Controlled Release of Thyrotropin Releasing Hormone from Microspheres: Evaluation of Release Profiles and Pharmacokinetics after Subcutaneous Administration

TOSHIRO HEYA*x, YASUSHI MIKURA‡, AKIHIRO NAGAI‡, YASUTOMO MIURA‡, TOMOMICHI FUTO‡, YOSHINAO TOMIDA‡, HISAYOSHI SHIMIZU‡, AND HAJIME TOGUCHI*

Received January 28, 1993, from the *DDS Research Laboratories and ‡Pharmaceutics Research Laboratories, Takeda Chemical Industries, Ltd., 2-17-85 Jusohonmachi, Yodogawa, Osaka 532, Japan. Accepted for publication January 10, 1994®.

Abstract □ The drug-release kinetics of thyrotropin releasing hormone (TRH) containing copoly(*dl*-lactic/glycolic acid) (PLGA) microspheres were evaluated both *in vitro* and *in vivo*. The drug was encapsulated in PLGA using an in-water drying method through a water in oil in water emulsion. The drug release from the PLGA microspheres *in vitro* correlated well with that *in vivo*, and pseudo-zero-order release kinetics were observed. The pharmacokinetics of TRH following administration of this controlled-release parenteral dosage form have been also examined in rats. Following a transient increase in the plasma level due to an initial burst, steady-state plasma levels were observed. The duration of drug release estimated from the plasma level was comparable with the results in the *in vitro* and *in vivo* release studies. The steady-state plasma levels correlated well with the levels predicted from the pharmacokinetic parameters following a single subcutaneous or intravenous injection of TRH solution. The results of this study confirm the previously reported *in vivo* sustained release of TRH achieved with this drug-delivery system.

Introduction

Thyrotropin releasing hormone (TRH) plays a variety of roles in the central nervous system (CNS) and has already been developed as a CNS-stimulating drug (Hirtonin). Long-term daily injection is often required for clinical use of TRH in the treatment of CNS dysfunction, such as spinocerebellum degeneration or unconsciousness. Therefore, we have developed injectable microspheres prepared with a biocompatible and biodegradable polymer, copoly(*dl*-lactic/glycolic acid) (PLGA), to produce a sustained-release dosage form of TRH. Recent investigation has revealed that the continuous TRH treatment using TRH microspheres causes shortening of pentobarbitone-induced sleeping time at doses lower than those required using bolus injection[1] in rats.

Many studies on the release kinetics of drug-containing PLGA microspheres have been reported and reviewed.[2,3] However, only a few studies have dealt with the sustained release of highly water-soluble drugs except for lutenizing hormone-releasing hormone analogues,[4-6] probably due to the difficulty in controlling the release rate.

In a previous study,[7] we clarified that the sustained release microspheres of TRH could be prepared in spite of the high water solubility of TRH. The ionic interaction between the basic functional groups of TRH and the carboxylic end terminal of PLGA during preparation was found to be crucial in the preparation of microspheres. The rate of TRH release has been shown to be dependent on the molecular weight and the copolymer ratio of PLGA.[8]

In the present study, the correlation between *in vitro* and *in vivo* release of the drug from the PLGA microspheres was investigated. In addition, the pharmacokinetics of TRH after a single injection of the microspheres were evaluated.

® Abstract published in *Advance ACS Abstracts*, March 15, 1994.

Experimental Section

Materials—TRH synthesized in the Pharmaceutical Production Research Laboratories of Takeda Chemical Industries, Ltd (Osaka, Japan) was used. PLGA was supplied by Wako Pure Chemical Ind. Ltd. (Osaka, Japan). PLGA with a copolymer ratio of 75/25 (lactic acid/glycolic acid) and weight-average molecular weight of 10 000 was used. The molecular weight was determined by gel permeation chromatography.

Preparation of PLGA Microspheres—The PLGA microspheres were prepared using an in-water drying method as described in our previous paper.[7] In brief, 7 g of TRH was dissolved in water, and 63 g of PLGA was dissolved in 78.4 mL of dichloromethane. These solutions were vigorously homogenized for a few minutes to make a water in oil emulsion. The emulsion was poured into an aqueous 0.1% polyvinyl alcohol solution with stirring by a homogenizer to make water in oil in water emulsion. To evaporate the dichloromethane, the water in oil in water emulsion was further stirred for 3 h. After removing large particles by sieving, the resulting microspheres were collected by centrifugation and then lyophilized into a powder with mannitol. The shape and surface of the microspheres were observed with a scanning electron microscope (Model JSM T-300, Jeol-Technics Co. Ltd., Tokyo, Japan). The size of the microspheres was determined by a Coulter TA-II.

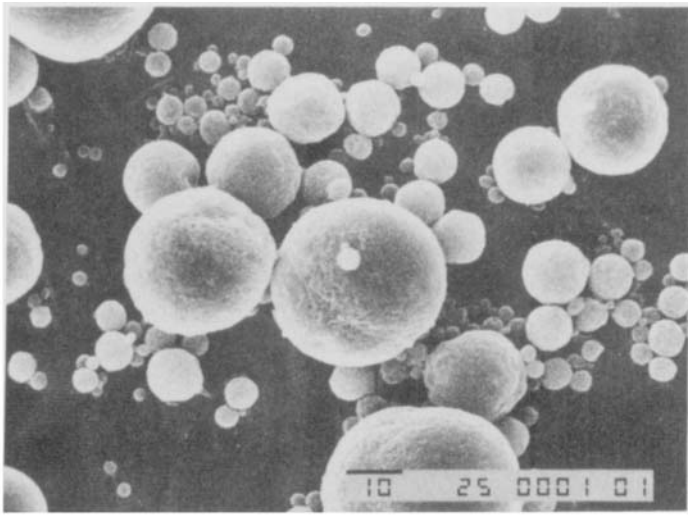
Determination of the TRH Content in the Microspheres—Fifty milligrams of the microspheres was dissolved in 10 mL of dichloromethane and 20 mL of $^1/_{30}$ M phosphate buffer, pH 6.0, and the TRH in the buffer layer was assayed by high-performance liquid chromatography (HPLC, Shimadzu LC-3A) with ultraviolet (UV) detection as follows: column, Nucleosil 5-C_{18} (150 mm in length, 4.6 mm i.d.); mobile phase, a mixture of 10 mL of methanol and 250 mL of 0.02 M $NH_4H_2PO_4$ buffer, pH 3.65; flow rate, 0.6 mL/min; wavelength, 215 nm.

***In Vivo* Release Studies**—*In vivo* release characteristics were evaluated by determining the residual amount of TRH at the injection site in male rats (JCL Wistar, 11 weeks old). The microspheres were injected sc into rats (8 mg/kg as TRH, $n = 5$) after dispersion in a vehicle, pH 5.5–7.0 (adjusted with HCl), containing 2.5% sorbitol, 0.9% NaCl, 0.1% Tween 80, and 0.07% Na_2HPO_4. The microspheres were periodically excised and homogenized in 10 mL of $^1/_{30}$ M phosphate-buffered saline (PBS) containing 0.02% Tween 80, pH 6.0, using a Polytron homogenizer (Kinematica GmbH, Luzern, Switzerland). The homogenate was shaken with an additional 10 mL of the PBS solution and 10 mL of dichloromethane. The resultant was then centrifuged and the drug content in the aqueous layer was determined by HPLC.

***In Vitro* Release Studies**—Fifty milligrams of the microspheres was suspended in 10 mL of $^1/_{30}$ M, pH 7, phosphate buffer containing 0.02% Tween 80 in 17-mL vials. The vials were incubated in a horizontally reciprocating water bath at 37 °C. The amount of residual TRH in the microspheres was determined using the analytical method described above after filtering the microspheres with a 1.2-μm Millipore filter.

Pharmacokinetics in Rats—Male Wistar rats (11 weeks old) supplied from Clea Japan Inc. were used for the pharmacokinetic study. The kinetics of TRH (0.5 mg/kg in 1.0-mL of a saline solution) was studied after either intravenous (iv) and subcutaneous (sc) injection in five rats, respectively. The iv injection dose was administered through the femoral vein. The sc dose was injected into the back of the rats. In a separate study, the bioavailability of the microspheres were studied following sc injection into the back of the rats at doses of 0.5, 2, and 8 mg/kg as TRH. Blood was periodically obtained from the tail vein for 4 weeks and the plasma TRH levels were determined. The sampled blood was immediately mixed with the TRH degradation enzyme

0022-3549/94/1200-798$04.50/0



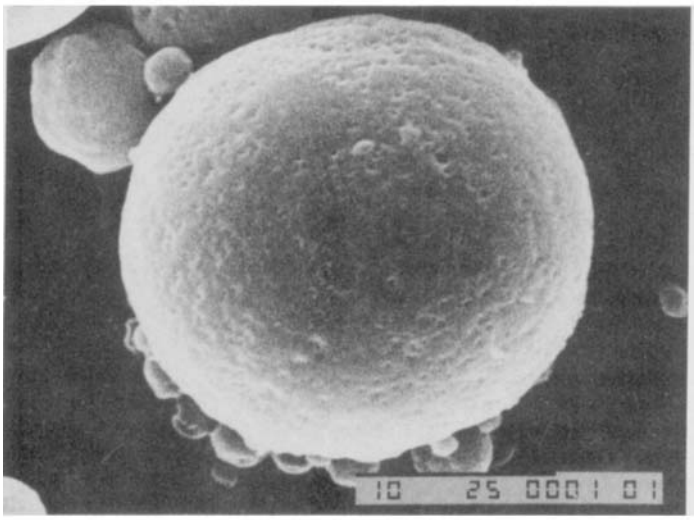


Figure 1—Scanning electron photomicrographs of PLGA microspheres containing TRH.

inhibitor containing 8-hydroxyquinoline, Tween 20, and EDTA-2Na obtained from Mistubishi Yuka BCL Ltd. (Tokyo, Japan). Plasma was separated by centrifuge and stored at less than −40 °C until assay of TRH. The TRH in the plasma was determined at Mitsubishi Yuka BCL Ltd. by a radioimmunoassay method (RIA). RIA was conducted by a method similar to that of Kamijo[9] et al.

Results and Discussion

1. **Characteristics of the Microspheres**—It is generally difficult to encapsulate a water-soluble drug in microspheres at a high entrapment ratio and to prevent a large initial burst of the drug from the microspheres. However, in previous experiments we have succeeded in preparing microspheres which provide constant drug release over a long period by utilizing the ionic interaction between a basic drug and the carboxylic end groups of PLGA.[7,8] TRH was encapsulated as its free base instead of the salt to increase the interaction with the PLGA.

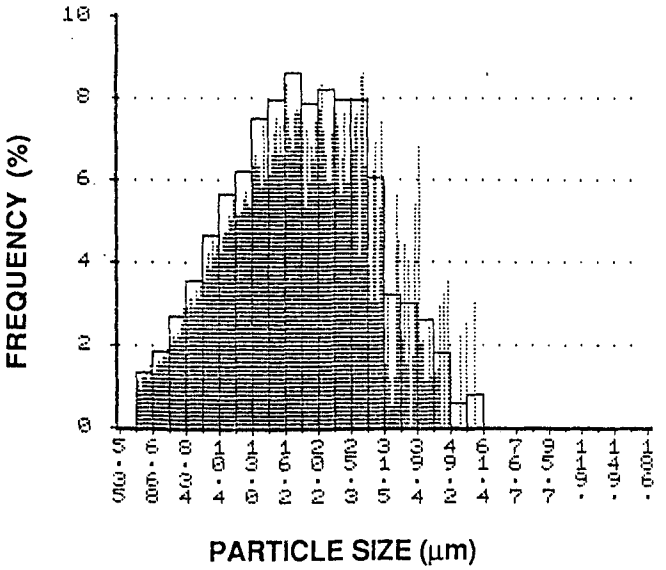


Figure 2—Particle size distribution of PLGA microspheres containing TRH.

Table 1—Content and Particle Size of PLGA Microspheres Containing TRH with Different Lots

Lot No.	TRH Content (%)	Diameter[a] (μm)
E04	7.81	18.1
E06	7.22	18.6
E07	7.41	20.8

[a] Weight-average diameter.

The TRH microspheres prepared by the in-water drying method with a loading amount of 10% were spherical particles with a mean diameter of about 20 μm and many fine pores on the surface (Figure 1). The pores are thought to be formed when the water and dichloromethane are removed during preparation. Figure 2 shows the particle size distribution of the microspheres. The distribution is relatively narrow, and the microspheres are considered to be homogeneous because the TRH content of microspheres sampled at random were uniform (data was not shown). Table 1 shows the content and the mean diameter of the microspheres of three lots. It was found that there is little interlot variability and that reproducible microspheres are being produced. Microspheres of this size are easily injected using a 23-gauge needle after dispersion in the vehicle for injection. Although these microparticles can also be called microcapsules, they are referred to as microspheres in this paper.

2. ***In Vitro* Release in Comparison with *in Vivo* Release**—The *in vivo* release estimated from the TRH remaining at the injection site correlated well with the *in vitro* release of TRH from PLGA microspheres in $^1/_{30}$ M, pH 7 phosphate buffer. This buffer composition was considered to be most appropriate for the estimation of *in vivo* release.[10] Figure 3 shows the *in vivo* and *in vitro* release profiles of the PLGA microspheres containing TRH (mean of three lots). Pseudo-zero-order release kinetics were observed both *in vitro* and *in vivo*, and the *in vivo* release pattern was comparable to that in the *in vitro* study. It was confirmed that the *in vivo* release can be conveniently estimated by the *in vitro* release.

3. **Pharmacokinetics of TRH—Dosed as Iv or Sc Solutions**—TRH pharmacokinetics after administration of the microspheres were evaluated for further clarification of the *in vivo* drug-release pattern. To examine the pharmacokinetics following sc injection of the microspheres, the pharmacokinetic parameters after a single injection of a TRH saline solution were determined. Figure 4 shows the plasma levels of TRH in male rats after iv and sc injection of the drug solution at a dose of 0.5

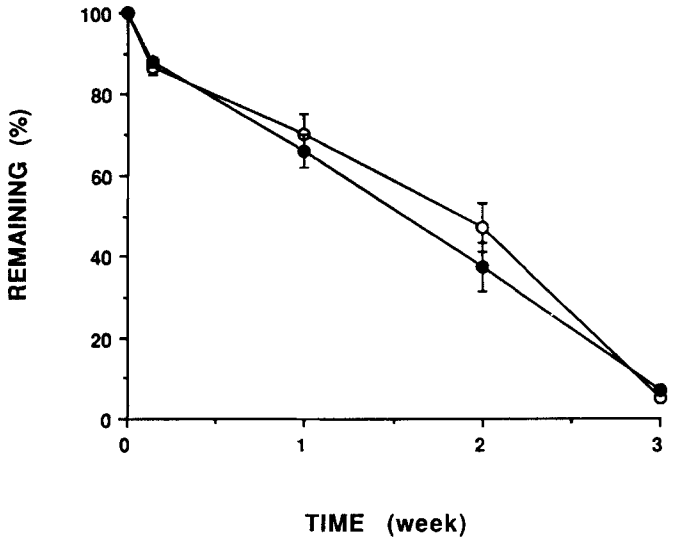


Figure 3—Profiles of TRH release from PLGA microspheres: (○) *in vitro*, (●) *in vivo* (mean ± SE, $n = 3$).

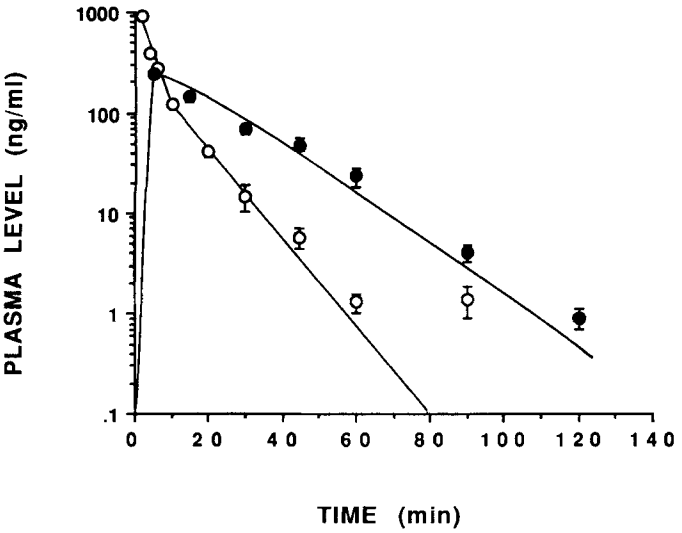


Figure 4—Plasma TRH levels in rats after intravenous and subcutaneous injection of TRH saline solution at a dose of 0.5 mg/kg (mean ± SE, $n = 5$): (○) intravenous injection, (●) subcutaneous injection.

Table 2—Pharmacokinetic Parameters of a TRH Saline Solution in Rats after a Single Iv and Sc Injection[a]

Parameter	Value	Parameter	Value
A (μg/mL)	2.22	F	0.883
B (μg/mL)	0.327	k_a (min^{-1})	0.061
α (min^{-1})	0.618	Cl_{total} (mL/min kg)	73.0
β (min^{-1})	0.100		

[a] Plasma level of TRH (C_t) at time t (iv), $C = Ae^{-\alpha t} + Be^{-\beta t}$; F, absorption fraction (sc); k_a, absorption rate constant (sc); Cl_{total}, total body clearance (iv).

mg/kg. The pharmacokinetic parameters were calculated by simulating both iv and sc data with simultaneous model fitting procedures using MULTI[11] (Table 2). Simulation was carried out by the damping Gauss–Newton method. The residual sum of squares[11] was 25.4, which was small enough to prove the validity of the simulation.

The F value was 0.88, indicating that metabolism in subcutaneous tissue was not extensive, and the k_a value was 0.06, indicating that TRH is rapidly transferred into the blood after sc injection. The k_a was smaller than β, indicating the existence of the flip–flop phenomenon.[12]

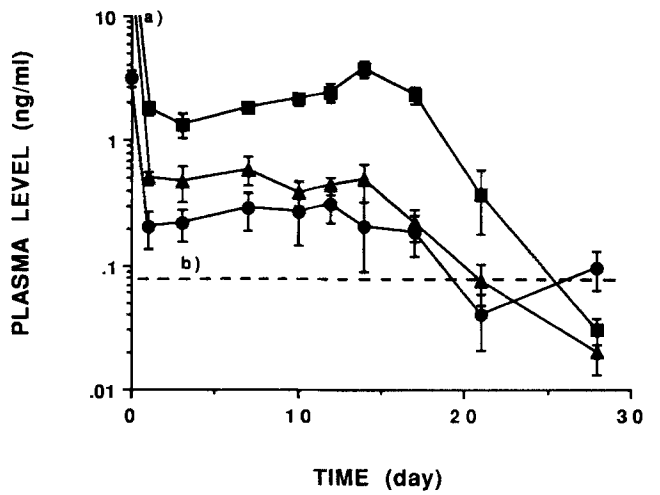


Figure 5—Plasma TRH levels in rats after subcutaneous injection of PLGA microspheres containing TRH (mean ± SE, $n = 5$). Dose: (●) 0.5 mg/kg, (▲) 2 mg/kg, (■) 8 mg/kg. (a) Plasma level at 30 min: (▲) 35.9 ± 2.27 ng/mL, (■) 85.8 ± 8.14 ng/mL. (b) Mean plasma endogeneous TRH level before administration of the microspheres.

Dosed as Microspheres—Figure 5 shows the plasma levels of TRH after a single sc injection of the microspheres. A sharp increase in the plasma level was observed due to the initial burst, but this was followed by a sustained constant plasma level for 17 days, and essentially the same pattern was observed at different doses. The duration for which the steady-state plasma level was maintained was comparable to that estimated from the *in vitro* release study, indicating that the pattern of drug release from the TRH microspheres after administration to rats can be conveniently estimated *in vitro* using an appropriate buffer.

The TRH levels tended to decrease below the baseline after 3 weeks. This might be due to the feedback effects of TRH regulation. Further study is required to elucidate the involvement of the regulation.

To evaluate the extent of TRH release *in vivo*, we predicted the plasma level of the drug at steady state using the kinetic parameters for the TRH saline solution. If the system is assumed to obey linear kinetics with this low plasma TRH level, the relationship can be represented as follows:[5]

$$Cl_{total} = \text{dose/AUC}$$
$$= 500\ \mu\text{g/kg}/6.846\ (\mu\text{g min/mL})$$
$$= 73.0\ \text{mL/min kg}$$

$$R_{iv} = R_{sc}F$$
$$= (8 \times 10^6 \times 0.039\ \text{ng/kg per day})(0.883/24\ \text{h}/60\ \text{min})$$
$$= 191.3\ \text{ng/min kg}$$

$$C_{ss} = C_e + R_{iv}/Cl_{total}$$
$$= 2.70\ \text{ng/mL}$$

where C_{ss} represents the steady-state drug level for 8 mg/kg, C_e represents the endogenous plasma TRH level, R_{sc} represents the subcutaneous constant infusion rate, R_{iv} represents the intravenously available constant infusion rate, Cl_{total} represents the total body clearance of the drug after iv injection, and AUC is the area under the curve after iv injection. R_{sc} is obtained from the regression line of the *in vivo* release data: R_{sc} (% of

Table 3—Steady-State Plasma Levels (C_{ss}) of TRH after Sc Injection of a TRH Solution

Dose (mg/kg as TRH)	C_{ss} (ng/mL)	
	Predicted[a]	Observed[b]
0.5	0.24	0.24
2.0	0.74	0.44
8.0	2.70	2.24

[a] This was corrected by adding the observed endogeneous TRH level of 0.08 ng/mL to the calculated value. [b] Mean plasma level (1–17 days).

drug remaining) = 86.3 − 3.9T (time in day). The correlation coefficient was 0.989. These results indicate that the release of TRH from the microspheres at the injection site in rats followed the zero-order kinetics of 3.9% of the dose released per day after an initial release of 13.7%. Since TRH levels before administration are considered to be endogenous, C_{ss} was calculated by adding the endogenous level (0.08 ng/mL) to the calculated value. The calculated and the observed C_{ss} are shown in Table 3. The predicted C_{ss}, calculated by assuming linear kinetics, agreed relatively well with the observed C_{ss}. The bias between the predicted value and the observed value at doses of 2, and 8 mg/kg may be attributed to the difficulty in determining the exact TRH clearance at each doses because of the dose-dependent nonlinear kinetics.[13]

In conclusion, the PLGA microspheres containing TRH released the drug continuously following a small initial burst; therefore, they should have long-acting effects. The rate of TRH release from the microspheres in rats could be estimated conveniently from the results of the *in vitro* release study. The steady state plasma levels of TRH after administration of the microspheres were consistent with the levels calculated using the pharmacokinetic parameters after a single injection of TRH solution, indicating the valid extent of drug release from the microspheres *in vivo*. Although there is concern with respect to receptor sensitivity and down-regulation, the continuous TRH treatment using microspheres was more effective than the repeated daily injection of TRH solution, which causes pentobarbitone-induced sleeping time in rats,[1] indicating the usefulness of this dosage forms. This depot formulation should provide a more convenient method for treating brain diseases with TRH than the daily injection of an aqueous solution.

References and Notes

1. Hashimoto, T.; Wada, T.; Fukuda, N.; Nagaoka, A. *J. Pharm. Pharmacol.* **1993**, *45*, 94.
2. Tice, T. R.; Cowsar, D. R. *Pharm. Technol.* **1984**, *8*, 26.
3. Wise, D. L. *Biopolymeric Controlled Release Syst.* **1984**, *1*, 3.
4. Okada, H.; Heya, T.; Ogawa, Y.; Shimamoto, T. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 744.
5. Ogawa, Y.; Okada, H.; Heya, T.; Shimamoto, T. *J. Pharm. Pharmacol.* **1989**, *41*, 439.
6. Okada, H.; Inoue, Y.; Heya, T.; Ueno, H.; Ogawa, Y.; Toguchi, H. *Pharm. Res.* **1991**, *8*, 787.
7. Heya, T.; Okada, H.; Tanigawara, Y.; Ogawa, Y.; Togichi, H. *Int. J. Pharm.* **1991**, *69*, 69.
8. Heya, T.; Okada, H.; Ogawa, Y.; Toguchi, H. *Int. J. Pharm.* **1991**, *72*, 199.
9. Kamijo, K., Sato, M., Taniuchi, A., Watanabe, Y., Kurimoto, f., Sakurai, H. *Clin. Endocrinol. Jp.* **1989**, *37*, 333–336.
10. Heya, T.; Okada, H.; Ogawa, Y.; Toguchi, H. *J. Pharm. Sci.* In press.
11. Yamaoka, K.; Tanigawara, Y., Nakagawa, T., Uno, T. *J. Pharm. Dyn.* **1981**, *4*, 897.
12. Ronfeld, R. A.; Benet, L. Z. *J. Pharm. Sci.* **1977**, *66*, 178.
13. Nagai, Y.; Yokohama, S.; Hirooka, Y.; Nihei, N. *J. Pharm. Dyn.* **1980**, *3*, 500.

Acknowledgments

The authors are indebted to Mitubishi Yuka BCL for radioimmunoassay of TRH. We are grateful to Dr. Yasuaki Ogawa, Dr. Hiroaki Okada, Dr. Naoru Hamaguchi, and Mr. Jeffrey Hogan for valuable comments on the manuscript and also grateful to Mrs. Mika Hori and Mrs. Katsumi Yano for their experimental assistance.