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Pituitary and Serum Levels of Prolactin (PRL), Thyroid Stimulating Hormone (TSH) and Serum Thyroxine (T4) in Hereditary Dwarf Rats (*rdw/rdw*)

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Female and male hereditary dwarf mutation rats (*rdw*), offspring of those found by Koto et al. in a breeding colony of Wistar-Imamichi rats, were decapitated to collcet blood and pituitary glands at 10 weeks of age. Levels of pituitary prolactin (PRL), thyroid stimulating hormone (TSH) and serum PRL, TSH and thyroxine (T4) in *rdw* rats were measured and compared with levels in normal rats (N). The hormone levels were measured with radioimmunoassay (RIA). It was found that the levels of pituitatry and serum PRL were considerably lower in *rdw* rats and that the level of serum T4 was also significantly lower than that in normal rats. TSH per pituitary was significantly lower in *rdw* rats but TSH per mg pituitary was not different between *rdw* and normal rats. The levels of serum TSH in *rdw* was not different from that in normal rats, but was rather higher in female *rdw* rats than in normal female rats. When the results of previous research were combined with the present results, it was clear that *rdw* rats are characterized by hypoplasia of GH and PRL synthetic cells of the pituitary and that *rdw* rat is possibly useful as a model animal with endocrinological defects in pituitary PRL and GH.
——KEY WORDS : dwarf, PRL, TSH, T4, rat

Koto *et al*. found a new strain of dwarf rat in a colony of CSK : Wistar-Imamichi rats and named it *rdw* [10]. They histologically examined the anterior pituitary of this rat and reported it to be especially characterized by a decrease in cell number and hypoplasia of secretory cells of growth hormone (GH), prolactin (PRL) and thyroid stimulating hormone (TSH) [10]. These rats seem to be different from the dwarf rats which were recently found genetically only GH deficient [18,5]. Umezu *et al*. previously reported that both female and male *rdw* rats showed remarkably low levels of GH in the blood and pituitary after the estimation of GH, follicle stimulating hormone (FSH) and luteinizing hormone (LH) with radioimmunoassay (RIA) using kits for measuring levels of rat pituitary protein hormones [24]. More information can be expected to be forthcoming on *rdw* rats when levels of some remaining horomones are determined.

In the present study, we examined the blood and pituitary levels of PRL and TSH and the blood levels of thyroxine(T4) in *rdw* and normal rats.

Materials and Methods

Animals : Adult female and male rats which had been confirmed to bear pups including *rdw* offsprings were mated to obtain *rdw* rats. Pups of *rdw* and normal litter mates (Normal : N) were fed until 10 weeks of age. A total of 64 rats, including 18 female dwarfs and 15 male dwarfs, were subjected to PRL, TSH and T4 assay.

Feeding conditions: Animals were placed in polycarbonated cages (25 x 40 x 20 cm) with

Table 1. Pituitary and plasma levels of PRL and TSH in *rdw* and normal rats

Items	Female		Male	
	rdw	Normal	*rdw*	Normal
No. of Animals	13	13	9	5
Body wt. (g)	77.7± 4.8***[a)]	191.6± 3.5	95.2± 7.4***	253.4± 8.0
Pituitary wt. (g)	4.9± 0.4***	9.0± 0.4	5.1± 0.5**	10.1± 1.1
PRL : serum (ng/mℓ)	6.3± 0.4**	16.7± 2.7	5.4± 0.6*	23.9± 5.2
PRL : pit (ng/pit)	290 ± 55***	10,487 ±1,009	119 ± 32**	2,961 ± 342
PRL : pit/mg (ng/mg·pit)	61.4 ± 12.0***	1,150.4± 108.2	22.6± 4.9**	316.7± 62.2
TSH : serum (ng/mℓ)	14.8 ± 0.9***	4.6± 1.0	12.5± 0.7	9.3± 1.9
TSH : pit (ng/pit)	3,648 ±228***	9,754 ±1,260	4,586 ± 529**	12,772 ±1,732
TSH : pit/mg (ng/mg·pit)	913.1±108.7	1,069.7± 122.3	941.2± 115.8	1,286.0± 116.5

[a)] Mean±S.E. * P<0.05 ** P<0.01 *** P<0.001 Rats were autopsied with decapitation at 10 weeks of age and hormone levels were estimated with RIA.

wood shavings on the floor and fed in a room maintained at a temperature of 24±2℃ and humidity of 65±5 %. They were given bullet-type commercial feed (Super pig-gold : Zen-nou) and tap water *ad libitum*. Light was turned on at 6 : 00 A.M. and off at 6 : 00 P. M.. Normal animals were weaned at 3 weeks but *rdw* rats were weaned at 5 weeks after birth according to the earlier report [10]. Blood and pituitary sampling: Animals were decapitated to collect blood at 1:00-3:00 P.M. at 10 weeks of age and the blood was immediately separated into serum by centrifugation. In the case of female rats, vaginal smears were taken beginning over a week before decapitation, and both *rdw* and N rats were confirmed to be in a diestrous state. The pituitary was dissected for weighing and then homogenated with an ultrasonic homogenizer by adding 1mℓ of 0.01 M phosphate buffer (pH 7.5) and centrifuged. The supernatant was designated "pituitary extract". Serum and pituitary extracts were kept in a freezer at -60℃ until use.

RIA : The levels of PRL and TSH were assayed using RIA kits for rat pituitary hormones employing a double antibody system, the kits being supplied by NIAMDD, Bethesda, Maryland, USA. These hormone kits included rPRL-I-6 and rTSH-I-9 of pure hormone for iodination, rPRL-S-9 and rTSH-S-5 for antibody and rPRL-RP-3 and rTSH-RP-2 for standard of hormone. Hormone content of the pituitary was represented as per pituitary and per mg pituitary. The minimum detectable dose of PRL and TSH was 1ng and the devia tions of intra-assay coefficient were 4.3 and 1.0%, respectively. The total thyroxine (T4) levels were assayed with RIA according to polyethylene glycol(PEG) method [14].

Statistical analysis : Statistical analysis of differences between *rdw* and normal rats was made with Student's *t* test.

Results

Body and pituitary weights : As shown in Table 1, body weight of *rdw* was less than half that of N rats regardless of sex, indicating remarkable lower values. Pituitary weight of *rdw* rat was about half that of normal rats (P <0.01-0.001).

Pituitary and serum levels of PRL and TSH : Pituitary content of PRL of *rdw* rats was much less than that in N rats in both values per pituitary and per mg pituitary (P<0.01-

Table 2. Plasma Thyroxine (T4) levels in *rdw* and normal rats

Items	Female		Male	
	rdw	Normal	*rdw*	Normal
No. of Animals	6	6	6	6
Bodywt.(g)	68.3±4.0***[a)]	170.0±9.0	89.2±7.2***	221.5±13.1
T4 : serum (ng/mℓ)	24.2±1.2**	51.7±5.9	23.5±0.5***	51.9± 3.7

[a)] Mean±S.E. * P<0.05 ** P<0.01 *** P<0.001
Rats were autopsied with decapitation at 10 weeks of age and hormone levels were estimated with RIA.

Table 3. Percent of mean hormone level of *rdw* rat to that of normal rat (*rdw*/N × 100)

	Sex	PRL(%)	TSH(%)	T4(%)
Pituitary /pit	Female	2.7***	37.4***	—
(/pit. mg)		(9.4***)	(85.4)	
	Male	4.1**	35.9**	—
		(7.2**)	(73.2)	
Serum	Female	37.1**	321.7**	46.8**
	Male	22.5*	134.4	45.3***

* P<0.05 ** : P<0.01 *** P<0.001
Mean value of each pituitary hormone corresponds with the value quoted in Table 1 and 2.

0.001). Serum concentration of PRL of *rdw* rats were significantly lower than that in N rats (P<0.05-0.001), indicating a value of less than 40% of the control. In pituitary content of TSH, values per pituitary were significantly lower in *rdw* rats (P<0.01-0.001), but the difference in value per mg pituitary was not significant between *rdw* and N rats. Serum concentration of TSH was higher in *rdw* rats than N rats, especially in female *rdw* rats in which valuse were four times higher (P<0.001).

T4 levels in serum : In this case *rdw* animals were especially sampled. The body weight of *rdw* rats was lower than that of the control animals (less than 1/2 of control) (P<0.001). There was a similar trend as that shown in Table 1 (Table 2). In the concentration of T4, *rdw* rats showed significantly lower values than N rats (P<0.01-0.001).

Percent of values of *rdw* rats per that of normal control rats (*rdw*/N x 100) (Table 3) : The value of *rdw*/N x 100 of PRL per pituitary and PRL per mg pituitary was less than 7.2% and 9.4%, respectively, and the value of PRL was considerably smaller than that of TSH, in which values of less than 37.4 % per pituitary and 85.4% per mg pituitary, were seen. Also, the value of *rdw*/N x 100 was less than 37.1% in serum PRL, which showed smaller values than 46.8% in T4. The value of *rdw*/N x 100 of serum TSH of *rdw* rats was higher than that of N rats, indicating a value of more than 100%.

Discussion

The body weight of *rdw* rats was half to a third lower than that of normal litter mates (N) in both female and male rats at 10 weeks of age [10,24]. The result of the pituitary content of PRL of *rdw* rats was considerably lower and also, the serum PRL concentration was remarkably lower than that of N rats. Koto *et al*. [10] observed a decrease of acidophilic cells in the anterior pituitary in *rdw* rats, as well as a remarkable decrease in PRL-, GH-and TSH-positive cells in the rats, as shown by enzyme histochemical examination of the corresponding site of the cells. Present results on pituitary PRL obtained with RIA were consistent with those of Koto *et al*. [10]. The value of PRL of *rdw*/N x 100, less than 4.1 % per pituitary and less than 10% per mg pituitary, was consistent with previous results for pituitary PRL with blood sampling under ether anesthesia which showed values of 2.7 and 4.0% per pituitary in females and males, respectively, and 10.0% and 9.1% per mg pituitary in females and males, respectively (Umezu *et al*. : The 37th Scientific Meeting of JALAS,1990). Although we collected blood after decapitation in the present study, the value of pituitary PRL (*rdw*/N x 100) seems to be invariant regardless of the method of autopsy. The value of *rdw*/N x 100 of serum

PRL (37.1% in female, 22.5% in male) was slightly higher in the present experiment with decapitation than previous results with ether (5.1% in female, 8.4% in male) (Umezu *et al.* : The 37th Scientific Meeting of JALAS, 1990). Although a similar tendency was observed, namely that the value of *rdw*/N x 100 was remarkably low for the serum PRL, this slight discrepancy may be due to the method of blood sampling. [3,23].

TSH content of the pituitary in *rdw* rats was significantly lower than that in N rats when presented as per pituitary, that but not different when represented as per mg pituitary. Furthermore, serum TSH concentration was rather higher in *rdw* rats than in N rats with a significant increase in females. When we consider these phenomena by combining the results on pituitary and serum levels of TSH, it appears that the functions concerning pituitary synthesis and secretion of TSH in *rdw* rats may not be severely affected in the genetic phase. This result does not agree with the histochemical results reported by Koto *et al*, [10]. We are not able to explain the cause of this discrepancy. It might be due to the different experimental methods between histological examination and RIA.

It was clarified in the present experiment that serum T4 in *rdw* was significantly lower than that in N rats. Koto *et al*. [10] histologically examined the thyroid gland in N and *rdw* rats. They observed a considerably higher level of eosinophilic colloid in follicles of the thyroid glands and reported that T4 was secreted actively in N rats. In *rdw*, although the follicle structure existed, the structures consisted of small follicles and the level of secreted substance inside the structure was slight. We obtained the same result as previous one [10] in *rdw* and N rats at 10 weeks of age (unpublished data). The remarkably lower serum T4 in *rdw* supports these histological observations. Thus, the thyroid gland of *rdw* may possess inferior receptivity to TSH than that of N rats. When the present results were combined with the previous results, *rdw* rats were seen to be characterized by hypoplasia in synthesis and secretion of pituitary GH and PRL regardless of sex.

Genetic strain is established in mice regarding dwarfism such as *dw* (Snell dwarf) [21, 25], *df* (Ames dwarf) [20,6], *lit* [8], *pyg* [9], *hyt* [2], *cn*, *bm*, and *stb et al* [11]. In rats, Martin *et al*. [13] reported on Munich Wistar rats and O'Sullivan [19] reported on a small type of rat . Recently, Okuma [18,17] and Charlton [5] separately found genetic dwarf rats of (*dr*) and (*Dw*). Among these genetic dwarf mice and rats, strains that are characteristic of pituitary hormone deficiency are *dw*, *df* and *lit* in mice and *dr* and *Dw* in rats. It is known that the remaining rodents (*df*, *lit*, *dr* and *Dw*) except *dw* posess a limited deficiency in synthesis and secretion of GH in the anterior pituitary. Snell dwarf is characteristic of extremely low levels of GH, PRL and TSH with RIA [25]. Lower levels of pituitary GH and PRL in *rdw* rats compared with those of N rats corresponded well with findings for Snell dwarf. Regarding TSH, the level of this hormone was also extremely lower in *dw* such as GH and PRL when compared with the value in genetic dwarfs [25]. However *rdw* rats are not specific as to TSH deficiency. Serum T4 levels in *dw* mice of values per normal mice were extremely low at 5% [25], while the value in *rdw* rats was 40%. As described above, the aspect of pituitary deficiency in *rdw* was not completely consistent with that in *dw* mice. Thus, *rdw* rats are characteristic of genetic dwarfs which have not been reported.

In *rdw*, blood IGF-1 levels decreased remarkably accompanied by GH deficiency. When recombinant bovine somatotropin was administered to *rdw* rats, partial restoration of growth was significant (Umezu *et al*. : The 38 th Scientific Meeting of JALAS, 1991). Also, it has been observed that exogenous T4 administration restores growth in *rdw* (Umezu *et al*. : unpublished data). Van Buuel *et al*. [25] reported that administration of PRL to *dw* mice partially restored growth, although such a trial has not yet been made in *rdw* rats. When these data are considered together, the cause of retardation of growth is seen to be possibly due to single action or mutual actions among these hormones.

Research on the physiology of rats under condition of GH deficiency were vigorously carried out using Okuma rats (*dr*) [16,22] and Charltons'rats (*Dw*) [1,4,7] as model animals of genetic defects. The *rdw* rats herein reported is also considered to be a new model animal of genetic defects because it has a deficiency of PRL as well as of GH.

The mechanism in which the synthesis and release of GH and PRL remain considerably low in *rdw* rat is not clear at present. Nikitovitch-Winner *et al* [15] reported that a distinct cell type : MS which contains both GH and PRL within an anterior pituitary cell besides already known GH positive cell and PRL positive cell. We can not deny a possibility that pituitary GH and PRL positive cells and/or MS cells were genetically concentrated to be damaged in *rdw* rat.

Recently, Li *et al*. [12] conducted a gene analysis of the pituitary of Snell and Ames dwarf mice and reported that transcription of homeo-domain protein is suppressed because amino acid sequences were slightly different from those of the normal Pit-1 protein. This leads to hypoplasia of three types of cells in the anterior pituitary (GH, PRL and TSH) which causes a decrease of synthesis of these pituitary hormones. Further study is required in a level of molecular genetics on *rdw* rats as it is clear that the rats genetically express deficiency of GH and PRL synthesis in the pituitary.

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遺伝性侏儒症モデルラット(*rdw/rdw*)の下垂体および血中のプロラクチン(PRL), 甲状腺刺激ホルモン(TSH)と血中サイロキシン(T4)水準

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雌雄の遺伝性侏儒症ラット(*rdw/rdw*)を生後10週齢で血清と下垂体を採取するために断頭で屠殺した。下垂体のプロラクチン(PRL),甲状腺刺激ホルモン(TSH)と血中PRL, TSHと甲状腺ホルモン(T 4)をラジオイムノアッセイ(RIA)で測定し，表現型が正常な同腹子(N)と比較した。*rdw*ラットでは下垂体と血中のPRLはNラットに比べて非常に低い値を示し，血中T 4も有意に低い値を示した。下垂体当たりのTSH含量は*rdw*ラットで有意に低かったが，下垂体mg重量当りの*rdw*ラットのTSH含量はNラットに比較して差がなかった。*rdw* の血中TSH水準はNラットと比べて雄で差がなく，雌では有意に高い値を示した。前回[23]と今回の成績を合わせて考慮した時，*rdw*ラットは下垂体のPRLとGH合成細胞の形成不全が特徴的で，下垂体のPRLとGHの内分泌欠陥を持つモデル動物として有用と考えられた。