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Quantitative Analysis of Hypothalamic-Hypophyseal-Testicular System: Why Testosterone Can Act Under Negative Feedback Control

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Abstract. Quantitative analysis of the positive and negative feedback actions of testosterone (T) was carried out using intact rats, and orchidectomized rats implanted with T-filled Silastic capsules. Target organ weights and serum levels of LH and T were examined 7 days later, and response ratios of positive and negative actions were plotted against logarithm of serum T. From x-intercepts and slopes of regression lines, thresholds and responsiveness of reactions were calculated, respectively. Maintenance T levels necessary to maintain onset weights of the organ were also calculated from the regression lines. We compared 5 parameters at various ages, 1) thresholds for lowering serum LH, 2) mean T concentration of intact animals, 3) upper limit of serum T (mean + 2SD), 4) maintenance T levels, and 5) lower 95% limit of the thresholds for target organ response in orchidectomized animals. Though T can act between thresholds for target organ response and upper limit of serum T, the action range of T to induce organ growth over the onset organ weight (growth-inducing range) should be the area between the maintenance T level and the upper limit for serum T. At 3 weeks of age, the threshold for serum LH was very low, which makes the upper limit of serum T lower than maintenance T, allowing no growth-inducing range. From 5 weeks of age, the threshold for serum LH increased, and the serum T level with its upper limit also increased over the maintenance T level, allowing the presence of a growth-inducing range of T to make the growth of target organs possible.

Key words: Action range of testosterone, Negative feedback control, Threshold, Responsiveness.

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THOUGH RECENT progress in endocrinological studies is remarkable, the simple but critical question why the sex accessory organs can grow under the strict negative feedback control of testosterone (T) cannot be answered clearly. To answer this question, quantitative studies on the negative feedback system are essential, and the action ranges of serum hormone level should be clarified. But in general, such studies have been quite few in the field of endocrinology. In the hypothalamic-hypophyseal-gonadal system, T acts positively on sex accessory organs and levator ani, while it suppresses LH secretion as its negative feedback action, resulting in the reduction of T secretion. However, in most cases, such actions were discussed with reference to the results of a single or repeated administration of T, where blood levels of the hormone fluctuated widely [1, 2]. Such studies made it difficult to analyze quantitative relationships between serum levels of T and its positive and negative actions. In the present study, to make quantitative analysis possible, we tried a chronic replacement experiment by implanting Silastic capsules containing T in orchidectomized rats, which produced constant serum T levels. We then analyzed the quantitative relationships between the serum T levels and target organ weights or serum LH in orchidectomized rats of various ages and calculated the threshold levels of T to induce

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reactions and responsiveness of the reactions. We further calculated, with the serum T level-organ weight equation thus obtained, the serum T level necessary to maintain the target organ weight at the start of the experiment (maintenance T level), and through the comparison of these parameters with the actual serum T level and its upper limit in intact rats at corresponding ages, we could estimate the action ranges of T, showing why the target organ of T can grow during the sex maturation period under negative feedback control.

Materials and Methods

Animals

Male Wistar rats were purchased from Charles River Japan. All were housed in temperature controlled rooms on a lighting schedule of 12 h light: 12 h dark with lights on at 0600 h, and supplied with food and water *ad libitum*.

Testosterone implants

Constant-release capsules for T administration were made from Silastic medical grade tubing (1.57 mm id; 3.18 mm od; Dow Corning Corp.) and sealed with Silastic medical adhesive silicone type A (Dow Corning Corp.) according to the method of Legan *et al.* [3] and Zirkin *et al.* [4] with minor modifications. The amounts of T powder (Wako Pure Chemical Industries) to be inserted were 2, 5 and 10 mg for small, medium and large dosage groups of 10 weeks old rats, respectively. The amounts of T for other age groups were calculated from the ratio of their mean body weight to that of 10-week-old rats as listed in Table 1. The length of the capsules was roughly proportional to the amount of T, i.e. about 0.8 mm/mg T, however, for technical reasons, the minimum length was fixed at 1 mm. These capsules were incubated in saline for 24 h at room temperature prior to implantation.

Cannulation

Rats were anesthetized with diethyl ether, and Silastic medical grade tubing (0.64 mm id; 1.19 mm od; Dow Corning Corp.) was inserted into the right external jugular vein, and the distal portion of the catheter was passed below the skin and exteriorized dorsally between the ears [5, 6]. The cannula was filled with a viscous mixture of heparin (500 U/ml), 10% polyvinylpyrrolidone, and 0.9% NaCl, and flushed at least three times daily with the same mixture to prevent coagulation.

Experiment 1

In the first experiment, male Wistar rats were orchidectomized at 10 weeks of age, and at the same time, implanted with 5 or 10 mg T-filled capsules subcutaneously in the back (n= 7 for each group). Blood samples were obtained via the cannula from each rat starting from the next day until the 13th day after implantation at 48 h intervals, then stored overnight at 4°C. All sampling was done between 1400 and 1500 h. The sera obtained after centrifugation at 1000×g for 15 min were stored at −20°C until the assay.

Experiment 2

Male Wistar rats were orchidectomized and received either empty (n=7/age group) or T-filled (n= 21–26/age group) Silastic capsules as shown in Table 1 at 3, 5, 7, 10 and 32 weeks of age. At the time of operation, a Silastic cannula was inserted, and 7 days after cannulation, blood samples were collected and stored overnight at 4°C. As the onset control, blood samples were collected for intact animals (n=5/age group). All sampling was done between 1400 and 1500 h. The sera obtained after centrifugation at 1000 ×g for 15 min were stored at −20°C until the assay. The animals were then sacrificed and their seminal vesicles, ventral prostates, and levator ani were dissected out and immediately immersed in 10% formalin, and weighed 3 days later.

Table 1. Doses of testosterone implanted

Age (weeks at operation)	Dosage (mg)		
	Small	Medium	Large
3	0.24	0.60	1.20
5	0.72	1.80	3.60
7	1.12	2.80	5.60
10	2.00	5.00	10.00
32	3.31	8.28	16.55

Radioimmunoassay

The serum LH concentration was determined by double antibody RIA [7], with a NIDDK assay kit (NIDDK-rLH-I-9 for radioiodination, NIDDK-rLH-RP-3 as the standard and NIDDK-anti-rLH-S-10 as the antiserum) with 2nd antibody prepared in our laboratory (HAC-RBA2-03GTP86). The sensitivity and intra- and inter-assay coefficients of variation were 0.125 ng/ml, 2.1%, 15.0%, respectively. Serum T was measured with a DPC total testosterone kit (Diagnostic Products Corp.). The sensitivity and intra- and inter-assay coefficients of variation were 0.04 ng/ml, 6.6% and 16.3%, respectively.

Calculation of serum T level-response parameters

From the target organ weights and LH levels obtained at the end of experiment 2, the response ratios (RR) of individual animals in the experimental group were calculated as RR = (R−Ro)/Ro × 100, where R is the organ weight or LH level and Ro is the mean organ weight or LH level of the control orchidectomized animals implanted with empty capsules. Scatter diagrams were prepared by plotting these response ratios against logarithm of serum T levels of the corresponding animals, and the regression lines were calculated by the least squares method in two ways: one Y on X, where the sum of squares of the Y coordinate (i.e. RR) differences was made minimum; the other X on Y, where the sum of squares of the X coordinate (i.e. log T) differences was made minimum. The slope of the Y on X regression line indicates the responsiveness, while the x-intercept of the X on Y regression line shows the threshold of the response.

Estimation of maintenance T levels

From the onset target organ weights of intact animals and equations for the serum T level-organ weight relationship, we calculated the theoretical serum T levels which were necessary to maintain the onset organ weights at various ages. We call them the "maintenance T levels".

Results

Serum T concentration after implantation of T capsule

Figure 1 shows the serum T concentration measured at 48 h intervals during 13 days after orchidectomy and capsule implantation at 10 weeks of age. Serum T levels showed rather high values on the day after implantation, then de-

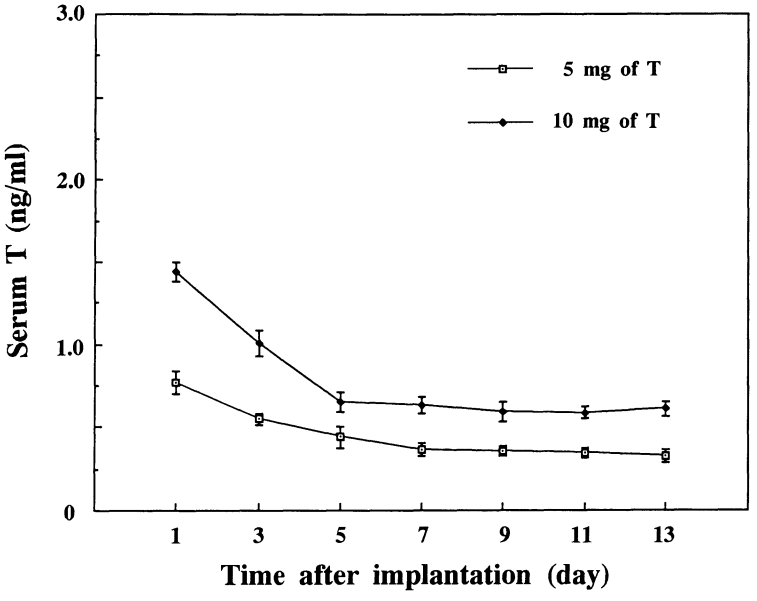

Fig. 1. Serum T concentration after implantation of T-filled capsules in orchidectomized 10-week-old rats. Mean for 7 animals and SEM.

creased until the 5th day and thereafter maintained constant values, indicating that the release of T from the capsule was quite constant.

Serum hormone levels and organ weights in age and dosage groups

The mean serum hormone levels and organ weights in onset intact control animals are shown in Table 2. Serum T levels which were very low at 3 weeks of age increased until 7 weeks of age, and remained almost constant thereafter. The mean+2SD (standard deviation) serum T levels in various age groups are thought to be the allowable maximum serum T levels for these age groups, because the probability of higher serum T levels over mean +2SD is only 2.3% in normal animals.

The mean serum hormone levels and organ weights of the experimental groups 7 days after orchidectomy and T-implantation are shown in Table 3. These doses of T could maintain nearly normal to subnormal serum T levels. Orchidectomy-induced increase in serum LH levels and weight loss of the target organs were blocked dose-dependently.

Table 2. Serum hormone concentrations and organ weights in intact males (onset control)

Age (weeks at sacrifice)	Number of rats	Serum T (ng/ml)	Serum LH (ng/ml)	Seminal vesicles (mg)	Ventral prostate (mg)	Levator ani (mg)
3	5	0.05 ± 0.01	0.61 ± 0.02	6.54 ± 0.37	12.64 ± 1.43	14.54 ± 0.66
5	5	0.19 ± 0.03	0.32 ± 0.05	23.10 ± 1.87	32.10 ± 2.98	30.76 ± 1.85
7	5	1.34 ± 0.21	0.48 ± 0.07	153.80 ± 22.10	81.02 ± 6.60	80.76 ± 1.44
10	5	1.28 ± 0.20	0.42 ± 0.12	626.06 ± 22.65	182.30 ± 12.34	196.10 ± 19.39
32	5	1.54 ± 0.27	0.38 ± 0.09	1427.50 ± 40.96	242.78 ± 22.47	380.02 ± 10.90

The results are shown as the mean ± SEM.

Table 3. Serum hormone concentrations and organs weights 7 days after orchidectomy and after receiving various doses in T-filled capsules

Age (weeks at operation)	Number of rats	Treatment	Serum T (ng/ml)	Serum LH (ng/ml)	Seminal vesicles (mg)	Ventral prostate (mg)	Levator ani (mg)
3	10	castrate	N.D.	4.83 ± 0.44	6.29 ± 0.70	4.76 ± 0.32	14.49 ± 1.67
	5	Small T	0.38 ± 0.03	1.15 ± 0.16	38.72 ± 1.35	32.58 ± 2.23	20.64 ± 0.89
	8	Medium T	0.53 ± 0.06	0.43 ± 0.16	47.34 ± 1.76	30.80 ± 1.94	26.51 ± 1.64
	10	Large T	0.93 ± 0.15	0.17 ± 0.05	65.59 ± 5.12	46.12 ± 4.17	33.55 ± 1.99
5	7	castrate	N.D.	5.95 ± 0.46	11.53 ± 0.56	12.90 ± 1.25	29.02 ± 3.38
	10	Small T	0.22 ± 0.04	4.29 ± 0.43	29.97 ± 2.28	43.00 ± 3.38	43.96 ± 3.43
	6	Medium T	0.23 ± 0.05	3.76 ± 0.39	31.45 ± 5.05	48.07 ± 1.60	48.05 ± 3.31
	8	Large T	0.92 ± 0.22	2.97 ± 0.22	48.35 ± 3.21	61.71 ± 3.30	57.46 ± 3.86
7	9	castrate	N.D.	5.04 ± 0.29	47.43 ± 1.24	23.40 ± 1.41	61.51 ± 3.49
	8	Small T	0.13 ± 0.03	4.56 ± 0.26	70.25 ± 2.43	53.78 ± 6.13	74.88 ± 3.30
	6	Medium T	0.40 ± 0.07	4.16 ± 0.54	84.60 ± 5.79	80.58 ± 4.88	84.23 ± 3.10
	7	Large T	1.54 ± 0.24	3.22 ± 0.24	144.71 ± 22.91	91.83 ± 4.36	95.43 ± 7.22
10	9	castrate	N.D.	5.06 ± 0.42	166.71 ± 5.24	47.78 ± 4.24	173.40 ± 8.33
	5	Small T	0.22 ± 0.05	4.36 ± 0.42	223.40 ± 29.13	86.86 ± 1.22	169.70 ± 5.48
	10	Medium T	0.56 ± 0.06	3.94 ± 0.19	415.27 ± 21.24	122.00 ± 5.58	205.41 ± 10.76
	11	Large T	0.86 ± 0.09	3.28 ± 0.36	523.13 ± 24.93	141.36 ± 5.64	219.97 ± 6.11
32	9	castrate	N.D.	2.77 ± 0.24	609.59 ± 66.29	125.91 ± 8.80	319.94 ± 11.13
	5	Small T	0.22 ± 0.03	2.51 ± 0.10	619.92 ± 81.79	190.68 ± 24.12	328.35 ± 9.44
	10	Medium T	0.47 ± 0.09	1.45 ± 0.26	1047.84 ± 52.46	259.15 ± 25.91	373.20 ± 13.13
	9	Large T	0.94 ± 0.10	0.20 ± 0.04	1363.80 ± 91.70	300.38 ± 13.34	416.68 ± 20.86

The results are shown as the mean ± SEM. N.D., not detectable .

Thresholds and responsiveness of positive and negative feedback action of T in age groups

An example of scatter diagrams, that of ventral prostate weights plotted against logarithm of serum T levels in 7 weeks old rats is shown in Fig. 2. Two regression lines, Y on X and X on Y, together with the threshold level of T (X-intercept of X on Y regression line) and responsiveness (slope of Y on X regression line) are also shown in the same figure.

Table 4 summarizes threshold levels of T and responsiveness of the target organs and serum levels of LH obtained for age groups by the same procedure as in the example.

The threshold of the serum T level sufficient to cause a serum LH reduction in orchidectomized rats was very low at 3 weeks of age. From 5 to 7 weeks of age, the thresholds were raised, reaching a peak at 7 weeks of age, and gradually fell thereafter. On the other hand, the threshold of the serum T level sufficient to cause a target organ weight increase in orchidectomized animals, was high at 3 weeks of age, then fell at 5 to 7 weeks of age, and again rose, showing a quite opposite tendency to the negative feedback threshold. When compared, the threshold for negative feedback action was lower than those for positive actions on sex accessory organs at 3 weeks of age, but was higher during 5–10 weeks of age, and again became lower at 32 weeks of age. The thresholds for levator ani, a target organ of anabolic action of T, was higher than that for the negative feedback action at 5, 10 and 32 weeks of age.

The responsiveness, i.e. the slopes of the regression lines which are interpreted as percent increases in the target organ weight or percent decrease in serum LH level in orchidectomized animals which is caused by a 10-fold increase of serum T level, also changed considerably. The responsiveness of serum LH to T, which was high at 3 weeks of age, decreased and stayed low during sexual maturation, then increased thereafter. The accessory sex organs were very responsive at 3 weeks of age but this responsiveness had also decreased at 5 weeks of age, but maintained 100–200% values thereafter. The responsiveness of the levator ani also showed a similar tendency, though the values were relatively low (Table 4).

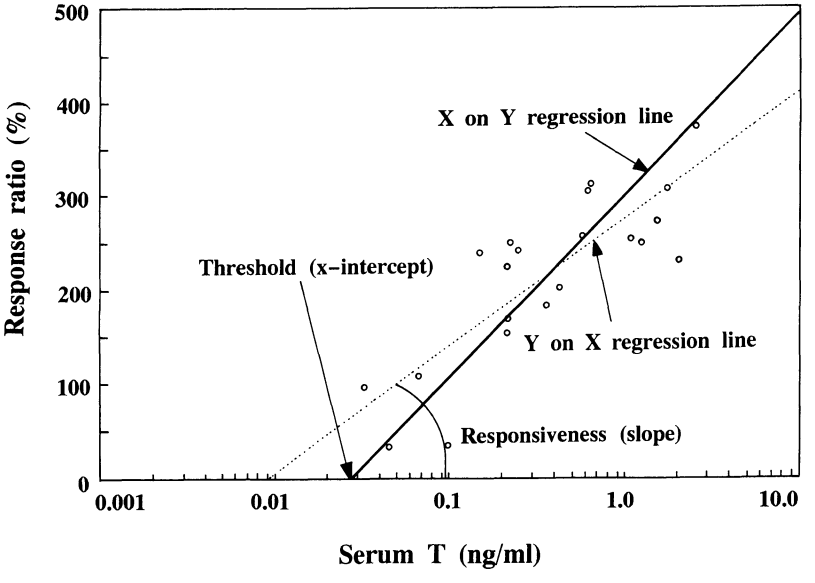

Fig. 2. A scatter diagram obtained by plotting response ratios against logarithm of serum T concentrations, regression lines, and estimation of threshold and responsiveness. An example of ventral prostates of 7-week-old rats.

Table 4. Thresholds and responsiveness of positive and negative feedback actions of testosterone

Age (weeks at operation)	Number of rats		LH	Seminal vesicles	Ventral prostate	Levator ani
3	23	R	0.83	0.44	0.57	0.62
		Xo	0.03 [0.01–0.08]	0.27 [0.13–1.58]	0.22 [0.11–0.44]	0.25 [0.15–0.43]
		Resp.	49.00 (7.50)	484.43 (219.42)	559.19 (178.45)	106.06 (30.12)
		Yo	102.63 (2.34)	948.99 (67.26)	845.38 (54.70)	126.27 (9.23)
5	24	R	0.56	0.75	0.83	0.37
		Xo	0.10 [0.04–0.22]	0.07 [0.04–0.14]	0.03 [0.01–0.06]	0.18 [0.08–0.36]
		Resp.	25.11 (8.12)	197.20 (39.47)	194.09 (29.06)	46.61 (26.01)
		Yo	51.65 (5.46)	320.02 (26.01)	393.80 (19.15)	101.36 (17.14)
7	21	R	0.46	0.81	0.82	0.73
		Xo	0.35 [0.14–0.89]	0.10 [0.06–0.18]	0.03 [0.01–0.07]	0.09 [0.04–0.19]
		Resp.	18.47 (10.40)	157.71 (26.02)	135.15 (21.34)	33.26 (7.08)
		Yo	27.71 (4.37)	199.90 (18.17)	274.78 (14.90)	52.14 (4.94)
10	26	R	0.72	0.82	0.51	0.58
		Xo	0.29 [0.21–0.40]	0.19 [0.13–0.27]	0.17 [0.07–0.43]	0.45 [0.33–0.62]
		Resp.	51.33 (10.97)	231.09 (36.48)	90.52 (34.33)	48.01 (15.84)
		Yo	41.12 (3.74)	219.61 (14.09)	199.98 (13.26)	32.66 (3.80)
32	24	R	0.91	0.71	0.58	0.49
		Xo	0.15 [0.11–0.20]	0.23 [0.14–0.38]	0.19 [0.10–0.37]	0.32 [0.19–0.52]
		Resp.	96.91 (9.89)	114.64 (27.18)	99.41 (31.29)	26.35 (10.75)
		Yo	91.48 (4.01)	120.57 (9.59)	147.86 (13.95)	29.89 (4.66)

R, correlation coefficient; Xo, threshold (x-intercept of X on Y regression line); [ ], 95% confidence limit; Resp, responsiveness (slope of Y on X regression line); Yo, intercept of Y on X regression line; ( ), SEM.

Action range of T estimated from parameters

In Fig. 3, we have summarized changes in various parameters for each target organ according to the age of the rat. These parameters are 1) threshold for serum LH reducing action in the orchidectomized rat, 2) mean actual serum T concentration in the intact rat, 3) mean+2SD serum T concentration, 4) T concentration to maintain the onset weight of the target organ (maintenance T level) calculated from the serum T level-organ weight equation, and 5) lower 95% confidence limit of the threshold for target organ growth in the orchidectomized rat. Fig. 3A is a schematic figure explaining the relationship between these parameters. The threshold for the serum LH reducing reaction (line 1) indicates serum T levels where negative feedback action would start. As the result of the negative feedback control, the serum T concentration (line 2) cannot increase over the mean+2SD (line 3). On the other hand, T action on the target organ weight would start from the lower limit of the threshold (line 5). However, in order to cause any growth of the target organ to over the onset weight, a higher concentration than the maintenance T level (line 4) is necessary. The entire action range of T would therefore be the area between lines 3 and 5. But "physiological growth-inducing range" which causes a further organ weight increase should be the area between lines 3 and 4. The two action ranges of serum T for the target organs are shown in Fig. 3B–D. At 3 weeks of age, the threshold for serum LH was quite low, so it made possible a very low serum T concentration. This T concentration could not exceed the T concentration necessary for the growth of the organ. In other words, a growth-inducing range of T does not exist. When the rats were in the sexually maturating period, the threshold for negative feedback action was raised up, and upper limit for serum T levels increased in parallel to the threshold. The actual serum T levels (line 2) increased to reach the growth-inducing range.

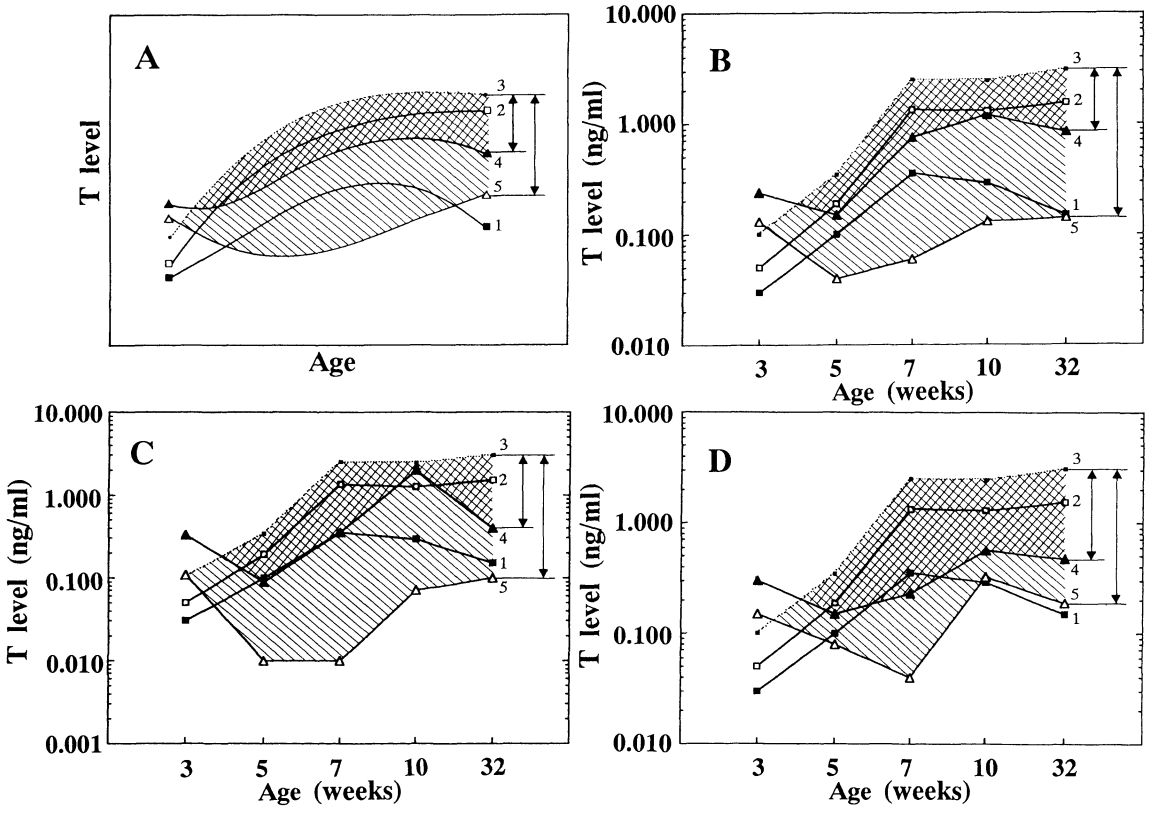

Fig. 3. Changes in various parameters of T action, and action range of serum T. A, schematic expression; B, seminal vesicles; C, ventral prostates; D, levator ani. Line 1 (—■—), thresholds for serum LH; Line 2 (—□—), mean T concentrations in intact animals; Line 3 (···■···), mean concentrations +2SD; Line 4 (—▲—), maintenance T concentrations; Line 5 (—△—), lower 95% limit of the threshold for organ response. Area between line 3–4, growth-inducing range of T; Area between 3–5, entire action range of T.

Discussion

In the present study, we attempted to quantitatively analyze T actions including positive actions on its target organs and negative feedback action on serum LH. For such analyses, together with the observation on intact rats, we carried out a T-replacement experiment in orchidectomized rats to estimate the serum T concentration-reaction relationship. In the experiment, it is preferable to keep the serum T concentration constant throughout the experimental period. We used constant T-releasing Silastic capsules which produce a stable serum T concentration in orchidectomized rats. It has been reported that the serum T level and the effects of T on the serum LH concentration and target organs [8, 9] varied depending on the dose of T administered and the length of the Silastic capsule [10].

In the first experiment, we examined the serum T concentration after T-filled capsule implantation, and found that the concentration seemed to be constant from 5 days after implantation (Fig. 1). We decided on a period of 7 days for the experiment because our preliminary observations showed that target organ weight decreases to the minimum within a week after orchidectomy and the effect of T on serum LH appears within 1 h. We also confirmed that the serum T concentration was proportional to the amount of T and the length of the capsule. From the purpose of our experiments, an unusually high serum T concentration is not suitable. We aimed to keep the serum T concentration subnormal to normal to calculate the

threshold as exact as possible. The dosage we chose, shown in Table 1, satisfied this demand. In our study, the dosage of T itself implanted into the animals is not important, but the serum T concentration produced by the implantation is the essential factor. So, we monitored the serum T concentration in all individuals. As mentioned above, the serum T concentrations after T-capsule implantation were considerably high for 3 days. But when we compared the target organ weights 5 days and 7 days after implantation in our preliminary experiment, we did not observe any significant differences. This indicates that we can ignore the possible influence of the serum T concentration for the first 3 days if we sacrifice the animals 7 days after implantation.

From the data for the T-replacement experiment, we calculated two parameters, threshold and responsiveness, of T action as described in the Materials and Methods section.

The relation of the thresholds for positive and negative actions is thought to be important for positive T actions. T can act positively within the range between the lower limit for the threshold for positive action and the upper limit for the serum T concentration which are allowed in the negative feedback control system. The upper limit for serum T would be primarily determined by the threshold for negative feedback action because at this point the serum LH reducing effect starts. However, the point where the negative feedback action works fully to stop further secretion of T must be determined by the responsiveness of the negative feedback system, i.e. the responsiveness of the pituitary to slow down LH release and the responsiveness of the testis to slow down T release in response to lowered LH. Practically, in order to estimate this point, we should observe the serum T concentration in intact animals, and the upper limit for serum T in intact animals must be the point. We adopted mean + 2SD as the upper limit.

Though the entire working range of T was determined in this way, if the concentration of T is below the maintenance T level for a target organ, the organ will lose weight. In order to cause any target organ growth, the action range of T must be between the maintenance T level and the upper limit for serum T as indicated in Fig. 3. We call this range the "physiological growth-inducing range".

Damassa *et al.* [11] reported that plasma T concentrations lower than 0.5 ng/m*l* did not influence the plasma LH concentrations in orchidectomized rats, and that the concentrations higher than 1.8 ng/m*l* could suppress the plasma LH to intact levels. Though they did not estimate the threshold by statistical treatment, this roughly corresponds to the area, in our experiment, between threshold for serum LH and upper limit for serum T in 7 weeks old rats as shown in Fig. 3.

As to the thresholds, it was very clear that the threshold of T for the serum LH lowering action, in other words the negative feedback action in the prepubertal (3 weeks of age) rats, was very low, which makes the serum T concentrations to stay low (Table 2 and Fig. 3). On the other hand, the thresholds for the positive actions and maintenance T levels were much higher. This means that there is no action range for T. From these facts we understand why the target organs remain small at this age. We observed that the pituitary itself had the ability to synthesize and release LH, because at 3 weeks of age orchidectomy induced a high serum LH concentration comparable to those observed in other age groups (Table 3).

At 5 weeks of age, the threshold for negative feedback action started to increase, reaching its maximum at 7 weeks of age, then gradually decreased (Fig. 3), while the threshold for target organ growth changed in quite the reverse way, i.e. the high threshold at 3 weeks of age lowered during sexual maturation, then increased again, though there was some variation depending on the organ. The actual serum T concentration can increase over the maintenance T level to cause organ growth during the sexual maturation period, and the T concentration stayed rather constant from 7 to 32 weeks of age, while the responsiveness of the target organ showed a tendency to decrease at 32 weeks of age. This would decelerate organ growth.

Smith *et al.* [8] reported on their T-replacement experiment that a given plasma T concentration produced a greater suppression of plasma LH in the prepubertal than in older animals. Though they did not distinguish threshold or responsiveness, their results support our present finding that the threshold is low and responsiveness is high at 3 weeks of age.

The responsiveness in the negative feedback action changed in the opposite way to that of the threshold (Table 4). This fact is possibly explained by the changes in the amount of androgen and estrogen receptors in the hypothalamus or those of

LHRH receptor in the pituitary gland. Kato [12] studied steroid receptors in hypothalamic tissue cytosols of developing male rats, and observed that androgen receptor, which appeared as early as 7 days after birth, continue to increase until 28 days, though no information was obtained after this age. It is also possible that estrogen receptors together with aromatizing enzyme would influence the threshold and responsiveness of the negative feedback action. The androgen receptor in the ventral prostate was reported to increase from 16 to 50 days of age, and decrease thereafter [13, 14]. This explains the decrease in the threshold for T action on the prostate during 5 to 7 weeks of age, and its increase thereafter. However the changes in the responsiveness of the target organs cannot be explained exclusively by the changes in the androgen receptor. There must be other factors involved, because an increase in the amount of the receptor should also cause an increase in responsiveness. These questions should be clarified in future.

So far we have only studied rats until 32 weeks of age, but the changes in these parameters for T actions as a function of age are interesting. Throughout the trial to analyze the action and negative feedback action of T, we were able to construct a model for endocrine system analysis. This kind of analysis is really important because most studies in endocrinology so far have been qualitative, and no quantitative simulation based on the results of qualitative studies is possible. Our present analytical method should be useful in the analysis of other endocrine controlling systems also.

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