

## Hypothyroidism and Pituitary Contents of Immunoactive Met-enkephalin and $\beta$ -Endorphin in Male Rats of Different Ages (42710)

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**Abstract.** A comparison of the effect of PTU-induced hypothyroidism on the contents of immunoactive met-enkephalin and  $\beta$ -endorphin in the pituitaries from rats aged 2½, 12, and 18 months was made. In all ages, there was significant reduction of IR-met-enkephalin content in the anterior lobe and IR- $\beta$ -endorphin content in the neuro-intermediate lobe after PTU treatment. There was a significant age-related decrease in IR- $\beta$ -endorphin content in the anterior lobe. Rats of all three ages responded to PTU treatment with an increase in serum TSH level and a drastic reduction in serum T<sub>3</sub> and T<sub>4</sub> levels. The results indicate that there was no age-related difference in the change of pituitary opioid peptide contents in response to hypothyroidism. © 1988 Society for Experimental Biology and Medicine.

Although Sprague-Dawley rats can have a life span of more than 30 months, a number of changes in the response of hormonal secretion take place as early as 18 months of age. For instance, the response of plasma ACTH levels to ether stress in 18-month-old rat decreases (1). So does the response of serum TSH to ether stress (2) and cold (3). Many other age-related studies in our laboratory have been done with rats up to 18 months old (4-6). Therefore, rats aged 2½, 12, and 18 months were used in this study.

It has been reported that hypothyroidism results in a decrease in  $\beta$ -endorphin in the pituitary (7). In our laboratory, propylthiouracil (PTU)-induced hypothyroidism has led to a decrease in both  $\beta$ -endorphin and met-enkephalin contents in the rat pituitary (8). These experiments show that thyroid hormone may be involved in the regulation of synthesis and/or release of  $\beta$ -endorphin and met-enkephalin in the pituitary. This study has therefore been undertaken to see whether hypothyroidism will have similar effects on the opioid peptide contents in the pituitaries of rats of different ages. There are many examples in which the basal levels are unchanged in rats of relatively old age but the responses to manipulation are changed (1-3).

Age is known to modify the response to thyroid hormone status in the hypothalamus and striatum so that the decrease in  $\beta$ -endorphin levels in the young as a result of hypothyroidism is no longer observable in rats of

relatively old age (9). It is therefore of interest to see whether a similar situation exists for pituitary  $\beta$ -endorphin and met-enkephalin contents.

**Materials and Methods.** Male Sprague-Dawley rats aged 2, 11½, and 17½ months were used. They were obtained from the specified-pathogen-free breeding colony of the Laboratory Animal Unit of the University of Hong Kong. Microbiological monitoring of the colony, carried out at three-month intervals, has shown it to be of Category 4 status (10) and free of pathogenic bacteria by cultural methods, of ectoparasites by direct examination, and of intestinal parasites by wet preparation and concentration methods. Examination of rat serum by enzyme-linked immunosorbent assay has been negative for *Mycoplasma pulmonis* antibodies but positive for those of Sendai virus, although without associated clinical or sub-clinical disease. Gross postmortem examinations of rats from the breeding colony and histological examination of the lungs and other major target organs have not shown evidence of pathological change associated with infection.

Hypothyroidism was induced in some of these rats by giving them 0.05% propylthiouracil to drink. Rats were given food and water *ad libitum* in a room with a photoperiod of 12 hr light and 12 hr dark (light on at 6:00 AM). After 2 weeks of treatment when the rats were 2½, 12, and 18 months old, both the control and the PTU-treated rats were

decapitated in the morning, and the pituitaries were separated into anterior and neuro-intermediate lobes. All samples were stored at  $-70^{\circ}\text{C}$  until extraction. Trunk blood was also obtained and serum TSH,  $T_3$ , and  $T_4$  levels were determined by radioimmunoassays as described previously (3). Lobes of pituitaries were extracted with 2 *N* acetic acid and the extracts were heated at  $100^{\circ}\text{C}$  for 10 min before centrifugation. The supernatants were lyophilized and later reconstituted in assay buffer. The clear supernatants were used for measurement of  $\beta$ -endorphin and met-enkephalin according to the method of Hong *et al.* (11). The antiserum for met-enkephalin (a gift from Dr. J. Hong and the Research Triangle Institute) cross-reacted 0.36% with leu-enkephalin, less than 0.06% with dynorphin A (1–8), approximately 2% with met<sup>5</sup>-enkephalin-Arg<sup>6</sup>-Phe<sup>7</sup>, and less than 0.2% with  $\beta$ -endorphin. The antiserum for  $\beta$ -endorphin (Milab, Sweden) cross-reacted 102% with human  $\beta$ -lipotropin, less than 0.01% with human  $\alpha$ -endorphin, met-enkephalin, leu-enkephalin, human  $\beta$ -lipotropin 61–69,  $\beta$ -lipotropin 88–91, 75% with human  $\beta$ -lipotropin 61–87, and 184% with human  $\beta$ -lipotropin 68–91. The amounts of protein in the pituitary samples were determined according to the method of Lowry *et al.* (12). All data were subjected to analysis of covariance fol-

lowed by Student's *t* test. Results were considered to be significant when the means differ at  $P < 0.05$ . All values were expressed as means  $\pm$  SE.

**Results.** As expected, after PTU treatment, there was a drastic decrease in serum  $T_3$  and  $T_4$  levels, and a significant increase in serum TSH levels (Table I). The age-related decreases in serum  $T_3$  and  $T_4$  levels were like those reported previously (3, 5). However, in hypothyroid rats the serum TSH level was significantly lower in the 18-month-old than in the 2½-month-old rats (Table I).

There was no age-related change in IR-met-enkephalin contents in either the anterior lobe or the neuro-intermediate lobe when the values are expressed on a per-milligram-protein basis although there was a progressive increase in the neuro-intermediate lobe on a per-lobe basis (Fig. 1). In addition, there was a slight but significant decrease in IR- $\beta$ -endorphin in the anterior lobe on a per-milligram-protein basis (Fig. 2). On the whole, there was no aged-related change in IR-met-enkephalin or IR- $\beta$ -endorphin in the pituitary.

As to the effect of hypothyroidism, there was a marked decrease in IR-met-enkephalin content in the anterior lobe, and in IR- $\beta$ -endorphin content in the neuro-intermediate lobe in the PTU-treated rats of all ages (Figs. 1 and 2). The PTU effect on neuro-interme-

TABLE I. EFFECT OF PTU TREATMENT ON THE SERUM LEVELS OF TSH,  $T_3$ , AND  $T_4$  IN RATS OF DIFFERENT AGES

	2½ months	12 months	18 months
Serum TSH (ng/ml)			
Control	3.69 $\pm$ 0.39 (10)	4.58 $\pm$ 0.73 (8)	3.84 $\pm$ 0.71 (8)
PTU	13.08 $\pm$ 1.68 (9) <sup>b</sup>	10.74 $\pm$ 1.22 (9) <sup>b</sup>	8.95 $\pm$ 1.19 (7) <sup>a,c</sup>
Serum $T_4$ ( $\mu\text{g}/100$ ml)			
Control	5.258 $\pm$ 0.332 (10)	3.918 $\pm$ 0.255 (8) <sup>c</sup>	3.723 $\pm$ 0.402 (8) <sup>c</sup>
PTU	0.09 $\pm$ 0.025 (10) <sup>b</sup>	0.248 $\pm$ 0.034 (9) <sup>b</sup>	0.422 $\pm$ 0.089 (8) <sup>b</sup>
Serum $T_3$ (ng/100 ml)			
Control	169.51 $\pm$ 7.88 (10)	122.87 $\pm$ 13.17 (8) <sup>c</sup>	117.46 $\pm$ 14.53 (8) <sup>c</sup>
PTU	32.02 $\pm$ 2.78 (10) <sup>b</sup>	25.64 $\pm$ 2.79 (9) <sup>b</sup>	30.22 $\pm$ 3.39 (8) <sup>b</sup>

Note. Analysis of covariance. TSH: treatment effect,  $df = 1/45$ ,  $F = 69.178$ ,  $P < 0.01$ .  $T_4$ : treatment effect,  $df = 1/47$ ,  $F = 474.923$ ,  $P < 0.01$ ; age effect,  $df = 2/47$ ,  $F = 3.292$ ,  $P < 0.05$ ; interaction,  $df = 2/47$ ,  $F = 7.644$ ,  $P < 0.01$ .  $T_3$ : treatment effect,  $df = 1/47$ ,  $F = 280.589$ ,  $P < 0.01$ ; age effect,  $df = 2/47$ ,  $F = 6.135$ ,  $P < 0.01$ ; interaction,  $df = 2/47$ ,  $F = 4.125$ ,  $P < 0.05$ .

<sup>a</sup>  $P < 0.01$  compared with control.

<sup>b</sup>  $P < 0.001$  compared with control.

<sup>c</sup>  $P < 0.05$  compared with 2½-month-old rat.

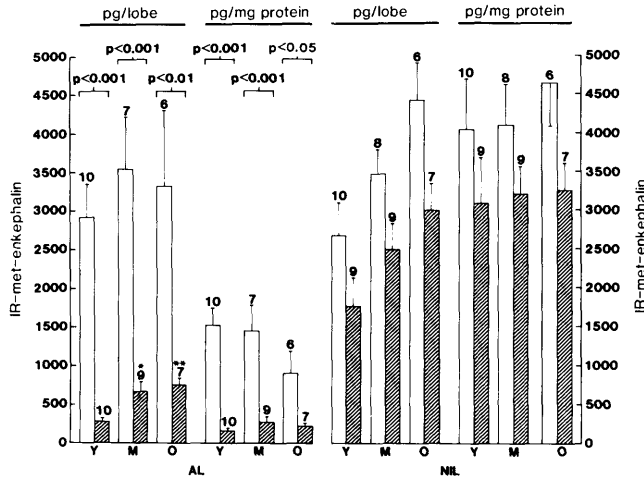


FIG. 1. The IR-met-enkephalin contents in the anterior lobes (AL) and neuro-intermediate lobes (NIL) of the pituitaries of 2½-month-old (Y), 12-month-old (M), and 18-month-old (O) rats. (□) Control; (▨) PTU-treated. \**P* < 0.05 and \*\**P* < 0.01 compared with 2½-month-old rats. Analysis of covariance for the anterior lobe. Met-enkephalin per lobe: PTU effect, *df* = 1/43, *F* = 65.889, *P* < 0.01. Met-enkephalin per milligram protein: PTU effect, *df* = 1/43, *F* = 57.172, *P* < 0.01. Analysis of covariance for neuro-intermediate lobe. Met-enkephalin per lobe: PTU effect, *df* = 1/43, *F* = 14.402, *P* < 0.01. Age effect, *df* = 2/43, *F* = 9.157, *P* < 0.01. Met-enkephalin per milligram protein: PTU effect, *df* = 1/43, *F* = 6.680, *P* < 0.05.

diate lobe met-enkephalin content was also significant by covariance analysis. On a per-lobe basis, the IR-met-enkephalin contents

in the anterior lobe of hypothyroid rats aged 12 and 18 months were higher than that of rats aged 2½ months.

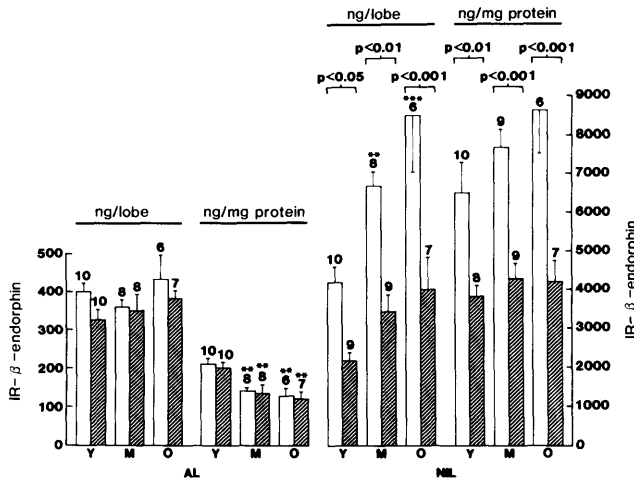


FIG. 2. The IR-β-endorphin contents in the anterior lobes (AL) and neuro-intermediate lobes (NIL) of the pituitary of 2½-month-old (Y), 12-month-old (M), and 18-month-old (O) rats. (□) Control; (▨) PTU-treated. \*\**P* < 0.01, and \*\*\**P* < 0.001 compared with 2½-month-old rats. Analysis of co-variance for the anterior lobe. β-Endorphin per milligram protein: age effect, *df* = 2/43, *F* = 18.632, *P* < 0.01. Analysis of covariance for the neuro-intermediate lobe. β-Endorphin per lobe: PTU effect, *df* = 1/43, *F* = 46.855, *P* < 0.01. Age effect, *df* = 2/43, *F* = 14.159, *P* < 0.01. β-endorphin per milligram protein: PTU effect, *df* = 1/43, *F* = 52.397, *P* < 0.01.

**Discussion.** In the PTU-treated rats, the decreases in serum  $T_4$  levels in the 12- and 18-month-old rats seem to be less marked than that in the 2½-month-old rats. It is interesting to note that the serum TSH level in the 18-month-old hypothyroid rats was significantly lower than that in the 2½-month-old hypothyroid rat, probably as a result of a change in sensitivity to the negative feedback signal in the old rats. A decrease in pituitary secretion of TSH in response to cold in old rats has been reported (3).

There is a dissociation between the changes in the serum thyroid hormone levels and in the IR-met-enkephalin and IR- $\beta$ -endorphin contents in the pituitary. Whereas there was an age-related decrease in serum  $T_3$  and  $T_4$  levels, there was no difference in the pituitary IR-met-enkephalin and IR- $\beta$ -endorphin contents in the control rats of different ages. The finding in the pituitary opioid peptide contents confirms and extends our previous findings (13). The previous reports of an increase in opioid peptide contents (14, 15) in aged rats come from studies of animals older than those used in the present investigation.

The effect of hypothyroidism on pituitary contents of IR-met-enkephalin and  $\beta$ -endorphin was similar in rats of all three ages, contrary to the finding of a lack of the effect of hypothyroidism on hypothalamic  $\beta$ -endorphin content in the 20- to 24-month-old rats (9). There was a marked decrease in IR-met-enkephalin content in the anterior lobe, and in IR- $\beta$ -endorphin content in the neuro-intermediate lobe in the PTU-treated rats. The lack of an age effect of hypothyroidism indicates the importance of thyroid hormones in the synthesis and/or release of opioid peptides even up to a relatively old age.

As to the mechanism for the decrease in IR-met-enkephalin content in the anterior lobe, Yoshikawa and Hong (16) have shown that a blockade of dopaminergic mechanism may decrease the met-enkephalin content in the anterior lobe. In the neuro-intermediate lobe, the release of IR- $\beta$ -endorphin is inhibited by dopaminergic (17) and stimulated by  $\beta$ -adrenergic (17-19) mechanisms. However, a relationship between hypothyroidism and dopaminergic and  $\beta$ -adrenergic mechanisms has yet to be established. Furthermore, the

protein contents in the pituitaries of rats of different ages were not decreased by PTU treatment. For the anterior lobe, the protein contents for the 2½-month-old rats were  $1.907 \pm 0.085$  and  $1.618 \pm 0.075$  mg, for the 12-month-old rats were  $2.558 \pm 0.152$  and  $2.569 \pm 0.098$  mg, and for the 18-month-old rats were  $3.357 \pm 0.290$  and  $3.526 \pm 0.633$  mg (the control values precede the PTU-treated values). Similarly, for the neuro-intermediate lobe, the values for the 2½-month-old rats were  $0.697 \pm 0.069$  and  $0.559 \pm 0.055$  mg, for the 12-month-old rats were  $0.877 \pm 0.041$  and  $0.813 \pm 0.064$  mg, and for the 18-month-old rats were  $0.973 \pm 0.078$  and  $0.936 \pm 0.075$  mg. This could mean that thyroid hormone may specifically affect the enzymes involved in the synthesis of these neuropeptides (20).

To the best of our knowledge, this is the first study that clearly demonstrated that hypothyroidism decreases IR-met-enkephalin and IR- $\beta$ -endorphin contents in the pituitaries of rats up to 18 months old. Differing from the results of Gambert (9) on the hypothalamus, age did not modify the response of the pituitary to hypothyroidism in  $\beta$ -endorphin and met-enkephalin contents. It is of interest to note that the changes in the two neuropeptides are in different lobes of the pituitary, which must mean that they are under different control mechanisms which can be modified by thyroid hormone levels.

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