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Inhibition of growth of a prolactin-secreting pituitary tumor in rats by analogs of luteinizing hormone-releasing hormone and somatostatin

(pituitary tumor/tumor weight and volume reduction/analogs of hypothalamic hormones/somatostatin analogs)

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ABSTRACT **We investigated the effects of [D-Trp^6]LH-RH [agonistic analog of luteinizing hormone-releasing hormone (LH-RH)], *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 and corticotropin-secreting pituitary tumor 7315a in female Buffalo rats. Chronic administration of [D-Trp^6]LH-RH in a dose of 25 μg/day, starting 18 days after inoculation with the tumor, inhibited the growth of the pituitary tumor. Tumor weight and volume also were reduced when this agonist was administered 3 days after inoculation. The antagonistic LH-RH analog, injected in a dose of 50–100 μg for 14–24 days, also significantly inhibited the growth of pituitary tumor. Chronic administration of the somatostatin analog in a dose of 25 μg twice a day likewise decreased tumor weights in comparison with controls. The inhibition of pituitary tumor growth by LH-RH agonist, LH-RH antagonist, and somatostatin analog was accompanied by a decrease in serum prolactin levels. It was concluded that LH-RH agonist, LH-RH antagonist, and somatostatin analog can inhibit the growth of estrogen-dependent prolactin/corticotropin-secreting pituitary tumor in rats.**

Chronic administration of superactive long-acting analogs of luteinizing hormone-releasing hormone (LH-RH) induces a paradoxical inhibition of reproductive functions in both female and male animals (1–3). In human beings, repeated administration of agonistic LH–RH analogs can also decrease responsiveness of the pituitary gland and inhibit the secretion of gonadal steroids (4–6). These paradoxical inhibitory effects, induced by prolonged administration of LH-RH agonists, have been linked with a regression of the growth of mammary carcinomas (7, 8) and endocrine-dependent prostate tumors (9) in rats. In addition, Lamberts *et al.* (10) showed recently that chronic administration of an LH-RH agonist inhibited the growth of estrogen-dependent prolactin (PRL)- and corticotropin (ACTH)-secreting pituitary tumor 7315a in rats. It also has been demonstrated that antagonistic analogs of LH-RH suppress gonadotropin release in animals and human beings (11, 12) and decrease the growth of prostate tumors in rats (13).

Various basic and clinical studies have established that somatostatin and its analogs suppress the secretion of growth hormone, thyrotropin, and, under certain experimental or clinical conditions, PRL and ACTH (14, 15). In the present study, we investigated the effects of chronic administration of [D-Trp^6]LH-RH, an agonist of LH-RH, *N*-Ac-[D-*p*-Cl-Phe^{1,2},D-Trp^3,D-Phe^6,D-Ala^{10}]LH-RH, an antagonist of LH-RH, and a somatostatin analog ([D-5-methoxy-Trp^8]somatostatin) on the growth of the estrogen-dependent pituitary tumor 7315a in the rat.

MATERIALS AND METHODS

All three analogs were synthesized by solid-phase methods in our laboratory and repurified as reported (16, 17).

Female Buffalo rats (body weight, 120–180 g) were inoculated subcutaneously in the scapular region with PRL/ACTH-secreting pituitary tumor 7315a (obtained from R. M. MacLeod, Univ. of Virginia, Charlottesville). Three experiments were carried out. In experiment I, the rats were treated after they developed fully grown tumors. In experiments II and III, the rats were treated before they developed tumors.

Experiment I. Eighteen days after inoculation, 90% of the rats had developed the tumors. Tumor growth was measured with microcalipers, and volume was calculated according to the formula $L \times W \times H \times 0.5236$ described by Janik *et al.* (18). Tumor volume varied between 158 and 4,915 mm^3. Tumor-bearing rats were randomized according to tumor volume and divided into four groups of five to seven rats each. Group I (controls) received 0.2 ml of 10% polyvinylpyrrolidone (PVP) in 0.9% saline containing 0.01 M acetic acid. Group II was injected with 25 μg of [D-Trp^6]LH-RH in 10% PVP. Group III received 50 μg of inhibitory LH-RH analog (*N*-Ac-[D-*p*-Cl-Phe^{1,2},D-Phe^6,D-Ala^{10}]LH-RH) in 40% propylene glycol saline solution. Group IV was treated twice a day (b.i.d.) with 25 μg of [D-5-methoxy-Trp^8]somatostatin in saline containing 10% propylene glycol and 10% PVP. The analogs were administered subcutaneously, and the treatment was continued for 14 days.

Experiment II. The effect of early administration of analogs was investigated. The injections were started three days after inoculation with the tumor and continued for 24 days.

Experiment III. This experiment was similar to experiment II except that the dose of inhibitory analog of LH-RH was increased to 50 μg b.i.d. The treatment lasted 20 days.

The rats were sacrificed 60–240 min after the last injection. Trunk blood was collected and centrifuged, and serum was kept at −40°C. Uterus, ovaries, and adrenals were removed, cleaned, and weighed. Tumors were cleaned of adhering tissue, weighed, and frozen on dry ice. Serum levels of PRL were measured by a double antibody radioimmunoassay with materials supplied

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Abbreviations: LH-RH, luteinizing hormone-releasing hormone; PRL, prolactin; ACTH, corticotropin; PVP, polyvinylpyrrolidone; b.i.d., twice a day.

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Table 1. Effect of chronic administration of analogs of hypothalamic peptides* on tumor volume and weight and on cell doubling time of female rats bearing pituitary tumor 7315a

Treatment	Tumor volume, mm^3		% increase	Cell doubling time, days	Tumor weight, g
	Initial	Final			
Control, 10% PVP in saline	1,252 ± 531	17,007 ± 6,625	1,446 ± 171	3.79 ± 0.15	27.11 ± 4.04
[D-Trp^6]LH-RH, 25 μg/day	3,034.3 ± 887	9,619 ± 2,654	361.3 ± 97	9.11 ± 1.59	9.15 ± 0.67
P	NS	NS	<0.01	<0.05†	<0.01
LH-RH antagonist, 50 μg/day	3,048 ± 956	12,395 ± 4,173	510 ± 140.0	7.54 ± 1.76	11.9 ± 3.87
P	NS	NS	<0.01	NS	<0.01
Somatostatin analog, 25 μg b.i.d.	1,937 ± 932	7,038 ± 2,451	412 ± 120	9.63 ± 2.92	9.25 ± 2.80
P	NS	NS	<0.01	<0.05	<0.01

NS, not statistically significant.
*[D-Trp^6]LH-RH, inhibitory LH-RH analog (*N*-Ac-[D-*p*-Cl-Phe^{1,2}, D-Trp^3, D-Phe^6, D-Ala^{10}]LH-RH), and [D-5-methoxy-Trp^8]somatostatin for 14 days.
†Student's *t* test. All other *P* values were obtained by Duncan's new multiple range test.

by the National Hormone and Pituitary Program. All data were expressed as means ± SEM. Statistical analyses were made by using Duncan's new multiple range test (19) or Student's *t* test.

RESULTS

The effect of administration of [D-Trp^6]LH-RH, LH-RH antagonist, and somatostatin analog on the growth of the PRL-secreting pituitary tumor 7315a was first evaluated by starting the treatment 18 days after inoculation and continuing it for 14 days. Table 1 shows initial and final tumor volumes and weights. The percentage increase in tumor volume was significantly reduced ($P < 0.01$) by all three substances as compared with controls. Calculation of the estimated cell doubling time revealed that tumors of rats treated with [D-Trp^6]LH-RH required 9.11 ± 1.59 days ($P < 0.05$), and those treated with somatostatin analog required 9.63 ± 2.92 days ($P < 0.05$), compared with 3.79 days for tumors in control rats. Cell doubling time of tumors in rats treated with LH-RH inhibitor also was increased, but this change was not statistically significant. Tumor weights were significantly reduced by treatment with all three analogs, in comparison with controls. Table 2 shows that serum PRL levels in all treated animals were significantly lower ($P < 0.05$) as compared with those of control tumor rats. Body, pituitary, adrenal, and uterus weights were not affected, but ovarian weights of animals treated with [D-Trp^6]LH-RH and LH-RH antagonist were decreased ($P < 0.01$) (Table 2).

The effect of early treatment with [D-Trp^6]LH-RH, *N*-Ac-[D-*p*-Cl-Phe^{1,2}, D-Trp^3, D-Phe^6, D-Ala^{10}]LH-RH, and [D-5-methoxy-Trp^8]somatostatin was investigated in experiment II by starting injections 3 days after inoculation with the pituitary tumor. The incidence of tumors, final tumor volume and weights, ovarian and uterine weights, and PRL levels in every group are recorded in Table 3. In rats treated with [D-Trp^6]LH-RH, no tumors were palpable; however, some tumoral tissue was found in the scapular region, and the mean tumor weight was 0.12 ± 0.05 g ($P < 0.01$ vs. controls). The weights of the ovaries and uterus also were similarly diminished in the [D-Trp^6]LH-RH-injected group. Tumor weights also were decreased significantly in rats treated with LH-RH antagonist and somatostatin analog ($P < 0.05$ vs. control) (Table 3). In addition, administration of the LH-RH antagonist reduced ovarian weights. Body weights as well as pituitary and adrenal weights again were not affected by treatment with these analogs. Serum PRL levels

Table 2. Effect of chronic administration of analogs of hypothalamic peptides* on body and organ weights and serum prolactin levels in female rats bearing pituitary tumor 7315a

Treatment	Weights				Serum PRL, ng/ml
	Body, g		Ovaries, mg	Uterus, mg	
	Initial	Final			
Control, 10% PVP in saline	228.2 ± 6.81	241 ± 7.3	128.42 ± 20.75	406.96 ± 62.5	1,196.2 ± 83.7
[D-Trp^6]LH-RH, 25 μg/day	228.5 ± 5.2	239 ± 7.3	66.5 ± 7.15	327.0 ± 38.68	773.8 ± 37.2
P	NS	NS	<0.05†	NS	<0.05†
LH-RH antagonist, 50 μg/day	235.3 ± 5.8	244 ± 15.1	73.0 ± 7.95	307.15 ± 31.2	843.6 ± 105.4
P	NS	NS	<0.05†	NS	<0.05†
Somatostatin analog, 25 μg b.i.d.	223 ± 3.1	242 ± 7.8	110.7 ± 11.7	329.05 ± 43.02	889.8 ± 96.5
P	NS	NS	NS	NS	<0.05†

NS, not statistically significant.
*[D-Trp^6]LH-RH, inhibitory LH-RH analog (*N*-Ac-[D-*p*-Cl-Phe^{1,2}, D-Trp^3, D-Phe^6, D-Ala^{10}]LH-RH), and [D-5-methoxy-Trp^8]somatostatin for 14 days.
†*P* values were obtained by Duncan's new multiple range test.

Table 3. Effect of early administration of analogs of hypothalamic peptides* for 24 days on tumor incidence and volume, tumor and organ weight, and serum prolactin levels of female rats inoculated with pituitary tumor 7315a

Treatment	Incidence of tumors†	Final tumor volume, mm^3	Weights			Serum PRL, ng/ml
			Tumor, g	Ovaries, mg	Uterus, mg	
Control, 10% PVP in saline	70%	3,986 ± 1,875	5.20 ± 1.85	105.96 ± 8.58	297.64 ± 40.58	311 ± 97
[D-Trp^6]LH-RH, 25 μg/day	38%	0	0.12 ± 0.05	75.27 ± 6.47	130.6 ± 5.1	9.3 ± 0.0
P		<0.01‡	<0.01	<0.05	<0.01	<0.01
LH-RH antagonist, 50 μg/day	57%	1,515.3 ± 860.8	1.84 ± 0.88	78.5 ± 1.41	277.1 ± 38.9	73.3 ± 35
P		NS	<0.05	<0.05	NS	<0.05
Somatostatin analog, 25 μg b.i.d.	63%	954.3 ± 196	1.75 ± 0.60	100.32 ± 3.47	329.8 ± 47.19	76.0 ± 37
P		NS	<0.05	NS	NS	<0.01

NS, not statistically significant.
*[D-Trp^6]LH-RH, inhibitory LH-RH analog (*N*-Ac-[D-*p*-Cl-Phe^{1,2}, D-Trp^3, D-Phe^6, D-Ala^{10}]LH-RH), and [D-5-methoxy-Trp^8]somatostatin.
†Percentage of animals which developed the tumor.
‡*P* values vs. corresponding control by Duncan's new multiple range test.

were markedly decreased in animals treated with the LH-RH antagonist and the somatostatin analog ($P < 0.05$ and < 0.01, respectively). The lowest prolactin levels were encountered in the group treated with [D-Trp^6]LH-RH, presumably because the growth of pituitary tumor 7315a was inhibited in those rats.

In experiment III, [D-Trp^6]LH-RH, somatostatin analog, and higher concentrations (50 μg b.i.d.) of LH-RH antagonist were again administered 3 days after inoculation of rats with the tumor. The results are shown in Table 4. Tumor volume and tumor weight of animals treated with LH-RH antagonist were significantly decreased ($P < 0.05$) as compared with controls. Ovarian weights were also reduced ($P < 0.01$). [D-Trp^6]LH-RH and somatostatin analog again decreased tumor volume and weight ($P < 0.05$) compared with controls. Ovarian and uterine weights were diminished in rats treated with [D-Trp^6]LH-RH ($P < 0.01$), but body weight, pituitary, and adrenal weights were not changed by any of the three analogs. Serum prolactin levels were again significantly reduced in the groups treated with LH-RH agonist, LH-RH antagonist, and the somatostatin analog.

DISCUSSION

The results of the present study demonstrate that chronic administration of [D-Trp^6]LH-RH inhibits the growth of transplantable prolactin-secreting pituitary tumor 7315a if the injections are started 18 days after tumor inoculation. Tumor growth also can be reduced markedly if this analog is administered early after implantation. The mechanism by which the LH-RH agonist inhibits the growth of the PRL-secreting rat pituitary tumor appears to be related to a suppression of sex steroid levels. Chronic administration of [D-Trp^6]LH-RH or related LH-RH agonists lowers plasma estrogen and testosterone levels in animals and human beings (2, 3, 6). The inhibition of the levels of sex steroids by LH-RH agonists may be the main mechanism for the regression of mammary carcinomas (7, 8) and of prostate tumors in rats (9). Recently we also have demonstrated tumor growth inhibition in patients with prostatic carcinoma treated with LH-RH agonists (20). A potential usefulness of LH-RH analogs for treatment of sex-steroid-dependent tumors has been postulated (9, 20, 21).

It has been reported that the growth of a transplanted estrogen-dependent prolactin-secreting pituitary tumor 7315a can be diminished by administration of an LH-RH agonist (10). Tamoxifen, an antiestrogen, similarly inhibited the growth of this tumor (10, 22). The mechanisms by which LH-RH agonists and tamoxifen inhibit pituitary tumor growth seem to be different. Chronic administration of the LH-RH agonist results in chemical castration as evidenced by lowered estrogen levels and

Table 4. Effect of early administration of analogs of hypothalamic peptides* for 20 days on tumor volume, tumor and organ weight, and serum prolactin levels in female rats inoculated with pituitary tumor 7315a

Treatment†	Final tumor volume, mm^3	Weights			Serum PRL, ng/ml
		Tumor, g	Ovaries, mg	Uterus, mg	
Control, 10% PVP in saline	1,923.6 ± 566	3.46 ± 0.83	88.7 ± 6.7	282 ± 15	488 ± 27
[D-Trp^6]LH-RH, 25 μg b.i.d.	630.8 ± 411	1.38 ± 0.64	53.2 ± 3.8	170.9 ± 21	89.6 ± 27
P	<0.05‡	<0.05	<0.01	<0.01	<0.01
LH-RH antagonist, 50 μg b.i.d.	811.8 ± 350	1.47 ± 0.64	61.2 ± 8	293.6 ± 31	145 ± 61
P	<0.05	<0.05	<0.01	NS	<0.01
Somatostatin analog, 25 μg b.i.d.	763.8 ± 270	1.60 ± 0.48	75.9 ± 6.6	304.4 ± 19	144 ± 50
P	<0.05	<0.05	NS	NS	<0.01

NS, not statistically significant.
*[D-Trp^6]LH-RH, inhibitory LH-RH analog (*N*-Ac-[D-*p*-Cl-Phe^{1,2}, D-Trp^3, D-Phe^6, D-Ala^{10}]LH-RH), and [D-5-methoxy-Trp^8]somatostatin.
†Five to seven animals per group.
‡*P* values vs. corresponding control by Duncan's new multiple range test.

atrophy of the ovaries and uterus (10). Tamoxifen-treated animals did not show castration signs. Lamberts *et al.* (10) suggested that Tamoxifen inhibited tumor growth by blocking estrogen receptors.

Our studies show that antagonistic LH-RH analogs can also inhibit the growth of pituitary tumor 7315a. In addition, the LH-RH antagonist reduced the ovarian weights. Acute administration of LH-RH antagonist leads to an inhibition of LH and follicle-stimulating hormone release (11, 12, 14), similar to that which can be obtained by chronic therapy with LH-RH agonists (1–6, 20). Recently we have shown that antagonists of LH-RH can suppress the growth of prostate tumors in rats (13). The inhibition of prostate and pituitary tumors by LH-RH antagonists may be mainly due to a decrease in testosterone and estrogen levels, respectively, in analogy to the action of LH-RH agonists (9).

Experimental and clinical investigations have shown clearly that somatostatin and its analogs inhibit the release of growth hormone and thyrotropin and, under certain conditions, also the secretion of PRL and ACTH (14, 15, 23, 24). Although this suggests that somatostatin and its analogs might affect pituitary adenoma cells, the observations reported here that somatostatin analogs can inhibit the growth of PRL- and ACTH-secreting pituitary tumor 7315a are original. This phenomenon could perhaps be explained in part by suppressive effects of somatostatin or its analogs on normal and pathological pituitary cells and manifested by inhibition of growth hormone, PRL, and ACTH secretion. In any case, this mechanism is likely to be different from that of LH-RH analogs. Although bromocriptine has been used with some success for treatment of acromegaly (25), not all patients respond to this therapy. It is possible that the findings reported in this paper could be of clinical importance. It cannot be excluded that a new therapy based on analogs of LH-RH alone or combined with somatostatin analogs could be of value in the treatment of patients with pituitary tumors who failed to respond to surgery, irradiation, and bromocriptine treatment.

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1. Sandow, J., Von Rechenberg, W., Terzabek, G. & Stoll, W. (1978) *Fertil. Steril.* **30**, 205–209.
2. Cusan, L., Auclair, C., Belanger, A., Ferland, L., Kelly, P. A., Sequin, C. & Labrie, F. (1981) *Endocrinology* **104**, 1369–1376.
3. Vilchez-Martinez, J. A., Pedroza, E., Arimura, A. & Schally, A. V. (1979) *Fertil. Steril.* **31**, 677–682.
4. Bergquist, C., Nillius, S. J., Bergh, T., Skarin, G. & Wide, L. (1979) *Acta Endocrinol. (Copenhagen)* **91**, 601–608.
5. Bergquist, C., Nillius, S. J. & Wide, L. (1979) *Lancet* **ii**, 215–216.
6. Tolis, G., Mehta, A., Comaru-Schally, A. M. & Schally, A. V. (1981) *J. Clin. Invest.* **68**, 819–822.
7. DeSombre, E. R., Johnson, E. S. & White, W. F. (1976) *Cancer Res.* **36**, 3830–3833.
8. Danguy, A., Legros, N., Heuson-Stiennon, J. A., Pasteels, J. L., Atassi, G. & Heuson, J. C. (1977) *Eur. J. Cancer* **13**, 1089–1094.
9. Redding, T. W. & Schally, A. V. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6509–6512.
10. Lamberts, S. W. J., Uitterlinden, P., Zuiderwizk-Roest, J. M., Bons-Van Evelingen, E. G. & DeJong, F. H. (1981) *Endocrinology* **108**, 1878–1884.
11. Schally, A. V., Arimura, A. & Coy, D. H. (1980) *Vitam. Horm. (N.Y.)* **38**, 258–324.
12. Zarate, A., Canales, E. S., Sthory, I., Coy, D. H., Comaru-Schally, A. M. & Schally, A. V. (1981) *Contraception* **24**, 315–320.
13. Redding, T. W., Coy, D. H. & Schally, A. V. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1273–1276.
14. Schally, A. V., Coy, D. H. & Meyers, C. A. (1978) *Biochemistry* **47**, 89–128.
15. Hall, R., Besser, G. M., Schally, A. V., Coy, D. H., Evered, D., Goldie, D. J., Kastin, A. J., McNeilly, A. S., Mortimer, C. H., Phenekos, C., Turnbridge, W. M. G. & Weightman, D. (1973) *Lancet* **ii**, 581–584.
16. Hirst, B. H., Shaw, B., Meyers, C. A. & Coy, D. H. (1980) *Regul. Pept.* **1**, 97–113.
17. Erchegyi, J., Coy, D. H., Nekola, M. V., Coy, E. J., Schally, A. V., Mezo, I. & Teplan, I. (1981) *Biochem. Biophys. Res. Commun.* **100**, 915–920.
18. Janik, P., Briand, P. & Hartmann, N. R. (1975) *Cancer Res.* **35**, 3698–3704.
19. Steel, R. G. D. & Torrie, J. H. (1960) *Principles and Procedures of Statistics* (McGraw Hill, New York), p. 114.
20. Tolis, G., Ackman, D., Stellos, A., Mehta, A., Labrie, F., Fazekas, A., Comaru-Schally, A. M. & Schally, A. V. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1658–1662.
21. Corbin, A., Rosanoff, E. & Bex, F. J. (1980) in *Progress in Cancer Research and Therapy: Hormones and Cancer*, eds. Iacobelli, S., King, R. J. B., Lindner, H. R. & Lippman, M. E. (Raven, New York), Vol. 14, pp. 533–540.
22. de Quijada, M., Timmermans, H. A. T. & Lamberts, S. W. J. (1980) *J. Endocrinol.* **86**, 109–116.
23. Besser, G. M., Mortimer, C. H., Carr, D., Schally, A. V., Coy, D. H., Evered, D., Kastin, A. J., Tunbridge, W. M. G., Thorner, M. O. & Hall, R. (1974) *Br. Med. J.* **1**, 352–355.
24. Rodriguez-Arnao, M. D., Gomez-Pan, A., Rainbow, S. J., Woodhead, S., Comaru-Schally, A. M., Schally, A. V., Meyers, C. A. & Coy, D. H. (1981) *Lancet* **i**, 353–356.
25. Besser, G. M. & Wass, J. A. H. (1978) *Med. J. Aust.* Sp. Suppl., November 4, 32–33.