CY 208–243 is centrally active in the dose range used in the present experiments. This is attested to by its effects in rats with unilateral 6-hydroxydopamine-induced lesions of the substantia nigra, and by its induction of sniffing and grooming behaviors[4].

CY 208–243 failed to influence the HHG axis in all of the various experimental models investigated herein. No evidence for either stimulatory or inhibitory effects of the drug on LH secretion or on ovulation was observed. Thus, CY 208–243, in therapeutically and pharmacologically relevant doses, does not affect the HHG axis in rats.

The present data should be considered in the light of previous reports. Local injections of the D-1 antagonist SCH 23390 into the zona incerta (Z.I.) of the hypothalamus suppressed ovulation in cycling female rats; dose-dependence could not, however, be demonstrated. A local stimulatory effect of the D-1 agonist SKF 38393 on LH secretion in estrogen-primed ovariectomized rats was also reported, but the duration of action was very short[3]. Those results were taken as evidence for the existence of a discrete D-1 neurotransmitter system located within the Z.I. which regulates the secretion of LH in female rats. The present study, in which our novel and selective D-1 agonist failed, in systemic doses which have been demonstrated to have clear central effects, to affect the HHG axis in various experimental paradigms, suggests that this discrete D-1 neuronal system is probably not of major importance in the normal function of the HHG axis. Rather, its previously demonstrated involvement in LH secretion regulation is probably minimized by other (e.g. opiate, serotoninergic) transmitter systems, acting in a counter-regulatory fashion.

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Release of 3,5,3′-triiodothyronine, thyroxine and thyroglobulin from TSH-stimulated mouse thyroids in the perifusion system

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Summary. We established a perifusion system using mouse thyroid glands. In this system, TSH increased the release of T_3 and T_4 significantly, and the response of thyroglobulin to TSH was delayed in comparison with that of T_3 and T_4.
Key words. Thyroglobulin; TSH; T_3; T_4; mouse; thyroid; perifusion system.

Thyroglobulin (Tg) is synthesized in the follicle epithelial cells of the thyroid gland and secreted into the blood circulation in normal subjects[1]. Although the precise mechanism for the secretion of Tg still remains unknown, TSH is one of the factors which control Tg secretion[1].

Previously, we reported the response of thyroid hormones to TSH in a perifusion system with rat thyroid glands[2,3]. In the present study, the same perifusion and the specific RIA system for mouse Tg were employed in order to clarify Tg release from TSH-stimulated mouse thyroid glands.

Materials and methods. Perifusion systems: Mouse thyroid glands were used in perifusion systems as previously described[2,3]. After ether anesthesia, thyroid glands were removed from male BALB/c mice (8–10 weeks old) and bisected. The lobes obtained were preincubated in Krebs-Ringer bicarbonate buffer containing 0.1% glucose and 0.3% BSA (KRBG) at 37 °C for 90 min, and twenty pieces were placed in a chamber with a capacity of 0.25 ml. The thyroid pieces were then perifused with the same buffer at a flow rate of 1.9 ml/20 min. TSH (10 mU/ml) was added 60 min after the start of perifusion, and the procedure was continued for 3 h. Perifusates were collected at 20-min intervals and stored at – 20 °C until assay of contents.

T_3, T_4 and Tg RIA: We used a previously described RIA method for measurement of T_3 and T_4[2]. Tg concentration in each of the perifusates was measured using a double-antibody RIA method, following the procedure of Kawamura et al.[4]. Mouse Tg was prepared by the method of Tarutani et al.[5]. Antiserum against Tg was obtained from albino rabbits which had been initially injected intracutaneously with 750 µg of mouse Tg in complete Freund's adjuvant, and 28 days later with 500 µg of mouse Tg. Antiserum was used at 20,000-fold dilution. Amounts of T_3 and T_4 up to 5000 ng per tube did not interfere with the binding of ^{125}I Tg. Each assay was done in duplicate.

For the morphological observation, the thyroid tissues after 3-h stimulation were fixed in 10% formaldehyde solution, embedded in paraffin, and processed for light microscopy. The tissues were stained with hematoxylin-eosin.

TSH was obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents used were of analytical grade. The statistical significance of the data obtained was determined by Student's t-test.

Results. Figure 1 shows the profiles of the release of T_3, T_4 and Tg from the mouse thyroid specimens. TSH (10 mU/ml) gradually increased the release of T_3 for 3-h stimulation. T_3

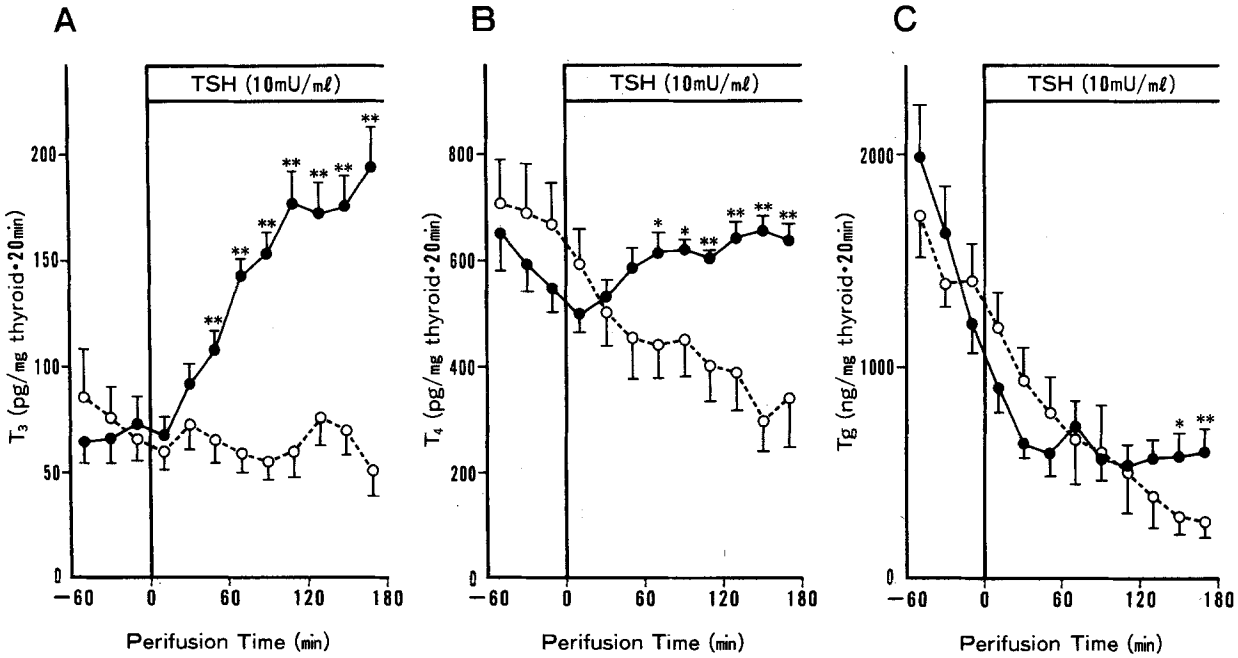

Figure 1. Profile of T_3, T_4 and Tg release from perifused mouse thyroids under continuous stimulation with TSH. KRBG was used as an infusion buffer for the initial period of 60 min, and was then changed to a buffer containing 10 mU/ml TSH (●—● n = 6). In the control group (○---○ n = 6), KRBG was infused for the whole 4-h period. Values are given as mean ± SE. * $p < 0.05$; ** $p < 0.01$ vs control.

release was increased significantly ($p < 0.01$) at 40 min after TSH stimulation. After 3-h stimulation with TSH, T_3 release was 194.3 ± 19.4 pg/mg thyroid · 20 min (mean ± SE), being markedly higher than that without TSH (51.5 ± 10.2). TSH-stimulated T_4 release was also increased significantly after 60 min of stimulation. T_4 release after 3-h stimulation with TSH was 638 ± 30 pg/mg thyroid · 20 min, which was higher than that without TSH (345 ± 98). An increase of Tg was observed at 140 min after stimulation with TSH, which was clearly delayed in comparison with that of T_3 and T_4.

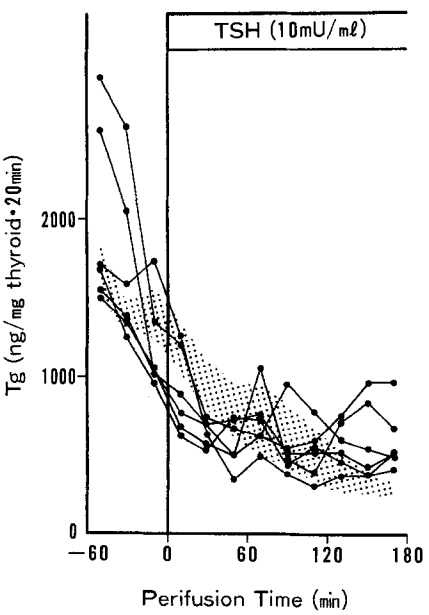

Figure 2. Tg secretion curves to TSH stimulation for each of the 6 preparations in fig. 1 C. The shaded area represents Tg release of the control group (mean ± SE).

After 160 min of stimulation with TSH, Tg release was 600 ± 70 ng/mg thyroid · 20 min, which was significantly higher than that without TSH (280 ± 50). Figure 2 shows the Tg-secretion curves for each of the 6 preparations in figure 1 C.

After 4-h perifusion, the thyroid pieces were observed by light microscope. As shown in figure 3 (a, b), the structure was well preserved in both groups and follicle epithelial cells after stimulation with TSH showed slight increase in cell height.

Discussion. We studied the release of thyroid hormones and Tg using a mouse thyroid perifusion system. The perifusion system enables us to observe dynamic effects of various substances on the thyroid gland in vitro and allows the exclusion of any influence of released thyroid hormones or other accumulated metabolites present in the medium [6]. In the present perifusion system, using mouse thyroid glands, a marked increase of T_3 and T_4 release was observed in response to TSH. The release of T_3 and T_4 was 3.8 times and 1.8 times higher, respectively, after 3-h stimulation with TSH in comparison with that without TSH. Furthermore, the structure of mouse thyroid pieces after 4-h perifusion was well preserved. Therefore, this perifusion method using mouse thyroids is very useful to study the function of the thyroid gland in vitro.

The response of serum T_3, T_4 and Tg to TSH or TRH administration in vivo has been reported by several authors [7–10]. In normal subjects, Belfiore et al. reported that serum T_3 and T_4 concentrations reached maxima at 4 h after TRH administration and that the peak of Tg occurred 6 to 72 h after TRH [7]. In all other studies as well, the response of serum Tg level was also delayed in comparison with increase in T_3 and T_4 [8–10]. With regard to in vitro studies, several reports of Tg release in response to TSH stimulation have been observed [11–13]. To our knowledge, there has been no report concerning the comparison between the release of thyroid hormones and that of Tg in vitro. In the present study, we observed the release of TSH-stimulated thyroid hormones

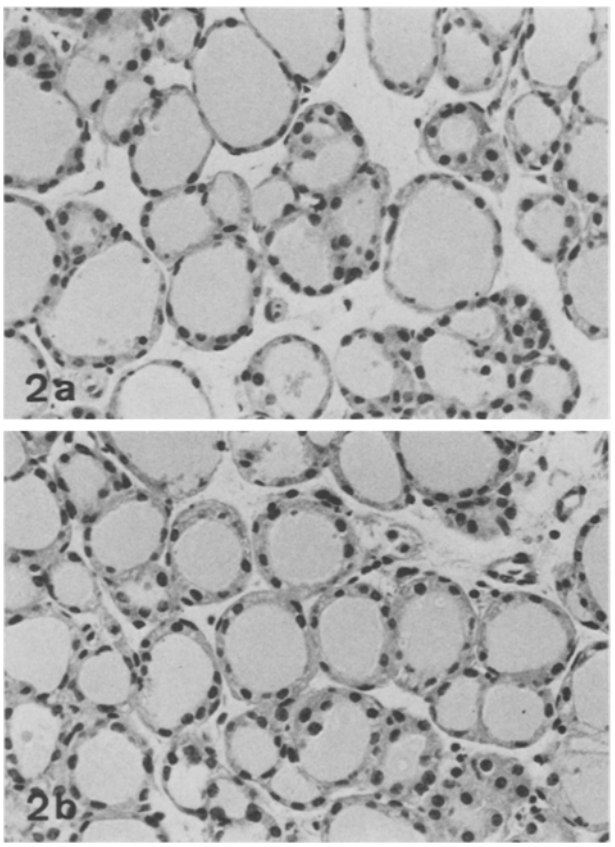

Figure 3. Mouse thyroid tissue after 3-h stimulation without TSH (*a*) or with 10 mU/ml of TSH (*b*). ×280

and Tg from perifused mouse thyroids. It is of interest that the TSH-induced release of Tg is delayed when compared with that of T_3 and T_4. For the mechanisms by which Tg crosses the closed follicular wall and reaches the circulation as an intact molecule, several possibilities have been discussed in the past: inflammation of the thyroid, temporary relaxation of tight junctions [1] and transepithelial vesicular transport of Tg [14]. Although the precise mechanism of TSH-induced Tg release has not been clarified yet, it is strongly suggested by the present study that the TSH-induced release mechanism is quite different between Tg and T_3 or T_4. This perifusion system may contribute to further elucidation of the mechanism of secretion of Tg.

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Spasmin-like proteins in various ciliates revealed by antibody to purified spasmins of *Carchesium polypinum*

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Summary. It was found that some ciliates, *Stentor, Spirostomum* and *Blepharisma,* which can contract rapidly like the stalks of Vorticellidae, have Ca^{2+}-binding proteins that are very similar to spasmins, in the immunological sense. The presence of spasmins in other Protozoa and in some Metazoa was also investigated.
Key words. Spasmin-like proteins; *Carchesium; Vorticella; Stentor; Spirostomum; Blepharisma;* immunoblotting.

Peritrich ciliates (*Vorticella, Carchesium* and *Zoothamnium*) have contractile stalks which are mainly composed of the spasmoneme and the surrounding sheath. Through investigations of the glycerinated spasmoneme [1–4], it has been shown that contraction of the spasmoneme is brought about by Ca^{2+} binding to some contractile elements composed of Ca^{2+}-binding proteins in the organelle. However, the molecular mechanism of the contraction is not known yet. Amos et al. [5] extracted two Ca^{2+}-binding proteins from isolated spasmonemes of *Zoothamnium geniculatum* and termed them spasmin a and spasmin b. Yamada and Asai [6, 7] reported that there are at least three kinds of Ca^{2+}-binding proteins in the stalks of *Carchesium polypinum,* and these Ca^{2+}-binding proteins are entirely different from calmodulin or troponin-C. Thus, the stalks contain new types of Ca^{2+}-binding proteins, and some of them are directly related to the contraction of the organelle. In this paper we use the term spasmins to refer to the Ca^{2+}-binding proteins extracted from the stalks of *C. polypinum.*

To elucidate the contractile mechanism of the spasmoneme, it is important to know which of the Ca^{2+}-binding proteins is essential to the spasmoneme-type contraction. It is not