Effects of photoperiod, temperature and testosterone-treatment on plasma T_3 and T_4 levels in the Djungarian hamster, *Phodopus sungorus*

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Summary. The effects of photoperiod, temperature and testosterone treatment on plasma T_3 and T_4 levels were investigated in the Djungarian hamster. Plasma T_3 level was affected by temperature (25 °C < 7 °C) but not by photoperiod. Plasma T_4 level was affected by photoperiod (short day < long day) at 25 °C. Administration of testosterone increased plasma T_4 level under short photoperiod at 25 °C. Thus, higher plasma T_4 level under long photoperiod at 25 °C might be induced by testosterone.
Key words. Djungarian hamster; photoperiod; temperature; T_3; T_4; testosterone.

In the Djungarian hamster, seasonal changes in various physiological parameters such as body weight, reproductive functions, fur color and daily torpor are mainly regulated by photoperiod[2-7] with minor effects of temperature[7]. Recently we found that plasma testosterone (Tsutsui et al., in preparation) and follicle stimulating hormone (FSH) levels and testicular gonadotropin receptors were also influenced mainly by photoperiod, though there were some effects of temperature on plasma FSH level[8]. Thyroid hormones in the Djungarian hamster might also be affected by photoperiod and temperature; it has been shown that these environmental factors have a substantial influence on T_3 (triiodothyronine) and T_4 (thyroxine) levels in Syrian hamsters[9-13]. However, to the best of our knowledge, there is only one report, published recently by Seidel et al.[14], on the Djungarian hamster under natural photoperiod and constant temperature. Therefore we investigated the effects of photoperiod and temperature on the plasma T_3 and T_4 of the Djungarian hamster. In addition, we studied the effect of testosterone administration on the plasma T_3 and T_4 levels, since some relationships between gonad and thyroid hormones have been suggested[15,16].

Materials and methods. Male Djungarian hamsters were obtained from our colony, which was derived from animals provided by Nissei Ken Co. (Tokyo). The animals had been raised from birth until 3–6 months of age under conditions of long-day photoperiod (about 16 h light and 8 h dark) and room temperature before the experiments started. Food (CE2, Nihon Haigo Shiryo Co., Tokyo) and tap water were given ad libitum. Plasma T_3 (triiodothyronine) and T_4 (thyroxine) were measured by radioimmunoassay using RIA kits (T-3 and T-4 RIABEASE; Dinabot Co., Tokyo). Cross-reactivity of the antibody of T-3 RIABEASE with L-thyroxine was 0.097% and that of T-4 RIABEASE with L-triiodothyronine and diiodotyrosine was 1.5% and <0.1%, respectively. Radioactivity was counted by a liquid scintillation counter with γ-vials (Packard Model 460C Tri-Carb, Ill.).

In experiment 1, adult male Djungarian hamsters kept under long-day conditions at room temperature (about 18 °C) were divided into 5 groups of 8 hamsters each. Four groups were subjected to different ambient temperatures and photoperiods; LD (16 h light and 8 h dark) at 25 °C, LD at 7 °C, SD (8 h light and 16 h dark) at 25 °C and SD at 7 °C. The remaining group served as the initial control. The hamsters of four groups were sacrificed by decapitation between 13.00 and 18.00 h after 8 weeks in the experimental conditions. Trunk blood was collected into heparinized glass tubes and centrifuged at 1800 × g for 20 min at 4 °C. Plasma samples from pairs of animals were pooled for the purpose of measurement of several hormones[8] (Tsutsui et al., in preparation), and stored at −20 °C until assayed.

In experiment 2, 28 hamsters previously maintained in LD at 25 °C for 12 weeks were divided into three groups. The 1st group (8 hamsters) was maintained in LD (LD control). The 2nd group (10 hamsters) was transferred to SD (SD control). Pieces of silastic tube (1.3 cm in length, 1.47 mm i.d. and 1.96 mm o.d.) filled with testosterone (Nakarai Chem. Co., Kyoto) were implanted s.c. into the hamsters of the 3rd group (10 hamsters). These testosterone implanted animals were transferred to SD (SD-T group). All groups were sacrificed 19 weeks later and plasma samples were obtained as in experiment 1.

The results on the organ weights (testes, epididymides and prostates), other plasma hormone levels (follicle stimulating hormone, testosterone) and testicular receptors for follicle stimulating and luteinizing hormones were published by Tsutsui et al.[8] or will be published elsewhere (Tsutsui et al., in preparation). The thyroid was fixed in 2.5% glutaraldehyde and 1% OsO_4, and embedded in Spurr resin, then semi-thin sections (1 μm) were made. Histological observation was done after toluidine blue staining, and epithelial cell height of the thyroid was measured with a digital ocular micrometer (OSM-D4, Olympus, Tokyo). Statistical analysis was done by analysis of variance, Student's t-test and Duncan's multiple range test.

Results. The result of experiment 1 is shown in figure 1. In LD (long photoperiod), plasma T_3 level at 7 °C was significantly higher than that at 25 °C ($p < 0.05$, fig. 1A). In SD (short photoperiod), plasma T_3 level at 7 °C was also higher than that at 25 °C ($p < 0.01$). However, there was no difference between LD and SD photoperiods at either high or low temperature. On the other hand, the plasma T_4 level at 25 °C in LD was higher than that in SD ($p < 0.01$, fig. 1B). Plasma T_4 level at 7 °C showed no statistical difference between LD and SD. Plasma T_4 level in LD was reduced by low temperature (by an amount close to the statistically significant level) but that in SD was not affected by low temperature, indicating that there was no additive effect of photoperiod and temperature. Histological observation revealed higher cell height in the follicle epithelium of the thyroid under low

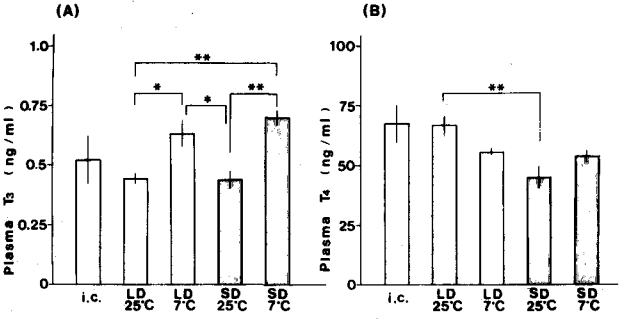

Figure 1. Effects of photoperiod and temperature on the plasma T_3 level (A) and T_4 level (B) in male Djungarian hamsters. i.c., initial control; LD, long photoperiod; SD, short photoperiod. Vertical bars in each column represent SEM. Significant difference (N = 4): *$p < 0.05$, **$p < 0.01$.

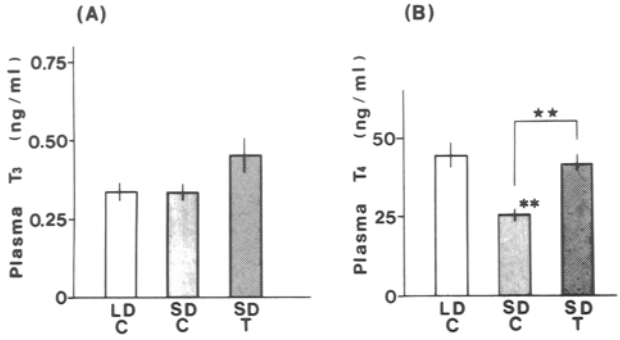

Figure 2. Effects of photoperiod and testosterone administration on the plasma T_3 level (A) and T_4 level (B) in male Djungarian hamsters. LD, long photoperiod; SD, short photoperiod; C, control; T, testosterone treatment. Vertical bars in each column represent SEM. Significant differences (LD-C: N = 4; SD-C, SD-T: N = 5): ** $p < 0.01$ (LD-C vs SD-C), ** $p < 0.01$ (SD-C vs SD-T).

temperature (6.31 ± 0.35 µm for LD, 6.64 ± 0.25 µm for SD) than under high temperature (5.56 ± 0.04 µm for LD, 6.15 ± 0.41 µm for SD) ($p < 0.01$; LD 25 °C vs SD 7 °C). But there was no statistically significant difference in the cell height between LD and SD.

The result of experiment 2 is shown in figure 2. Plasma T_3 level was not different between LD and SD controls (fig. 2A), confirming the result in experiment 1. Plasma T_3 level of the testosterone-treated SD group was slightly higher than that in LD and SD controls, but the difference was not statistically significant. Plasma T_4 level in the LD control group showed a higher value than that in the SD control ($p < 0.01$, fig. 2B), which confirmed the result of experiment 1. Plasma T_4 value in the testosterone-treated SD group was higher than that in the SD control ($p < 0.01$) and similar to that in the LD control. There was no difference in the cell height of the follicle epithelium of the thyroid among LD (5.63 ± 0.40 µm), SD (5.94 ± 0.27 µm) and SD-T (6.04 ± 0.25 µm) groups.

Discussion. In the present study, cold exposure for 8 weeks increased plasma T_3 level in both LD and SD conditions. Since the basal metabolic rate of Djungarian hamsters at 7 °C is about twice as high as that at 25 °C (Heldmaier and Steinlechner[3], and our unpublished data), this increase of T_3 level under low temperature appears to be associated with the metabolic change needed to maintain normal body temperature. On the other hand, the plasma T_4 level was affected by photoperiod at 25 °C (higher in LD than in SD). The reason why temperature affects only T_3 level and photoperiod affects only T_4 level might be that the activity of 5'-monodeiodination or of thyroid stimulating hormone is differentially affected by temperature and photoperiod. Seidel et al.[14] reported that the plasma T_3 and T_4 levels of adult Djungarian hamsters changed seasonally under natural photoperiod and constant temperature (23 °C); that is, the T_3 level in winter and early spring was higher than that in other seasons and the T_4 level in spring was higher than that in fall and winter. These results suggest that both T_3 and T_4 levels are affected by photoperiod. Their results on T_4 level agree with ours, but there is an inconsistency in the results on the T_3 level, since our results indicated no effect of photoperiod on the T_3 level. The discrepancy might be due to differences in age, season or experimental conditions. In the Syrian hamster, similar results to ours have been reported, i.e., plasma T_4 level was higher in LD than in SD[10,11,13], and plasma T_3 level was higher in natural photoperiod and temperature in winter than in SD at 22 °C[12] and showed no difference between LD and SD[13], although some discrepancies exist in T_4 levels at low temperature[10,12].

Plasma testosterone concentrations in the LD group (4.70 ± 0.41 ng/ml for 25 °C, 5.10 ± 1.04 ng/ml for 7 °C) were significantly higher than those in the SD group (0.34 ± 0.07 ng/ml for 25 °C, 0.18 ± 0.04 ng/ml for 7 °C) in experiment 1, and testosterone implantation in SD increased the plasma testosterone to the level of LD in experiment 2 (6.16 ± 2.11 ng/ml, 0.36 ± 0.12 ng/ml and 7.35 ± 0.69 ng/ml for LD, SD and SD-T, respectively) (Tsutsui et al., in preparation). These results for testosterone levels, together with those for T_4 levels described in the present report, strongly suggest a relationship between testosterone and T_4. In this connection, the results for the Syrian hamster reported by Vriend et al.[15] are interesting. They showed that blindness and melatonin injection induced reduction of plasma T_4 level as well as gonadal atrophy. But gonadectomy with or without blindness did not induce a reduction of plasma T_4 level. From these results they suggested that gonadectomy activated the pituitary-thyroid axis, owing to reduced feed back inhibition. However, they stated that this reasoning did not support the hypothesis that melatonin-thyroid effects were secondary to changes in the gonadal hormones. Since we have not examined the effect of melatonin or gonadectomy on the plasma T_4 level, we do not know whether a similar mechanism is working in the Djungarian hamster. However, from our present results gonadal hormones seem to be involved in the regulation of photoperiodically-induced change of plasma T_4 level. Since the physiological significance of photoperiodically regulated plasma T_4 level is not known, we are now performing experiments to clarify this question.

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