PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 185, 69–75 (1987)

Inhibitory Effects of Somatostatin Analogs on Prolactin Secretion in Rats Pretreated with Estrogen or Haloperidol (42518)

TSUYOSHI KARASHIMA* AND ANDREW V. SCHALLY*,†

*Section of Experimental Medicine, Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana 70112, and †Endocrine, Polypeptide, and Cancer Institute, Veterans' Administration Medical Center, New Orleans, Louisiana 70146

Abstract. The effect on prolactin (PRL) secretion of acute administration of new octapeptide analogs of somatostatin (SS) with an enhanced and prolonged growth hormone inhibitory activity was investigated in rats under various pretreatment conditions with estrogen and antidopaminergic drugs. Analog D-Phe–Cys–Tyr-D-Trp–Lys–Val–Cys–Thr-NH_2 (RC-121), at a dose of 5 μg/100 g body wt, did not decrease basal PRL levels in thiopental-anesthetized female rats, untreated or treated with estrogen benzoate (EB) (8 μg/rat) for 5 days. When haloperidol was used to elevate PRL level, a single injection of RC-121 inhibited PRL release in EB-pretreated female rats or untreated female and male rats. Analog D-Phe–Cys–Trp-D-Trp–Lys–Val–Cys–Trp-NH_2 (RC-160), which has a potency similar to RC-121 in the tests on inhibition of GH, in a dose of 0.2 μg/100 g body wt, did not lower the elevated PRL level induced by α-methyl-*p*-tyrosine and/or pretreatment with EB (100 μg/rat, 3 and 6 days before) in pentobarbital-anesthetized male rats. However, both analogs RC-121 and RC-160, in doses of 0.2 μg/100 g body wt, decreased the PRL levels elevated by prolonged pretreatment with EB (100 μg/rat, twice a week for 3 weeks) in male rats. These results indicate that acute administration of these SS analogs can induce a prolonged inhibition of PRL release when PRL is acutely elevated by haloperidol or chronically elevated by 3 weeks of estrogen administration. Future additional studies are required to investigate the effects of chronic administration of these SS analogs on PRL levels.

Recently nearly 300 octapeptide analogs of somatostatin (SS)-14 have been synthesized in our laboratory. Among them, D-Phe–Cys–Tyr-D-Trp–Lys–Val–Cys–Thr-NH_2 (RC-121) and D-Phe–Cys–Tyr-D-Trp–Lys–Val–Cys–Trp-NH_2 (RC-160) have been found to be 177 and 113 times more potent, respectively, than SS-14 in tests for the inhibition of growth hormone (GH) release (1). These analogs possess antitumor activities as shown by the inhibition of growth of animal models in prostate, mammary, and ductal pancreatic tumors (1). Chronic treatment with 2.5 μg/day of analog RC-121 or RC-160 for 60–90 days lowered serum prolactin (PRL) levels in rat models of prostate cancer (2). Since these analogs are expected to be used clinically, it is important to characterize carefully their biological activities, including the effect on PRL secretion.

SS-14 decreases basal and stimulated PRL release from pituitaries of rats and humans in monolayer culture systems (3–7). Recent studies in man *in vivo* showed that SS-14 decreased the PRL release augmented by various stimuli (8, 9). Although in early studies (3, 4) the inhibitory action of SS-14 on PRL release was not observed in male rats primed with estrogen for a short period (less than 1 week), Cooper and Shin (10) found that the suppressive effect could be seen in male rats primed with estrogen for 3 weeks. They also indicated that this effect was not observed in pimozide-treated male rats that were not estrogen-primed (10). The same group (11) reported, based on electron microscopy studies, that SS-14 induces ultrastructural changes in the adenohypophysis of estrogen-primed male rats and suggested that pretreatment with estrogen creates a new environment for SS action through the alteration of receptors on the lactotrophs. Kimura *et al.* (7) indicated that 17β-estradiol acts on the pituitary lactotrophs, rendering them sensitive to SS-14, probably by increasing the number of receptor sites for SS-14. These results suggest that long-term estrogen priming plays a critical role in enhancing the inhibitory action of SS-14 on PRL release.

In the investigation of the effects of our new SS analogs on PRL levels, we first used RC-

0037-9727/87 $1.50

121 in male and female rats pretreated with haloperidol and/or a small dose of estrogen. Subsequently, we investigated whether long-term treatment with large doses of estrogen can augment the PRL-inhibiting response to both SS analogs.

Materials and Methods. *Animals.* Adult male and female Sprague–Dawley rats weighing 200–400 g were used in all experiments. Animals were allowed a standard rat diet and tap water *ad libitum* and were maintained under controlled conditions at 24 ± 2°C and a 12-hr-light, 12-hr-dark schedule. Groups of eight animals were used in each experiment. The weight difference between the rats in a single experiment was less than 40 g.

Treatment and experimental procedures. Intravenous (iv) injection and blood sampling were performed using the jugular vein. Blood was taken in a volume of 0.7 ml and the same volume of saline was injected after each blood sampling. In the first experiment, one group of female rats received 8 μg/rat of estradiol benzoate (β-estradiol-3-benzoate (EB; Sigma, St. Louis, MO) sc daily for 5 days before the experiment. Other groups of normal female and male rats were not treated with EB. Animals were anesthetized ip with 50 mg/kg body wt of sodium thiopental (Sigma) 30 min before the experiment. SS analog RC-121 (5 μg/100 g body wt) was administered iv to estrogen-treated and untreated female rats and a blood sampling was done after 0, 30, 60, 90, and occasionally 120 min. Some estrogen-primed and untreated female rats and untreated male rats received haloperidol (Haldol; McNeil Pharmaceutical Co., Spring House, PA), 0.1 mg/kg body wt iv, in saline, 1 min before administration of the SS analog, and samples were taken at the same time intervals. In the second experiment estrogen-untreated female rats were injected with 0.2 or 2 μg/100 g body wt of RC-121 after haloperidol treatment. In the third experiment, normal male rats, untreated or pretreated 6 and 3 days previously with 100 μg/rat of EB, were used under pentobarbital anesthesia (Nembutal, Abbott Lab., North Chicago, IL), 60 mg/kg body wt ip. α-Methyl-*p*-tyrosine (AMT; Sigma) was injected ip at a dose of 250 mg/kg body wt, 90 min before administration of saline or SS analog RC-160. AMT was first dissolved in 1.0 *N* NaOH and then adjusted to pH 10.0 by adding 1.0 *N* HCl (12). One control group that had received EB treatment was not pretreated with AMT. Thirty minutes after the initiation of anesthesia, the animals were injected iv with RC-160 (0.2 μg/100 g body wt) and blood samples were taken 0, 15, 30, and 60 min later. In the fourth experiment, normal male rats were primed with EB (100 μg/rat, twice a week, every 3–4 days for 3 weeks, the last injection being performed 3 days before the experiment). The animals were injected iv with RC-160 or RC-121 (0.2 μg/100 g body wt) or the vehicle under pentobarbital anesthesia.

Peptides. Analogs RC-121 and RC-160 were synthesized by solid phase methods and repurified by HPLC (1). Their effects on GH, glucagon, and gastric acid secretion are described elsewhere (1). Both analogs were 100–150 times more potent than SS-14 on inhibition of GH release (1). For the injection, the peptides were dissolved with saline containing 0.2% bovine serum albumin.

Radioimmunoassay (RIA). All blood samples were centrifuged and sera were kept at −20°C until assayed. RIAs for PRL and GH were carried out with materials supplied by the National Hormone and Pituitary Program of NIH. Interassay variation was less than 15% and intraassay variation was 10% or less for both RIAs. Data are expressed as means ± SEM. Statistical significance was assessed by Student's *t* test in the first and third experiment and by Duncan's new multiple range test (13) in the second and fourth experiment.

Results. The effect of SS analog RC-121 on the basal (Experiment 1) and elevated PRL level induced by haloperidol (Experiments 1 and 2) was investigated in EB-untreated and -pretreated female rats and in EB-untreated male rats. Administration of RC-121, in a dose of 5 μg/100 g body wt, did not decrease the basal PRL level in EB-untreated female rats and in female rats primed with 8 μg/rat of EB for 5 days (data not shown). Treatment with haloperidol increased the PRL levels to various degrees in male rats and estrogen-primed or untreated female rats (Fig. 1). Analog RC-121, in a dose of 5 μg/100 g body wt, inhibited the haloperidol-induced PRL release in female rats primed with EB ($P < 0.01$ vs saline control at 30, 60, and 90 min; Fig. 1a). A fall in PRL was also seen in female rats not treated with EB ($P < 0.01$ at 30 and 60 min; Fig. 1b). This

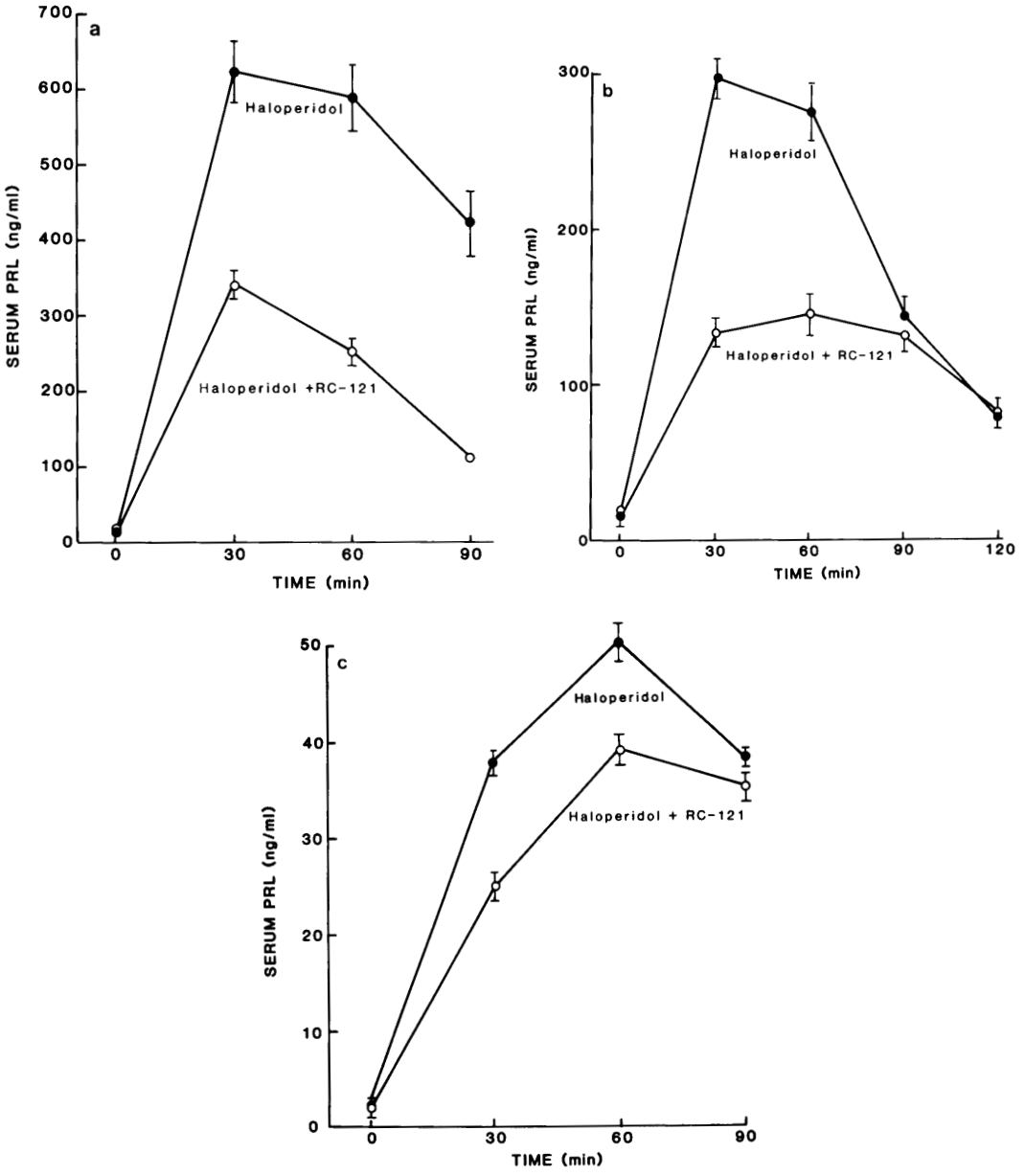

FIG. 1. The effects of SS analog RC-121 on the PRL release induced by haloperidol in female rats primed with estrogen (a), female rats not treated with estrogen (b), and male rats not treated with estrogen (c). The analog RC-121 was given in a dose of 5 μg/100 g body wt, iv, under thiopental anesthesia. The estrogen-primed group received EB (8 μg/rat) daily for 5 days.

analog also slightly but significantly inhibited PRL levels in haloperidol-treated male rats ($P < 0.01$ at 30 min; $P < 0.05$ at 60 min; Fig. 1c). In Experiment 2, analog RC-121, in a dose of 2 μg/100 g body wt, depressed the haloperidol-induced PRL release in female rats, but it was ineffective at a dose of 0.2 μg/100 g body wt (Fig. 2).

In Experiment 3, we investigated the effects of SS analog RC-160, which has a potency

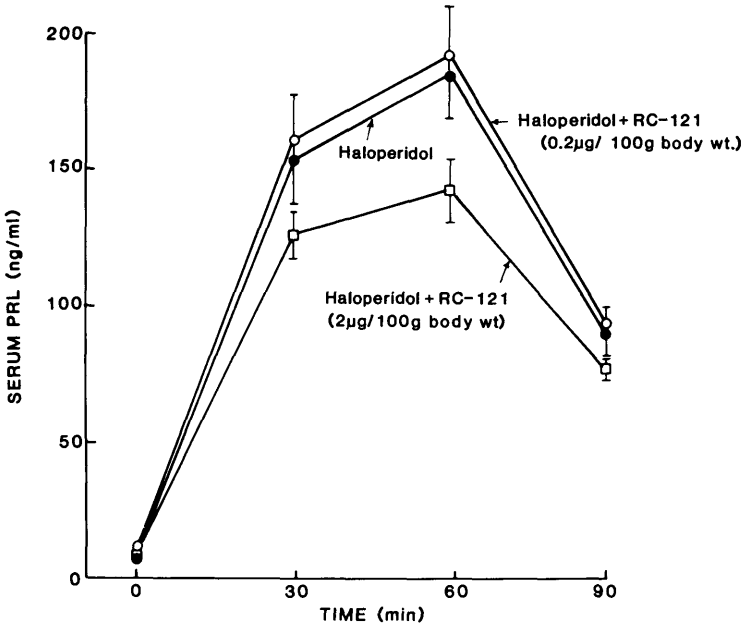

FIG. 2. The effects of different doses of SS analog RC-121 on the PRL release induced by haloperidol in estrogen-untreated female rats under thiopental anesthesia. The analog RC-121 was given in doses of 0.2 and 2 μg/100 g body wt, iv.

similar to RC-121 in the tests on inhibition of GH, insulin, glucagon, and gastric acid release, on PRL levels in male rats pretreated with a large dose of estrogen and/or AMT (Table I). Analog RC-160, in a dose of 0.2 μg/100 g body wt, did not decrease the PRL level in estrogen-primed male rats (Group I). This analog also failed to decrease the elevated PRL levels induced by AMT in estrogen-treated (Group III) or untreated male rats (Group II).

TABLE I. THE EFFECT OF RC-160 ON THE PRL LEVEL ELEVATED BY EB (GROUP I), α-METHYL-*p*-TYROSINE (AMT) (GROUP II), OR BOTH DRUGS (GROUP III) IN THE PENTOBARBITAL-ANESTHETIZED MALE RATS

	PRL level (ng/ml)			
Group[a]	0 min	15 min	30 min	60 min
I				
Control	36.3 ± 4.8	41.7 ± 9.0	38.3 ± 3.4	44.6 ± 10.7
RC-160 (0.2 μg)	53.4 ± 9.8	48.9 ± 9.2	34.6 ± 3.4	42.6 ± 12.0
II				
Control	21.0 ± 2.9	18.9 ± 2.7	17.8 ± 2.5	30.8 ± 4.6
RC-160 (0.2 μg)	27.8 ± 5.6	21.5 ± 2.4	24.0 ± 4.3	37.8 ± 3.4
III				
Control	81.2 ± 8.2	95.3 ± 16.8	91.8 ± 11.5	101.8 ± 9.8
RC-160 (0.2 μg)	91.6 ± 9.0	99.8 ± 8.9	102.8 ± 17.3	111.0 ± 16.1

Note. In Groups I and III, the animals were primed with EB (100 μg/rat), 3 and 6 days previously.
[a] Per 100 g body wt.

In Experiment 4, we prolonged the pretreatment with EB to 3 weeks. This prolonged priming with EB raised the basal PRL level about 25-fold as compared with values found in normal male rats, under pentobarbital anesthesia. The resulting PRL level was similar to that observed in rats treated with AMT in combination with estrogen in Experiment 3 (Table I, Group III). Both analogs RC-121 and RC-160, in doses of 0.2 μg/100 g body wt, inhibited PRL levels for more than 60–120 min ($P < 0.01$ at 15, 40, and 60 min for RC-121; $P < 0.01$ at 15, 40, and 60 min and $P < 0.05$ at 120 min for RC-160) (Fig. 3). The time course of PRL inhibition induced by both analogs was similar to that of GH (Fig. 1, Ref. (1) and other unpublished data).

Discussion. This work indicates that new superactive analogs of SS can decrease PRL levels in rats primed with estrogen or pretreated with haloperidol, and that the suppression of PRL release is protracted and follows a time course similar to inhibition of GH release. We found that our SS analog RC-121 in high doses inhibited the haloperidol-induced PRL release in estrogen-untreated and short-term estrogen-treated female rats and estrogen-untreated male rats. Cooper and Shin (10) did not observe the inhibitory action of SS-14 in pimozide-treated male rats without estrogen-priming. In addition to the difference in antidopaminergic drugs used and the much greater activity of the SS analog as compared with SS-14, the discrepancy between their results and ours may be due to a sex difference because in our study, estrogen-untreated male rats were less sensitive to the SS analog than were the intact females.

We could not detect the inhibitory action of SS analog on PRL release in rats primed with estrogen for less than 1 week, although AMT was used to elevate the PRL levels. However, we observed the inhibitory effect of SS analogs on PRL levels in male rats pre-

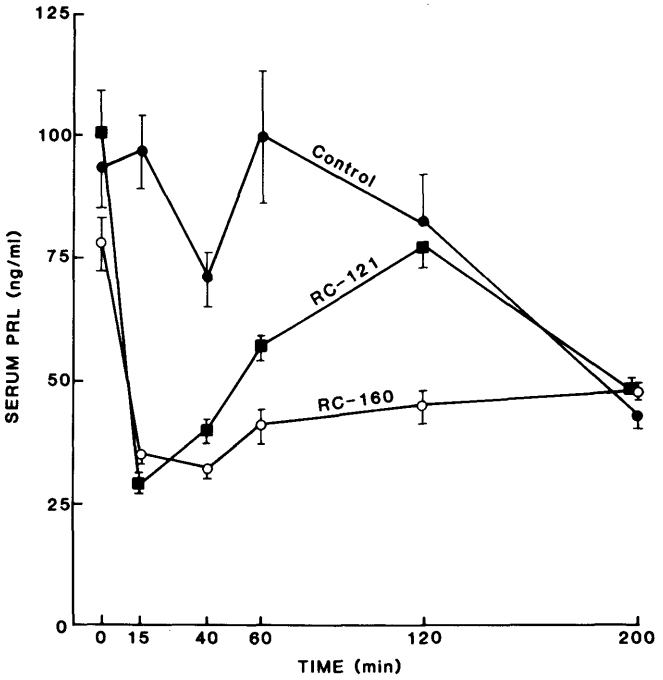

FIG. 3. Time course of inhibition by SS analogs of estrogen-stimulated PRL secretion in male rats under pentobarbital anesthesia. Analogs RC-121 and RC-160 were given in doses of 0.2 μg/100 g body wt, iv. The animals were primed with EB (100 μg/rat), twice a week (every 3 or 4 days) for 3 weeks. Pentobarbital (2 mg/100 g body wt) was injected again at 50 min for the continuation of anesthesia.

treated with estrogen for 3 weeks. These results are in agreement with a report by Cooper and Shin (10) on similar action exerted by SS-14, although the inhibition of PRL induced by the analogs was more prolonged than that of SS-14. These results indicate that the duration of estrogen-priming is important for enhancing the sensitivity of the inhibitory effect of SS or its analogs on PRL release.

Analogs RC-121 and RC-160 were shown to possess significant antitumor activity in animal models including the inhibition of growth of prostate cancer and breast cancers (1). Various studies support the view that PRL may play a role in the development and growth of breast cancer in rodents as well as in humans (14, 15). The presence of PRL receptors in mammary tumors, including human breast cancer cells, also lends support to the theory that a certain proportion of mammary tumors can be PRL dependent (15). If SS analogs suppress serum PRL on chronic administration, they could be used for inhibiting the growth of breast cancer and possibly also for augmenting the suppressive effects of LH-RH agonists on prostate cancer (2, 14–16). This appears to be the first report on the inhibitory effects of acute administration of new analogs of somatostatin on prolactin levels in animals subjected to some pharmacological manipulations. Development of delayed delivery systems for once-a-month administration, similar to microcapsules of poly(DL-lactide-co-glycolide) formulated for D-Trp-6-LH-RH (17), will permit investigation of chronic effects of SS analogs on levels of PRL and other hormones. These studies on chronic administration of various dosage regimens must be performed in several animal models in view of the planned clinical trials with these analogs of somatostatin in acromegaly and some hormone-sensitive cancers.

This work was supported by National Institutes of Health Grants AM07467 and CA 40003 (to A.V.S.) and the Medical Research Service of the Veterans Administration. We thank the National Hormone and Pituitary Program (NHPP) for the gifts of materials used in RIA. The participation of Dr. L. Debeljuk in earlier phases of this project is acknowledged. We also thank Miss Martha Sampson, Dr. Elsa Winsor, Dr. J. I. Paz, Dr. R. Lu, and Mr. Weldon Carter for their valuable experimental assistance.

1. Cai R-Z, Szoke B, Lu R, Fu D, Redding TW, Schally AV. Synthesis and biological activity of highly potent octapeptide analogs of somatostatin. Proc Natl Acad Sci USA **83**:1896–1900, 1986.
2. Schally AV, Redding TW, Paz-Bouza JI, Bajusz S, Cai R-Z, Comaru-Schally AM. Use of analogs of hypothalamic hormones for the treatment of hormone-dependent cancers. In: Proceedings of the 14th International Cancer Congress, Budapest, August 1986. Basel, Switzerland: Karger, in press.
3. Vale W, Rivier C, Brazeau P, Guillemin R. Effects of somatostatin on the secretion of thyrotropin and prolactin. Endocrinology **95**:968–977, 1976.
4. Drouin J, Leab AD, Rainville D, Lachance R, Labrie F. Characteristics of the interaction between thyrotropin-releasing hormone and somatostatin for thyrotropin and prolactin release. Endocrinology **98**:514–521, 1977.
5. Hanew K, Rennels EG. Effects of culture age on PRL and GH responses to bromocriptine and somatostatin from pituitary cultures of rat anterior pituitary cells (41470). Proc Soc Exp Biol Med **171**:12–18, 1982.
6. Ishibashi M, Yamaji T. Direct effects of catecholamines, thyrotropin-releasing hormone and somatostatin on growth hormone and prolactin secretion from adenomatous and nonadenomatous human pituitary cells in culture. J Clin Invest **73**:66–72, 1984.
7. Kimura N, Hayafuji C, Konagaya H, Takahasi K. 17β-estradiol induces somatostatin (SRIF) inhibition of prolactin release and regulates SRIF receptors in rat anterior pituitary cells. Endocrinology **119**:1028–1036, 1986.
8. Millar RP, Klaff LG, Barron JL, Levitt NS, Ling N. Somatostatin-28 and somatostatin-14 suppression of arginine-, insulin-, and TRH-stimulated GH and PRL secretion in man. Clin Endocrinol (Oxford) **18**:277–285, 1983.
9. Degli-Uberti EC, Trasforini G, Salvadori S, Margutti A, Teodori V, Rotola C, Tomatis R, Ransini R. Effect of somatostatin on growth hormone and prolactin response to dermorphin in man. Acta Endocrinol (Copenhagen) **180**:20–25, 1985.
10. Cooper GR, Shin SH. Somatostatin inhibits prolactin secretion in the estradiol-primed male rat. Canad J Physiol Pharmacol **59**:1082–1088, 1981.
11. Saunders SL, Reifel CW, Shin SH. Ultrastructural changes rapidly induced by somatostatin may inhibit prolactin release in estrogen-primed rat adenohypophysis. Tissue Res **232**:21–34, 1983.
12. Lu K-H, Amenomori Y, Chen C-L, Meites J. Effects of central acting drugs on serum and pituitary prolactin levels in rats. Endocrinology **87**:667–672, 1970.
13. Steel RGD, Torrie JH. In: Principles and procedures of statistics. McGraw-Hill, New York, 1960.

14. Schally AV, Redding TW, Cai R-Z, Paz JI, Ben-David M, Comaru-Schally AM. Somatostatin analogs in the treatment of various experimental tumors. In: Klijn J, Ed. International Symposium of Hormonal Manipulation of Cancer: Peptides, Growth Factors of New (Anti) Steroidal Agents. New York: Raven Press, in press.
15. Ben-David M, Kadar T, Schally AV. Micromethod for the determination of free and total prolactin receptors: Measurement of receptor levels in normal and malignant mammary and prostate tissues. Proc Natl Acad Sci USA **83**:8375–8379, 1986.
16. Schally AV, Cai R-Z, Torres-Aleman I, Redding TW, Szoke B, Fu D, Hierowski MT, Colaluca J, Konturek S. Endocrine, gastrointestinal, and antitumor activity of somatostatin analogues. In: Moody T, Ed. Neural and Endocrine Peptides and Receptors. New York: Plenum, pp73–88, 1986.
17. Redding TW, Schally AV, Tice TR, Meyers WE. Long-acting delivery systems for peptides: Inhibition of rat prostate tumors by controlled release of [D-Trp^6] luteinizing hormone-releasing hormone from injectable microcapsules. Proc Natl Acad Sci USA **81**:5845–5848, 1984.

Received September 29, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted January 27, 1987.