DETERMINATION OF COMPOSITION OF FREE AMINO ACIDS AND ENZYME ACTIVITY IN HUMAN NASAL POLYPS DURING STIMULATION OF SEROTONIN IN BIOSYNTHESIS

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The composition of free amino acids and activity of certain enzymes in human nasal polyps and their changes during incubation of the polyps with reserpine and compound 48/80, which stimulate serotonin biosynthesis, were studied. It is postulated that patterns of distribution of free amino acids and of enzyme activity discovered are evidently attributable to the biosynthesis of serotonin de novo which takes place under these conditions.

The nasal polyp is a benign human new growth which is allergic in its genesis and rich in the biogenic amines histamine and serotonin [2, 4]. The source of the serotonin in nasal polyps has been shown to be the orthochromatic granules of the mast cells [3]. It has also been shown [5] that if nasal polyps are incubated with chemical liberators of serotonin, such as reserpine and compound 48/80, and also with the serotonin precursor, tryptophan, they readily undertake the biosynthesis of serotonin. For instance, during incubation of a polyp with 2.5 mg reserpine its serotonin concentration rose from 5.5 to 1020 μg/g fresh tissue. Morphologically, serotonin formation was reflected in the appearance of numerous serotonin cells, or mast cells containing orthochromatic granules in their cytoplasm.

The purpose of this investigation was to study the composition of free amino acids and the activity of certain enzymes in nasal polyps before and after their incubation with substances stimulating serotonin formation: reserpine and compound 48/80. By comparing data for the amino-acid composition and activity of various enzyme systems in the same polyp it should be possible to obtain a clearer picture of the character of the specific changes in cell metabolism which are characteristic of the growth of tumors and which accompany biosynthesis of the biogenic amine serotonin.

EXPERIMENTAL METHOD

Immediately after their removal at operation the polyps were cut into three equal parts: reserpine (1 mg/ml) was injected into one part, compound 48/80 (5 mg/ml) into the second, and the third part acted as the control. The polyp was then incubated at 37°C, for as long as required, under oxygen saturation (see below).

The polyp fluid freely exuding from it during incubation for 1 h was used as the material for chromatographic fractionation. The composition of free amino acids in the polyp fluid was determined by ascending paper chromatography. For details of treatment of the chromatograms and their staining with ninhydrin, and of quantitative assay of the amino acids, the paper by Paskhina [1] may be consulted. The amino-acid concentrations were calculated in mg%. Altogether 39 experiments were carried out on polps taken from 18 patients; the total number of tests was 466.

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TABLE 1. Concentration of Free Amino Acids (in mg%) in Polyp Fluid in Relation to Composition of Incubation Medium

Amino acid	Polyp fluid	Polyp fluid after incubation	
		with compound 48/80	with reserpine
Crystine-cysteine	15,3±8,4	15,3±4,7	16,2±8,7
Lysine	22,3±2,8	14,0±6,8	7,2±2,6
Histidine	11,7±5,3	8,2±2,1	11,8±3,8
Glutamine	10,0±4,4	7,5±2,0	5,9±2,9
Aspartic acid	—	—16,3	—7,9
Serine	6,6±3,7	6,7±3,1	7,0±3,2
Glycine	10,9±5,9	5,6±1,1	10,4±5,7
Glutamic acid	11,2±4,1	7,1±3,5	6,6±3,7
Threomine	6,4±3,4	4,1±1,6	4,4±2,5
Alanine	8,8±3,7	5,6±1,4	5,8±2,9
Tyrosine	3,3±1,2	1,6±0,2	2,6±1,4
Tryptophan	73,1±7,5	25,1±6,7	33,7±7,7
Methionine	3,6±2,1	2,7±0,9	3,1±1,1
Valine	3,2±1,0	2,6±0,3	2,3±1,3
Phenylalanine	10,3±6,2	3,9±1,6	5,8±2,4
Leucine	5,3±2,3	3,6±1,7	5,1±2,8
Total	202,0	129,9	135,8

Activity of the enzymes was determined in a tissue homogenate from a native polyp and in equal parts of the same polyps incubated for 10 min and 1 h with reserpine and compound 48/80 respectively. The technique of assaying the activity of the various enzymes is indicated in Table 2.

EXPERIMENTAL RESULTS

As Table 1 shows, the polyp fluid is extremely rich in free amino acids. Qualitative analysis revealed 15 amino acids, including glutamine. The total content of free amino acids was 202 mg%.

The first fact to be noted when the results of analysis of the amino-acid composition of the intact polyp fluid are examined is its high tryptophan content. Since tryptophan is the only precursor of serotonin, the increased content of this amino acid can account for the strikingly high ability of nasal polyps to accumulate serotonin, as is observed during incubation of polyps with reserpine and compound 48/80 [5].

Of the other amino acids contained in the polyp fluid in high concentrations those which deserve special mention are glutamic acid, glutamine, and alanine and also the sulfur-containing amino acids cysteine and methionine. The high concentrations of glutamic acid, cysteine, and glycine suggest that polyps contain glutathione, a substance participating in trigger reactions initiating peptide and protein synthesis.

The results of measurements of the activity of certain enzymes in the polyps are summarized in Table 2 and they show that measurable activity of all the enzymes studied is present in nasal polyp tissue. The highest activity found was of transketolase, an enzyme of the pentose cycle of flucose oxidation participating in structural processes in the tissue, and of lactate dehydrogenase, a glycolytic enzyme participating in energy metabolism in the tissues. The activity of the acid hydrolases, evidently of lysosomal origin, namely acid phosphatase, acid deoxyribonuclease, and cathepsins, which are concerned in the hydrolysis of polymer molecules, also was considerable. It was found that the nasal polyp possesses marked aspartate aminotransferase and alanine aminotransferase activity.

The discovery for the first time in nasal polypi of comparatively high 5-hydroxytryptophan-decarboxylase activity must be specially emphasized. The enzyme 5-hydroxytryptophan decarboxylase catalyzes the biosynthesis of serotonin from its immediate precursor, 5-hydroxytryptophan.

During incubation of the polyp tissue with the stimulators of serotonin synthesis (reserpine and compound 48/80) substantial changes took place in the quantitative and qualitative composition of the free amino acids, accompanied by an increase in the activity of all the enzymes studied, affecting them all to different degrees and occurring at different periods of incubation (see Tables 1 and 2). Whilst the concentration of most free amino acids was reduced, a characteristic feature was the appearance of aspartic and γ-aminobutyric acids and also of a considerable amount of a peptide located in the zone of the glutamine spot.

TABLE 2. Dependence of Activity of Enzymes in Homogenate of Polyp on Duration of Incubation of Tissues with Reserpine and with Compound 48/80 (M±m)

Enzymes (per g tissue per min)	Method of assay of active enzyme	Polyp tissue homogenate				
		before incubation	after incubation			
			with reserpine		with compound 48/80	
			10 min	1 h	10 min	1 h
Lactate dehydrogenase	After Roman [11]	47,8±3,8	63,3±4,3	107,3±12,4	69,6±5,8	81,0±11,2
Transketolase	After Bruns [7]	405,7±50,1	675,7±100	690,0±47,7	421,0±65,7	508,0±68,4
Aspartate aminotransferase	After Umbreit, in Paskhin's modification [1]	51,0±5,8	106,0±9,6	68,6±3,8	79,0±9,9	68,0±7,3
Alanine aminotransferase		26,1±1,8	20,3±2,1	28,3±3,8	20,6±5,3	28,5±4,2
Cathepsins (in units/h)	After Homolka [10]	6,5±0,5	12,8±0,8	4,2±0,5	11,5±1,4	3,4±0,2
Acid phosphatase	After Bessey [6]	69,3±5,2	85,5±9,8	38,4±9,7	81,3±5,6	36,8±3,5
Deoxyribonuclease	After Byvoet [9]	1,2±0,2	2,9±0,4	0,6±0,1	1,6±0,1	0,5±0,1
5-Hydroxytryptophan decarboxylase	After Undenfriend and Buzard [8]	0,82±0,12	0,82±0,12	1,12±0,12	0,82±0,12	1,5±0,4

The study of enzyme activity showed that after incubation for 10 min there was activation of lactate dehydrogenase, transketolase, aspartate aminotransferase, cathespins, acid phosphate, and acid deoxyribonuclease. The only exception was 5-hydroxytryptophan decarboxylase, whose activity remained unchanged at this time. In the course of further incubation (1 h) the activity of the enzymes participating in energy metabolism and biosynthesis continued to increase (lactate dehydrogenase) or remained high (transketolase, aspartate aminotransferase). Activity of the acid hydrolase (cathepsins, acid phosphatase, deoxyribonuclease) fell sharply to much below the initial level. Activity of alanine aminotransferase showed no significant change throughout the period of incubation.

The enzyme 5-hydroxytryptophan decarboxylase occupies a special position. Marked activation of this enzyme was observed only after incubation for 1 h, which coincided with the time of an increase in the serotonin content in the nasal polyps under the influence of reserpine and of compound 48/80 and with a massive appearance of mast cells (serotonin cells), responsible for serotonin formation.

The results of these experiments thus suggest that a high metabolic reserve of amino acids exists in nasal polyps, including a surprisingly high content of the serotonin precursor, tryptophan. The characteristic dynamics of activation of the enzyme systems observed during incubation of the polyps with reserpine and compound 48/80, accompanied by changes in the content of most of the free amino acids, suggests that certain amino acids are utilized for peptide and protein synthesis which accompanies the biosynthesis of serotonin, and that the general principles governing the distribution of free amino acids and the enzyme activity observed in this investigation can be attributed to the serotonin biosynthesis taking place under these conditions.

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