MECHANISM OF THE ANABOLIC EFFECT OF NEUROBOLIL ON NORMAL AND THYROIDECTOMIZED RATS

A. P. Popov and A. E. Rabkina

UDC 612.432.014.46:615.357.631]-06:612.441-089

Administration of neorobolil to intact and thyroidectomized rats causes an increase over the normal gain in body weight. Thyroidectomy is followed by a slower gain in body weight and a decrease in the number of acidophilic cells, producing somatotropic hormone. The anabolic effect of neurobolil is accompanied by an increase in the number of acidophilic cells in the pituitary.

The anabolic action of the androgenic steroids is exhibited as stimulation of the increase in body weight, stimulation of RNA and protein synthesis in the tissues, and a decrease in the excretion of nitrogen in the urine [5, 8, 9, 13].

Pituitary somatotropic hormone (STH) has a similar action. However, the role of endogenous STH, if any, in the mechanism of the anabolic action of androgenic steroids has not yet been explained. Data in the literature on this matter are contradictory [1, 10, 12, 14-18].

It was therefore decided to study the effect of the androgenic anabolic steroid neurobolil on the cell composition of the anterior pituitary in normal animals and in rats subjected to bilateral thyroidectomy, paying particular attention to the state of the acidophilic cells, producing STH.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male rats weighing 120-140 g, divided into four groups: 1) control rats; 2) rats receiving neurobolil intramuscularly in a dose of 1 mg/100 g body weight daily for 1, 2, and 3 weeks; 3) rats undergoing bilateral thyroidectomy; and 4) thyroidectomized rats receiving neurobolil 24 h after the operation at the above times and doses. The body weight of the animals was checked each week. After sacrifice the endocrine glands were fixed in a 1:9 mixture of mercuric chloride and formol and in Zenker's mixture. Paraffin sections of the pituitary, 4 μ in thickness, were stained by Mallory's azan method and for glycoproteins (PAS reaction with triple counterstaining). In sections of the pituitary from three or four rats from each, stained by histochemical methods, the ratio between the various types of cells was determined (in five fields of vision, 1000×, immersion). Altogether 25,000 cells of different types were counted. The results were analyzed by statistical methods.

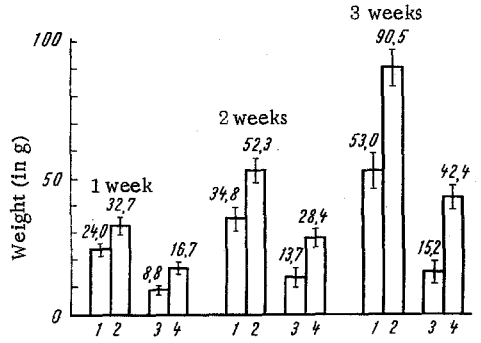

Fig. 1. Mean values of increase in weight of control and thyroidectomized rats receiving neurobolil for 1-3 weeks: 1, 2, 3, 4) Group number.

Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 11, pp. 99-103, November, 1970. Original article submitted June 12, 1970.

TABLE 1. Changes in Weight of Thymus, Testes, and Accessory Reproductive Organs of Control and Thyroidectomized Rats after Administration of Neurobolil for 1, 2, and 3 Weeks (M ± m)

Experimental group	Weight of endocrine glands (mg)								
	thymus			testes			seminal vesicles and prostate		
	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks
Control	262±58 (20)	255±22 (12)	282±28 (19)	1 319±41 (15)	1 770±105 (12)	1 976±58 (19)	155±18 (15)	441±106 (12)	522±55 (19)
Control + neurobolil	157±18 (16) $P<0,05$	104±5 (12) $P<0,001$	127±17 (4) $P<0,001$	1 443±133 (16) —	1 941±71 (12) —	1 615±82 (4) $P<0,01$	658±48 (16) $P<0,001$	1 483±72 (12) $P<0,001$	1 250±86 (4) $P<0,001$
Thyroidectomy	168±13 (10) $P<0,05$	156±30 (7) $P=0,01$	170±19 (9) $P<0,001$	1 322±98 (10) —	1 642±152 (7) —	1 500±115 (9) $P<0,01$	206±19 (10) —	166±75 (5) $P=0,05$	218±24 (7) $P<0,001$
Thyroidectomy + neurobolil	76±8 (13) $P<0,01$	72±16 (8) $P<0,05$	71±9 (14) $P<0,001$	1 450±112 (13) —	1 690±222 (8) —	1 926±82 (14) $P<0,01$	592±32 (13) $P<0,02$	1 275±97 (8) $P<0,001$	1 663±68 (14) $P<0,001$

Note. Number of animals in parentheses.

TABLE 2. Relative Proportions of Types of Cells in Anterior Pituitary (in %) in Control and Thyroidectomized Rats after Administration of Neurobolil for 3 Weeks*

Group of animals	Types of anterior pituitary cells†			
	acidophilic	basophilic	chromophobic	"thyroidectomy cells"
1	36,6±0,39	13,4±0,39	50 0±0,56	—
2	44,7±0,61	10,3±0,29	45,0±0,54	—
3	0,9±0,22	15,8±0,65	68,7±0,74	14,5±0,54
4	12,1±0,49	11,9±0,42	63,5±0,76	13,5±0,48

*Neurobolil given on second day after thyroidectomy.
† Data statistically significant when $P < 0.001$.

EXPERIMENTAL RESULTS

The anabolic effect of neurobolil was judged from the degree of increase in the gain in body weight of the animals. Neurobolil increased the gain in weight of both normal and thyroidectomized rats ($P < 0.01$; Fig. 1). The mean body weight of normal rats after administration of neurobolil was increased by 36% after 1 week, by 50% after 2 weeks, and by 70% after 3 weeks. The gain in weight of rats after thyroidectomy was considerably retarded. Delay in growth of the thyroidectomized rats, although not its complete arrest, can evidently be explained by the "acute" experimental conditions and the possibility of an extra thyroid pathway of formation of thyroid hormones or their precursors [1, 3, 11, 19, 20]. Administration of neurobolil to these rats for 1-3 weeks caused marked stimulation of their gain in body weight. The gain in weight of these rats was 45-74% greater than that of the thyroidectomized animals not receiving neurobolil.

Administration of neurobolil led to a change in weight of the endocrine glands. The weight of the pituitary was increased in control animals receiving neurobolil for 2 weeks, but after 3 weeks it was reduced. This can evidently be attributed to adaptation of the animal to prolonged administration of this dose of neurobolil.

Thyroidectomy caused hypertrophy of the pituitary as the result of increased secretion of thyrotropic hormone. Administration of neurobolil to these rats had no effect on the weight of the pituitary.

The greatest changes in weight of the thymus were found in animals of all the experimental groups. Neurobolil and thyroidectomy caused a considerable increase in weight of the thymus in the control rats. Administration of neurobolil to thyroidectomized rats stimulated involution of the thymus. The thymolytic action of neurobolil was due to its direct action on lymphopoiesis in the gland [3]. After administration of neurobolil the androgenic effect was exhibited as a result of the direct action of the substance on the accessory reproductive organs [9].

Hence, using animals with inhibited growth, the positive action of the anabolic steroid was observed: the gain in body weight was increased.

To study the role of endogenous STH in the anabolic effect of neurobolil, it was decided to study the morphological reaction of the anterior pituitary and to investigate the state of the acidophilic cells, with whose activity the secretion of STH is associated. The absence of thyroid hormones is known to stop growth of young animals and to inhibit differentiation of acidophilic cells, leading to a disturbance of STH formation in the pituitary [2, 4, 6].

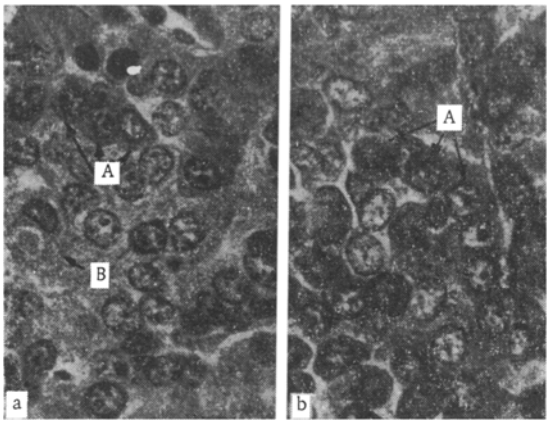

Fig. 2

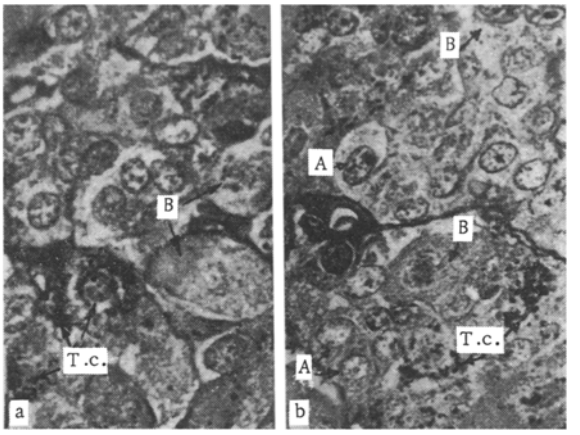

Fig. 3

Fig. 2. Anterior pituitary of control rat (a). Increase in number of acidophilic cells in pituitary of rat receiving neurobolil (b). A) Acidophilic cells; B) basophilic cells. Heidenhain's modification of Mallory's azan stain, 750×.

Fig. 3. Anterior pituitary of thyroidectomized rat (a). "Thyroidectomy cells" and a few acidophilic cells are visible in the pituitary of a thyroidectomized rat receiving neurobolil (b). T.c.) "Thyroidectomy cells;" A) acidophilic cells; B) basophilic cells. PAS reaction with triple counterstaining, 750×.

Determination of the relative proportions of different types of cells in the anterior pituitary of the control rats showed that following administration of neurobolil the number of acidophilic cells was increased (Table 2). Meanwhile, the number of basophilic and chromophobic cells was reduced compared with the control (Fig. 2). The increase in number of acidophilic cells evidently took place as a result of transformation of chromophobic cells, and it led to an increase in STH secretion.

After thyroidectomy (3 weeks), solitary acidophilic cells were found in the pituitary, while the number of chromophobic and basophilic cells was increased. Hypertrophy of the nuclei and degranulation of the cytoplasm were observed in the large, polygonal basophilic cells (thyrotrophs). Vacuoles were observed in some thyrotrophs containing a small number of large glucoprotein granules (Fig. 3). These cells are known as "thyroidectomy cells."

Following administration of neurobolil to thyroidectomized rats, acidophilic cells were found in the pituitary in larger numbers than in thyroidectomized rats not receiving neurobolil (Table 2). The number of basophilic and chromophobic cells in the pituitary of these animals was correspondingly reduced.

Hence, after administration of neurobolil, besides the anabolic effect (increase in body weight), the histological structure of the pituitary was changed and the number of acidophilic cells producing STH was increased in the control and thyroidectomized rats. This confirms the hypothesis that somatotropic hormone may play a role in the mechanism of action of neurobolil.

LITERATURE CITED

1. N. A. Zarubina and M. I. Balabolkin, Probl. Éndokrinol., No. 6, 44 (1967).
2. Ya. M. Kabak and E. B. Pavlova, Byull. Éksperim. Biol. i Med., 21, No. 4, 17 (1946).
3. Z. A. Kabalova, Relations of the Thymus with Glands of Internal Secretion, Candidate's Dissertation, Moscow (1969).
4. E. V. Pavlova, Probl. Éndokrinol., No. 1, 13 (1958).
5. B. V. Pokrovskii, Probl. Éndokrinol., No. 3, 112 (1966).
6. T. S. Pronina and E. B. Pavlova, Probl. Éndokrinol., No. 6, 58 (1967).
7. A. E. Rabkina, Byull. Éksperim. Biol. i Med., 23, No. 5, 364 (1947).
8. R. S. Tishenina and L. M. Gol'ber, Probl. Éndokrinol., No. 4, 64 (1968).
9. N. A. Yudaev and B. V. Pokrovskii, Vopr. Med. Khimii, No. 5, 527 (1966).

10. P. A. Dessaulles, Helv. Med. Acta, 27, 479 (1960).
11. D. M. Hegsted, Endocrinology, 81, 673 (1967).
12. U. Kadzuo, J. Wakayama Med. Soc., 17, 421 (1966).
13. C. D. Kochakian and I. R. Murlin, J. Nutr., 10, 437 (1935).
14. C. D. Kochakian, Vitam. and Horm., 4, 255 (1946).
15. C. D. Kochakian, Am. J. Physiol., 160, 66 (1950).
16. C. D. Kochakian and F. Jong, J. Endocrinol., 13, 90 (1955).
17. C. D. Kochakian, Proc. Soc. Exp. Biol. (New York), 103, 196 (1960).
18. M. Reiss et al., Acta Endocrinol. (Copenhagen), 49, 366 (1965).
19. J. Solamon and R. O. Greep, Endocrinology, 65, 158 (1959).
20. S. Werner (editor), The Thyroid Gland [Russian translation], Leningrad (1963).