AMINO ACID UPTAKE AND INCORPORATION INTO RAT PITUITARY AS AFFECTED BY THYROIDECTOMY AND THYROXINE ADMINISTRATION

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Since Hoskins proposed the 'feed-back' hypothesis in 1949, a homeostatic control between the pituitary and the thyroid has been extensively investigated to confirm the hypothesis. There have been many reports concerning the sites of action of thyroid stimulating hormone (TSH) in the thyroid (see Rall et al., 1964; Suzuki, 1966). Recently Raghupathy et al. (1963) described that TSH stimulates protein synthesis of the thyroid. On the other hand, the action of thyroid hormones on the pituitary has been studied only with the use of an over-all effect, e.g., thyroid changes, TSH release from or morphological changes of the pituitary (see Purves, 1964), and the sites of the action have not been elucidated.

Thyroid hormone has been known to enhance protein synthesis in the liver (Sokoloff and Kaufman, 1961; Tata et al., 1963; Buchanan and Tapley, 1966), heart and kidney (Michels et al., 1963) and skeletal muscle (Brown, 1966). In view of the peculiar interrelationship between the thyroid and pituitary, it can be presumed that thyroid hormone depresses protein synthesis of the

pituitary. The present experiment showed that the presumption is really the case. Both uptake and incorporation of amino acids into rat pituitary were stimulated by thyroidectomy and the stimulation was depressed by thyroxine (T_4) administration.

Material and Methods: Young Wistar rats of body weights of 130 to 180 g were used for all experiments. Thyroidectomy was performed surgically 2 to 3 weeks before experiments. T_4 was administered by a subcutaneous injection at a dose of 20 μg per 100 g body weight. Animals were sacrificed by decapitation and the pituitary was dissected, cleaned and incubated with amino acids.

Experiments on amino acid uptake were carried out after the method of Wool et al. (1965) with slight modifications. The whole pituitary was incubated with 0.25 μmoles of α-aminoisobutyric acid (AIB), which contained 0.2 μc of AIB-1-^{14}C (Radiochemical Center, Amersham). After incubation for 60 min at 37°C, AIB accumulated by the pituitary was extracted with water and the water extract was counted for radioactivity. The results were expressed in terms of AIB distribution ratio, that is, the ratio of radioactivity counts in an unit volume of tissue water to the counts in incubation medium of the same volume.

Experiments on amino acid incorporation into pituitary proteins were carried out after the method of Wool et al. (1965) with slight modifications. Sliced anterior pituitary was incubated with 0.5 μc of L-alanine-^{14}C(U)(5.88 mμmoles)(Radiochemical Center, Amersham). After incubation for 60 min at 37°C, proteins were extracted and the extract was counted for radioactivity. For the counting, 25 μg protein was applied to a planchet, therefore corrections for selfabsorption was unnecessary. The protein content of the extract was determined by measuring its

turbidity after the addition of trichloroacetic acid at a final concentration of 3%.

Results: A representative result of the effect of thyroidectomy and thyroxine supplement on the uptake of AIB is shown in Table 1. Thyroidectomy increased the uptake by 28%. This difference was statistically significant. When T_4 was

TABLE I

Effect of thyroidectomy and T_4 injection on AIB uptake by rat pituitary

Treatment	No. of animals	Weight of pituitary	Distribution ratio of AIB-1-^{14}C
Control	4	5.8	2.96 ± 0.08*
Thyroidectomy	4	7.0	3.57 ± 0.07
Thyroidectomy + T_4 injection			
3 hr	4	7.3	3.37 ± 0.07*
12 hr	4	7.0	2.68 ± 0.04*

* Difference from thyroidectomy, $p < 0.01$

administered to the thyroidectomized rats, the stimulated AIB uptake by thyroidectomy decreased significantly 3 hr after T_4 injection and the uptake was lower than the level of the normal rats 12 hr after T_4 injection.

Table 2 shows a representative experiment on incorporation of alanine into proteins of the pituitary. The alanine incorporation was stimulated significantly by thyroidectomy by 35%. The stimulation was significantly depressed 3 hr after T_4 injection. After 12 hr, alanine incorporation was the same as in control animals. These results were confirmed by repeated experiments.

TABLE II

Effect of thyroidectomy and T_4 administration on alanine incorporation into proteins of rat pituitary

Treatment	No. of animals	L-Alanine-^{14}C incorporation (cpm/mg protein)
Control	6	21960 ± 195*
Thyroidectomy	6	29518 ± 264
Thyroidectomy + T_4 injection		
3 hr	6	23210 ± 264*
12 hr	6	22001 ± 1048*

* Difference from thyroidectomy, $p < 0.01$

It should be noted here that both AIB uptake and alanine incorporation were very active in the pituitary when compared with other tissues. AIB uptake was about 5 times greater and alanine incorporation was about 70 times greater in the pituitary than in the liver and diaphragm, when compared in the same experimental conditions.

Discussion: The results described above show clearly that thyroidectomy causes a stimulation of uptake and incorporation of amino acids into the pituitary and that the stimulation is depressed by T_4 supplement.

It is well known that in hypothyroidism the discharge of TSH from the pituitary increases and in hyperthyroidism the discharge decreases (van Rees, 1966). However, the detailed mechanisms do not appear to have been made clear. The present results suggest strongly that the thyroid regulates the pituitary

function through protein synthesis. Concerning the effect of thyroid hormones on protein synthesis in tissues other than the pituitary, a stimulatory effect has been reported. The fact that the effect of T_4 upon the pituitary is inhibitory is consistent with the peculiar feed-back relationship between the thyroid and the pituitary, though the mechanisms of this reversed effect is not clear at present. High rates of amino acid uptake and incorporation of the pituitary indicate the high potency to synthesize its trophic hormones of protein or polypeptide nature.

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