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Influence of hypothyroidism on adrenal dopamine-beta-hydroxylase in the developing rat

D. Gripois and M. Valens

Laboratoire d'Endocrinologie, Université Paris XI, F-91405 Orsay Cédex (France), March 18, 1982

Summary. In young rats rendered hypothyroid from birth, adrenal dopamine-beta-hydroxylase activity increases more rapidly than in the control animals.

In a previous paper¹ we described the influence of the thyroid status of young rats on the catecholamine (CA) content of their adrenals, together with the activity of tyrosine hydroxylase (TH), the enzyme which catalyzes the initial and rate-limiting step in CA biosynthesis. In the present experiment, we attempted to explain the influence of neonatal hypothyroidism on the activity of another enzyme of CA biosynthesis, i.e. dopamine-beta-hydroxylase (DBH; EC 1-14-17-1). DBH catalyzes the conversion of dopamine to norepinephrine².

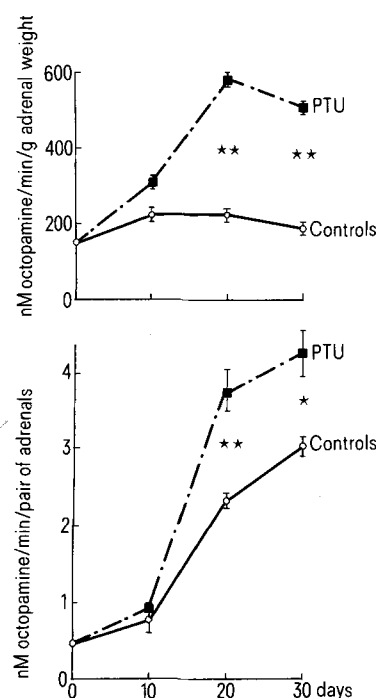
Materials and methods. Young rats of both sexes were rendered hypothyroid from birth by daily administration of propylthiouracil (PTU; 50 mg/day in 4 ml of water) to their mother by gastric intubation. Control animals received no treatment. The animals were studied from 0 to 30 days of postnatal life. They were killed between 09.00 and 11.00 h and the adrenals were rapidly dissected out and weighed; the glands were homogenized in ice-cold 0.1 M phosphate buffer pH 7.4 containing 0.13% triton X 100. The homogenate was mixed for 20 min at 4°C and centrifuged for 90 min at 7000 × g and the supernatant was assayed for enzyme activity. 2-4 pairs of adrenals were used for each determination. DBH activity was measured according to Kato et al.^{3,4}. The following concentrations of reagents in the final incubation mixture were used: pargyline: 1 mM; CuSO₄: 10 μM; N-ethylmaleimide: 30 mM. The results are expressed as nM of octopamine formed per min. The statistical analysis of the results was performed according to the U-test of Mann and Whitney.

Results. The body weight, adrenal weight and the relative weight of the adrenals are given in the table. From 10 days both body and adrenal weights were lower in the PTU-treated rats than in their controls. Furthermore, the eye-opening age was retarded by the PTU (19 days instead of 15 days in the controls).

The evolution of DBH activity is reported in the figure. The enzymatic activity is expressed both per pair of adrenals and per g of adrenal weight. In the control group, the enzymatic activity remained constant from 0 to 30 days if it is calculated per unit of adrenal weight, and increased per pair of glands. In the hypothyroid rats, using either way of expressing the results, DBH activity increased from 0 to 20 days and remained nearly constant between 20 and 30

days. At 20 and 30 days, the activity was 1.4-2.7 times higher in the hypothyroid rats than in the control animals.

Discussion. It has already been clearly established that PTU given p.o. to a suckling female rat results in the clinical signs of hypothyroidism in the pups, with a very low level of plasmatic thyroxin⁵⁻⁷. The different results we obtained concerning the growth and differentiation of our animals show that PTU induces a clearly identifiable state of hypothyroidism. In this respect, the variations in DBH



Evolution of adrenal dopamine-beta-hydroxylase activity in young rats. PTU, hypothyroid animals. Means \pm SEM. Statistical significance between treated animals and controls: * $p \leq 0.05$; ** $p \leq 0.01$. Number of determinations per group; 0 day: 8; 10 days: 5 and 7; 20 days: 8 and 8; 30 days: 9 and 14.

activity we observed between the 2 experimental groups might be due to a deficiency in thyroid hormone secretion. Furthermore, purified bovine adrenal DBH is directly inhibited by PTU⁸; the increase we find in the enzymatic activity obviously cannot be produced by a direct effect of PTU.

The evolution of adrenal DBH activity we found in the young control rats is similar to that described in previous reports^{9,10}. Male and female rats were used in natural proportions, because no sex-related variations in DBH activity have been found.

The present results show that hypothyroidism accelerates the post-natal evolution of adrenal DBH activity. In the adult rat, some authors studied the influence of a decreased thyroid activity on DBH; thyroidectomy fails to modify DBH activity^{11,12}. Comparing these different findings, it appears that adrenal DBH activity is more sensitive to hypothyroidism during development than in the adult. In the young rat, hyperthyroidism has been shown to slacken

the evolution of adrenal DBH activity¹⁰; hypo- and hyperthyroidism appear to have opposite effects on the development of the enzymatic activity.

Finally, the present results concerning DBH activity have to be compared with our previous findings about the adrenal TH activity and CA content¹; we found that neonatal hypothyroidism accelerates the increase in TH activity and in epinephrine and norepinephrine content. Thus, DBH responds to hypothyroidism just like TH. The consequent increase in CA biosynthesis could explain, at least partially, the increase in the CA content of the glands.

Age (days)	0	10	20	30			
		Cont	PTU	Cont	PTU	Cont	PTU
Body weight (g)	5.4 ± 0.1	18.8 ± 0.3	11.8 ± 0.4 **	37.2 ± 0.6	18.0 ± 0.3 **	77.8 ± 0.9	29.1 ± 0.5 **
Adrenal weight (mg/pair)	3.09 ± 0.04	3.57 ± 0.03	3.19 ± 0.12 *	10.54 ± 0.62	6.48 ± 0.31 **	16.18 ± 0.81	8.42 ± 0.42 **
Ratio: adrenal weight/body weight (mg/g)	0.57	0.18 a	0.27	0.28 a	0.36	0.21 a	0.29
Number of animals	26	17	22	20	18	18	28

Body weight and adrenal weight of young rats. Cont, control rats; PTU, hypothyroid rats. Means ± SEM. Statistical significance between the 2 groups: *p ≤ 0.05; **p ≤ 0.01. a, no statistical analysis.

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Oestrus induction in unisexually grouped mice by multiple short-term exposures to males¹

B.K. Gangrade and C.J. Dominic

Department of Zoology, Banaras Hindu University, Varanasi-221 005 (India), April 13, 1982

Summary. Oestrus induction and synchronization (the Whitten effect) were achieved in unisexually grouped female mice by short-term (10 and 30 min) exposure to conspecific males.

Female mice housed in large unisexual groups exhibit irregularities in the oestrous cycles². Continuous exposure of such females to conspecific males for at least 48 h results in the synchronization of oestrus 3 days later³. An olfactory, androgen-dependent urinary pheromone is involved in this synchronization of oestrus (the Whitten effect^{4,5}). In the present study the effect on oestrus induction of multiple short-term exposure of grouped females to males was evaluated.

Regularly cycling, 10–12-week-old virgin females of the Parkes (P) strain were divided into 5 unisexual groups of 30 each and housed in a male-free room for 28 days in cages, 48 × 34 × 11 cm. Females in groups I–III were exposed for varying periods of time (see table for protocol) to 3 confined (in an expanded metal corral) P males on days 29–35. The cages with the females were carried to another room for exposure to males and returned to the male-free room soon after exposure, remaining there until the next exposure. Females in group IV were taken to the room containing the males along with those in group II, but were not

exposed to males and kept at a distance of 1.8 m from the cages housing the males. They were returned to the male-free room along with the females in group II. Controls (group V) remained in the male-free room throughout. Vaginal smears were daily examined from all females. The days of oestrus return (vaginal cornification) in the table refer to days 29–35 (7 days) in all groups. Data were analyzed by the χ^2 -test.

The results are presented in the table. Unisexual grouping resulted in the incidence of prolonged cycles (10–16 days duration) in most females. Continuous (group I) or intermittent (groups II and III) exposure to males induced acceleration and synchronization of oestrus in females irrespective of the duration of the exposure. No significant difference in the percentage of oestrus return on day 3 between females in the 3 groups was found. Females in groups IV and V did not exhibit synchronized oestrus. Even though the occurrence of all-female groups of *Mus musculus* in nature is doubtful^{6,7}, our results suggest that brief but frequent male-female encounters in the natural