for providing the preparation of TPA, and to Dr. S. Murphy (Milton Keynes, England) for providing the 6,7-dimethyl-5,6,7,8-tetrahydropterine.

LITERATURE CITED

1. I. P. Ashmarin, Patol. Fiziol., No. 4, 13 (1982).
2. Zh. D. Bespalova, N. V. Korobov, M. I. Titov, and O. N. Chichenkov, Farmakol. i Toksikol., No. 2, 39 (1982).
3. N. S. Popova and E. L. Dovedova, Zh. Vyssh. Nerv. Deyat., 34, 768 (1984).
4. M. G. Uzbekov, Ontogenez, No. 1, 58 (1981).
5. M. G. Uzbekov, Byull. Éksp. Biol. Med., No. 2, 38 (1983).
6. M. G. Uzbekov, in: Proceedings of the 9th All-Union Conference on Biochemistry of the Nervous System [in Russian], Erevan (1983), p. 92.
7. D. Biesold, V. Bigl, and M. G. Uzbekov, Activ. Nerv. Sup. (Prague), 19, 154 (1977).
8. D. A. Brase, Adv. Biochem. Psychopharmacol., 20, 409 (1979).
9. E. De Robertis, A. R. Iraldi, G. R. L. Arnaiz, and L. Salganicoff, J. Neurochem., 9, 23 (1961).
10. G. Loew, G. Hashimoto, L. Williamson, et al., Mol. Pharmacol., 22, 667 (1982).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
12. R. Sneglar and M. Vogt, J. Physiol. (London), 314, 395 (1980).
13. S. Spampinato, V. Locatelli, D. Cocchi, et al., Endocrinology, 105, 163 (1979).

FUNCTIONAL STATE OF THE SEROTONINERGIC SYSTEM OF THE THYROTOXIC THYROID GLAND

E. S. Rom-Boguslavskaya, V. G. Gal'chinskaya, and M. R. Ozerova

UDC 616.441-008.61-07:[616.441-008.94:577.175.823]-018.92

KEY WORDS: serotonin; serotoninergic system; 5-hydroxytryptophan; monoamine oxidase; thyroid gland; thyrotoxicosis; K cells.

In thyrotoxicosis (diffuse toxic goiter) "escape" of the thyroid gland (TG) from thyrotrophic regulatory influences is observed. Under these conditions the role of activators of thyroid function is played primarily by thyroid-stimulating immunoglobulins [13, 15]. The problem of the role of other stimulators of thyroid activity, above all biogenic amines, under these circumstances remains unsolved. Yet the solution to this problem may be directly relevant to the elucidation of certain aspects of the pathogenesis of thyrotoxicosis.

In the investigation described below the functional state of the serotoninergic system of the thyrotoxic TG was studied. The mechanisms of the activating effect of serotonin on the follicular apparatus of TG are quite complicated [4, 8, 10-12]. It has been observed that the stimulating effect of serotonin on thyroid function is most clearly manifested during blocking of thyrotrophic influences [9].

EXPERIMENTAL METHOD

Concentrations of serotonin [2] and of its precursor 5-hydroxytryptophan (5-HTP) [1] and monoamine oxidase (MAO) activity [6], using serotonin as the substrate, were determined in thyroid tissue obtained during operations on 26 patients with manifest thyrotoxicosis and in the unchanged paranodal tissue of TG obtained from 14 patients with nodular euthyroid goiter (control group). The rate of incorporation and efficiency of the reaction of uptake of ^{3}H-5-HTP (specific activity 5 Ci/mmole, from Amersham Corporation, England), by the gland also

Laboratory of Biochemistry of Endocrine Diseases, Khar'kov Research Institute of Endocrinology and Hormone Chemistry. (Presented by Academician of the Academy of Medical Sciences of the USSR, L. T. Malaya.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 2, pp. 161-163, February, 1986. Original article submitted April 17, 1985.

 0007-4888/86/0002-0180$12.50

TABLE 1. Parameters of Activity of the Serotoninergic System in the Euthyroid and Thyrotoxic Human TG (M ± m)

Test object	Serotonin concentration, μmoles/g	5-THP concentration, μmoles/h	Parameters of 5-HTP uptake			MAO activity, $\mu moles \cdot g^{-1} \cdot h^{-1}$
			$K_M \cdot 10^{-8}$	V_{max}, m/g/h	$E_{max} \times 10^{-3}$	
Euthyroid TG (n=14)	1,48±0,06	3,79±0,07	3,80±0,19	1,00±0,05	1,20±0,06	2,16±0,17
Thyrotoxic TG (n=29)	2,23±0,06*	6,12±0,07*	4,10±0,22	2,20±0,06*	2,70±0,07*	1,69±0,07*

Legend. *P < 0.05 compared with euthyroid TG.

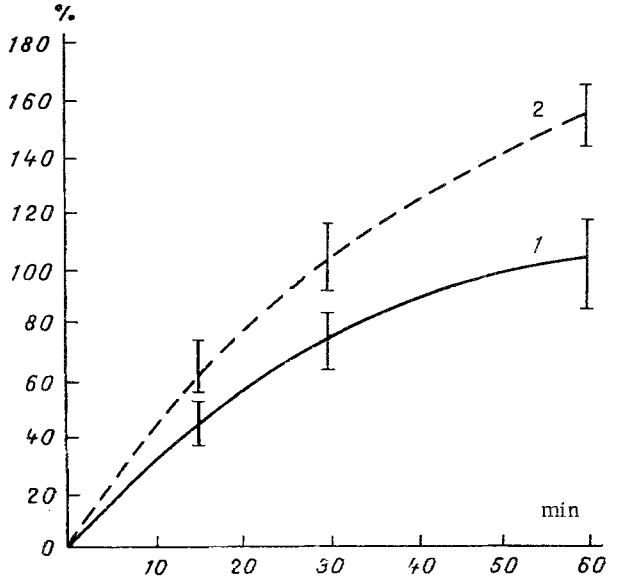

Fig. 1. Uptake of ^{3}H-5-HTP by TG tissue. Abscissa, incubation time (in min); ordinate, uptake (in % compared with initial values). 1) Euthyroid tissue; 2) thyrotoxic tissue.

were determined, for which purpose slices of thyroid tissue were incubated in Eagle's medium with Hanks' solution (1:1) at 37°C. Uptake of ^{3}H-5-HTP, with different concentrations in the incubation medium and different durations of incubation, was investigated after preincubation in the above-mentioned medium for 30 min. After rinsing five times with physiological saline the preparations were placed in scintillation cuvettes and covered with 1 ml of ethanol. After extraction for 18-20 h, the specimens in the cuvettes were treated with 10 ml of ZhS-7A scintillator and their radioactivity was measured on an SBS-2 scintillation counter.

EXPERIMENTAL RESULTS

As will be clear from Table 1, the serotonin concentration in thyrotoxic TG tissue was significantly higher than in the control. Differential analysis of the results showed that progression of thyrotoxicosis was accompanied by a rise of the serotonin level in the gland (in moderately severe thyrotoxicosis this parameter was 1.88 ± 0.06 μmole/g, in a severe form 2.73 ± 0.01 μmole/g, and in unchanged TG tissue 1.48 ± 0.06 μmole/g). The concentration of 5-HTP, the direct precursor of serotonin, in thyrotoxic TG tissue was more than 60% higher than in unchanged tissue. Analysis of the individual values showed that the maximal 5-HTP concentration in euthyroid TG tissue was 5.79 μmoles/g and in thyrotoxic TG tissue 10.13 μmoles/g.

Serotonin in the human TG is known to be located in K cells, which are part of the so-called APUD system [14]. Cells of this system contain not only oligopeptide hormones (in this case calcitonin and somatostatin), but also biogenic amines and their precursors, and they thus are able to take up the latter and to decarboxylate them. For this reason the rate of uptake of ^{3}H-5-HTP into TG tissue can be regarded as an indicator of the functional activity of serotonin-containing formations.

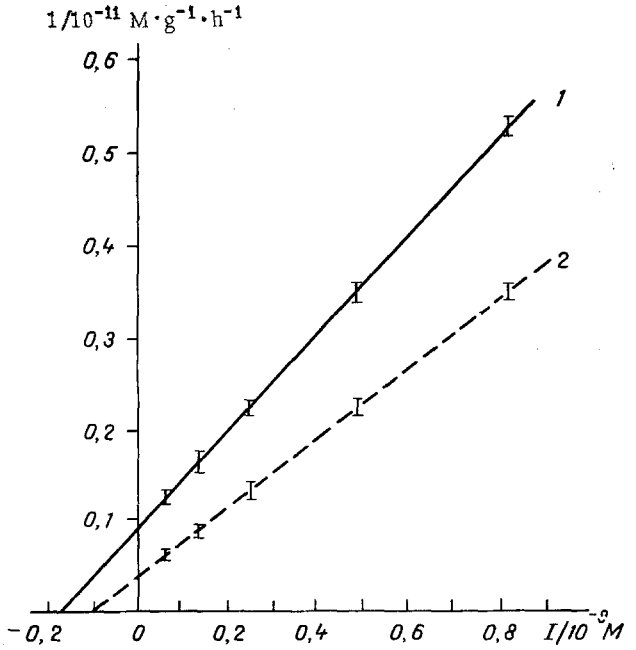

Fig. 2. Rate of uptake of ^{3}H-5-HTP by TG tissue. Legend as to Fig. 1.

It will be clear from Fig. 1 that differences between the quantity of ^{3}H-5-HTP taken up by unchanged and thyrotoxic TG tissues during the first 15 min of incubation are not significant. Later the intensity of uptake of the labeled serotonin precursor by thyrotoxic tissue increased significantly. The increase in the quantity of ^{3}H-5-HTP taken up by thyrotoxic gland tissue compared with the control was 30.8% at the 30th minute of the investigation and 56% at the 60th minute. The radioactivity of the TG tissue in the second and, in particular, in the third stage of the investigation was due not only to the quantity of ^{3}H-5-HTP incorporated into the gland tissue, but also to the quantity of labeled serotonin formed from it; however, the total radioactivity reflects the intensity of the process under analysis, and thus corresponds to the aim of the investigation.

Besides determining the ^{3}H-5-HTP uptake, we also studied the rate of uptake of ^{3}H-5-HTP by TG slices when its concentration in the incubation medium varied between 0.4 and 8.3 pM.

Graphs showing dependence of the rate of ^{3}H-5-HTP uptake on the substrate concentration in the incubation medium, plotted between double reciprocal coordinates, are given in Fig. 2. Table 1 shows that the rate of uptake of ^{3}H-5-HTP by thyrotoxic TG tissue was sharply increased, whereas the value of the Michaelis' constant (K_M) was virtually the same as in the control.

For a general description of the process of ^{3}H-5-HTP uptake, the so-called "efficiency" of the reaction of uptake of the labeled serotonin precursor by TG tissue was used. The efficiency of the reaction (E) is essentially an absolute characteristic of reactivity of the test system: $E = V_{max}/2K_M$. It will be clear from Table 1 that the efficiency of uptake of labeled 5-HTP by the thyrotoxic TG was significantly higher than by the normal TG.

Thus one way of increasing the serotonin concentration in thyrotoxic TG tissue is the more active uptake of 5-HTP and its decarboxylation. Accordingly, it is reasonable to analyze the conditions leading to an increase in the 5-HTP concentration in TG of patients with thyrotoxicosis.

The writers observed previously [3, 5] that the serotonin level in the blood is sharply reduced (by about half) in patients of this category. This could not be explained by acceleration of metabolic inactivation of the monoamine, since the quantity of 5-hydroxyindoleacetic acid (the principal metabolite of serotonin) excreted was increased only very slightly. Meanwhile, the urinary excretion of 5-HTP, reflecting its blood level to a certain degree, rose in the patients studied in the present investigation (which was carried out in the preoperative period) from 1.10 ± 0.18 μmole/day in the control to 2.27 ± 0.026 μmole/day. Thus, serotonin formation at the periphery from its precursor was delayed; this led to accumulation

of the precursor, i.e., to a situation permitting increased uptake of 5-HTP by TG cells from the blood stream. An increase in activity of the serotoninergic neurons of the hypothalamus is known [7] to be accompanied by increased uptake of tryptophan, the precursor of the whole group of indoles, from the blood. The possibility cannot be ruled out that a similar situation holds good also during TG hyperfunction.

However, an increase in the serotonin concentration in thyrotoxic TG tissue may be the result not only of increased biosynthesis of the amine, but also of slowing of its oxidative deamination. It will be clear from Table 1 that MAO activity in the thyrotoxic tissue was significantly lower than in the control, and this was bound to affect the serotonin concentration. Nevertheless, when individual values of MAO activity were analyzed, it became clear that high levels of the amine in TG in thyrotoxicosis corresponded to both low and normal values of MAO activity. It can be concluded from a comparison of the 5-HTP concentration in TG and the level of MAO activity that in some cases the raised serotonin level in thyrotoxic TG tissue depended mainly on a reduction in the intensity of its metabolic conversions, and in other cases on a higher level of synthesis in the K-cells of TG.

Thus the functional state of the serotoninergic system in TG in patients with thyrotoxicosis undergoes considerable changes, as a result of which stronger activating influences can be brought to bear on the follicular apparatus than under normal conditions.

LITERATURE CITED

1. Ts. G. Gerasimova, Lab. Delo, No. 1, 14 (1971).
2. V. I. Kulinskii and L. S. Kostyukovskaya, Lab. Delo, No. 7, 390 (1969).
3. V. V. Natarov, E. S. Rom-Bugoslovskaya, and M. R. Ozerova, Probl. Éndokrinol., No. 1, 16 (1981).
4. E. V. Naumenko and N. K. Popova, Serotonin and Melatonin in Regulation of the Endocrine System [in Russian], Novosibirsk (1975).
5. E. S. Rom-Bugoslovskaya and M. R. Ozerova, in: Chemistry, Biochemistry, and Pharmacology of Indole Derivatives [in Russian], Tbilisi (1981), p. 169.
6. M. I. Chuiko, in: Current Problems in Theoretical and Clinical Medicine [in Russian], Minsk (1975), pp. 230-233.
7. M. D. Gerchon and C. F. Dreifus, Brain Res., 184, 229 (1980).
8. H. Holak, A. Baldys, B. Yarzab, et al., Acta Endocrinol. (Copenhagen), 87, 279 (1978).
9. A. Melander, Acta Med. Scand., 201, 257 (1977).
10. A. Melander, L. Ericson, F. Sundler, et al., Rev. Physiol. Biochem. Pharmacol., 73, 39 (1975).
11. A. Melander and F. Sundler, Endocrinology, 93, 193 (1973).
12. B. Mess and L. Peter, Endocrinol. Exp., 9, 105 (1975).
13. J. Orgiazzi and A. M. Mandec, Ann. Endocrinol., 43, 509 (1982).
14. A. G. E. Pearse, Nature, 262, 92 (1976).
15. A. Pinchera, G. F. Fenzi, A. E. Macchia, et al., Horm. Res., 16, 317 (1982).