from cyclophosphamide-induced testicular damage in mice (197). However, the conclusions were based on the survival of two treated mice (185, 197) and more work is needed to confirm and extend these findings.

Summary. A new approach to the treatment of endocrine-dependent tumors based on analogs of hypothalamic hormones is in the early stages of development, but appears promising and significant.

Administration of hypothalamic hormones can mimic hypophysectomy and gonadectomy, and is essentially devoid of side effects. A successful use of agonistic analogs of LH-RH for treatment of endocrine-dependent prostate cancer has been documented in several hundred patients. Experimental studies suggest that agonists and/or antagonists of LH-RH might be useful for treatment of breast cancer and pituitary tumors. Our work in animal models also indicates that analogs of somatostatin, alone or combined with LH-RH agonists, could be considered for therapy of chondrosarcomas, osteosarcomas, and pancreatic cancer. Experiments are in progress on the use of LH-RH analogs for treatment of ovarian cancer, neoplasms of the female genital tract, and for protection against gonadal damage during chemotherapy. These investigations should extend the concepts of endocrine treatment of cancers.

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