

EFFECT OF RESERPINE AND COMPOUND 48/80  
ON LIBERATION AND SECRETION OF SEROTONIN  
IN NASAL POLYPS

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In nasal polyps incubated in vitro with reserpine and compound 48/80, serotonin is formed and is liberated into the surrounding medium. The quantity of serotonin formed is directly dependent on the concentration of reserpine and compound 48/80 and on the incubation time.

Until recently it has been held that serotonin is present only in the mast cells of rats and mice. The mast cells of other animals, and of man, do not contain serotonin or, if they do, it is present in extremely small amounts [4, 7-12]. Only very recently [3, 5, 6] has it been shown for the first time that in diseases accompanied by an increase in serotonin formation (carcinoid tumors) or an increase in the number of mast cells in the tissues (diffuse mastocytosis of the skin), serotonin can also be found in human mast cells.

By means of fluorometric and fluorescence-microscopic methods the writers have shown that serotonin is also present in nasal polyps in concentrations of up to  $3.4 \mu\text{g/g}$  tissue homogenate [2], and that the source of serotonin in the polyp is the orthochromic mast cells [1].

In the investigation described below the ability of nasal polyps to synthesize serotonin was studied during incubation with the most powerful serotonin liberators known (reserpine and compound 48/80).

EXPERIMENTAL METHOD

Altogether 121 tests were carried out on nasal polyps removed at operations on 63 patients.

Immediately after removal of the polyp from the patient's nose, a known quantity of reserpine or compound 48/80, dissolved in physiological saline, was injected into it through a needle, and the polyp was then incubated at  $37^\circ$ . After the necessary time, the fluid escaping from the polyp was collected. The serotonin content was determined fluorometrically in the polyp fluid and in a homogenate of the polyp tissues. The results were expressed in micrograms per milliliter fluid or per gram tissue homogenate respectively. The quantity of serotonin liberated was calculated from the ratio between its concentration in the polyp fluid and the total content of serotonin in the polyp.

The Soviet compound 48/80, synthesized at the State Research Institute of Organochlorine Products and Acrylates by Doctor of Chemical Science V. S. Étlis, was used in these experiments.\*

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# EXPERIMENTAL RESULTS

The total serotonin content in the freshly removed polyp varied from 0.6 to 5.7  $\mu\text{g}$ , with a mean value of  $2.59 \pm 0.4$   $\mu\text{g/g}$  tissue homogenate. The quantity of serotonin liberated spontaneously from the fresh polyp into the polyp fluid ranged from 6 to 20% during the first 30 min, and did not exceed 40% at the end of the first hour, depending on the consistency of the polyp. In edematous polyps the percentage of serotonin liberated was higher than in solid polyps.

During incubation of the polyp with the two liberators the percentage of serotonin liberated into the polyp fluid increased, depending on the type of liberator used.

Liberation of serotonin by the action of compound 48/80 was "explosive" in character, and its action was similar to that of polyp antigen. The highest degree of liberation of serotonin occurred during the first 15 min of incubation of the polyp. The dose of liberator had no particular effect on the time of serotonin liberation under these conditions. The curve of serotonin liberation under the influence of reserpine was more drawn out. The liberation of serotonin was obviously dependent on the concentration of reserpine: low concentrations were associated with slight liberation of serotonin, high concentrations with more considerable liberation.

Incubation of nasal polyps with reserpine and compound 48/80 revealed an important feature distinguishing the action of these liberators. Both compound 48/80 and, in particular, reserpine, possessed the well marked property of inducing fresh serotonin formation in the polyps. The intensity of this fresh serotonin formation in the polyp was directly dependent on the concentration of liberator and the duration of incubation. The formation of fresh serotonin was also observed to a slight degree during incubation of the polyp with physiological saline.

The least concentration inducing a statistically significant increase in the absolute serotonin content in the polyps, and also an increase in the percentage of serotonin liberated into the polyp fluid, was 0.25 mg/ml for reserpine and 5 mg/ml for compound 48/80. The increase in serotonin during incubation of the polyp with reserpine in a concentration of 25 mg/ml was particularly striking (Table 1).

The dependence of the formation of fresh serotonin on the concentration of liberators was demonstratively revealed when different parts of the same polyp were incubated with different concentrations of reserpine and compound 48/80. A tenfold increase in the concentration of reserpine and compound 48/80

TABLE 1. Mean Serotonin Content in Nasal Polyp in Relation to Concentration of Reserpine and Compound 48/80

Index studied	Physiological saline	Reserpine (mg/ml)				Compound 48/80 (mg/ml)			
		0.25	0.5	1.0	25	5	10	20-40	
Absolute content of serotonin (in $\mu\text{g}$ )	$6.0 \pm 0.9$ (19)	$16.7 \pm 5.5$ (3)	$32.7 \pm 7.3$ (11)	$43.3 \pm 17.8$ (5)	$592.7 \pm 108$ (5)	$9.9 \pm 2.8$ (5)	$24.7 \pm 6.5$ (5)	$29.5 \pm 5.6$ (8)	
Percentage of serotonin liberated	$46.4 \pm 6.0$ (19)	$75 \pm 1$ (3)	$75.5 \pm 9.0$ (11)	$93.1 \pm 3.5$ (5)	$97.2 \pm 0.7$ (5)	$72.1 \pm 2.7$ (5)	$73.5 \pm 5.6$ (5)	$64.5 \pm 4.8$ (8)	

Note. Here and in Table 2, numbers in brackets refer to number of experiments.

TABLE 2. Mean Content of Serotonin in Nasal Polyps in Relation to Duration of Incubation and Concentration of Reserpine and Compound 48/80

Incubation medium	Concentration (mg/ml)	Duration of incubation					
		1/2 h		1 h		2 h	
		$\mu\text{g}$	% liberation	$\mu\text{g}$	% liberation	$\mu\text{g}$	% liberation
Reserpine	0.5-1	$21.2 \pm 3.0$ (4)	$95 \pm 3.0$	$16.2 \pm 3.5$ (6)	$90.8 \pm 1.4$	$36.0 \pm 2.3$ (7)	$94.3$
Compound 48/80	10-40	$17.8 \pm 3.3$ (11)	$71.8 \pm 4.8$	$33.3 \pm 8.4$ (5)	$72.9 \pm 6.6$	$9.0 \pm 2.6$ (2)	$77.2 \pm 6.4$ (2)
Physiological saline	—	$2.2 \pm 0.7$ (4)	$29.5 \pm 9$	$5.0 \pm 0.5$ (7)	$32.1 \pm 4.8$	$8.8 \pm 2.4$ (6)	$62.6 \pm 8.0$

TABLE 3. Mean Serotonin Content in Nasal Polyp in Different Incubation Media

Incubation medium	Number of tests	Duration of incubation (h)	Concentration	Variations of indices	Mean total content ( $\mu\text{g/g}$ )	Mean % liberation into polyp fluid
Fresh polyp	22	—	—	0.6-5.7	$2.59 \pm 0.4$	
Physiological saline	19	$\frac{1}{2}$ -4	—	1.4-17.3	$6.0 \pm 0.93$	$46.4 \pm 6.0$
Compound 48/80	33	$\frac{1}{2}$ -1	0.5-40	0.9-56.4	$17.6 \pm 2.4$	$65.9 \pm 3.6$
Reserpine	43	$\frac{1}{2}$ -4	0.01-25	3.1-1010.9	$51.5 \pm 32.2$	$88.4 \pm 2.4$
Tryptophan	4	1	1.0	6.1-97.9	$44.8 \pm 21.8$	$94.4 \pm 2.6$

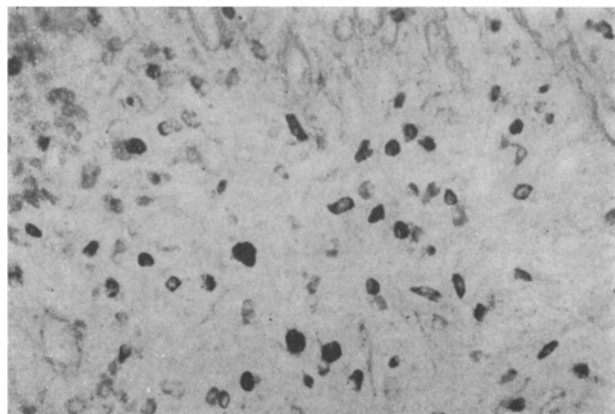


Fig. 1

Fig. 1. Accumulation of metachromatic substance in undifferentiated polyp cells. Incubation for 1 h with 10 mg/ml compound 48/80. Fixation in solution containing 0.6 % formaldehyde and 0.5 % acetic acid. Toluidine blue, 200 $\times$ .

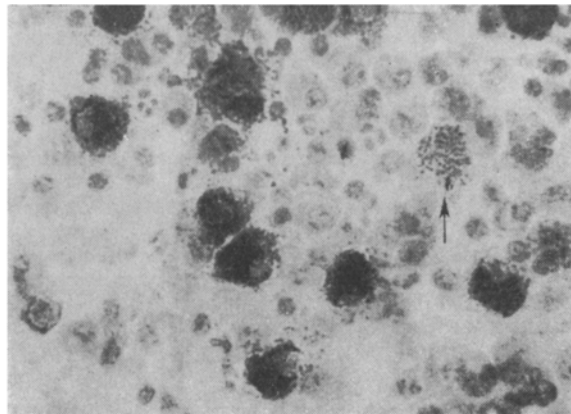


Fig. 2

Fig. 2. Cluster of serotonin cells undergoing degranulation. Incubation for 45 min with 0.5 mg/ml reserpine. Toluidine blue, 400 $\times$ .

led to a considerable increase in the formation of fresh serotonin in the polyps and to an increase in the proportion of serotonin liberated into the polyp fluid.

With an increase in the duration of incubation with reserpine and compound 48/80, the absolute content of serotonin in the polyps increased, as also did the percentage of serotonin liberated into the polyp fluid (Table 2). A particularly marked increase in serotonin content was observed during incubation of polyps with reserpine for 2-4 h. By contrast with reserpine, a high concentration of compound 48/80 had a cytotoxic effect, and the production of serotonin in the polyp came to an end if incubation continued for longer than 1 h.

Considering that serotonin is a product of tryptophan metabolism, and that the conversion of tryptophan into serotonin is a specific property of the tissue forming serotonin, it was decided to study in vitro the ability of polyp tissue to convert tryptophan, as serotonin precursor, into serotonin and also to determine the intensity of this conversion. It is known that only 1 % of the alimentary tryptophan entering the human body is converted into serotonin. However, in diseases accompanied by a marked increase in serotonin output, the percentage of tryptophan converted into serotonin may be increased. In carcinoid tumors, for instance, the cells may convert up to 60 % of the alimentary tryptophan into serotonin.

Altogether four experiments were carried out with tryptophan. A polyp was incubated for 1 h with 1 mg tryptophan. Serotonin was formed in amounts varying from 6 to 98  $\mu\text{g/g}$ , or 0.6-10 % (mean 4.5 %) of the original amount of tryptophan (Table 3). In edematous polyps this percentage was lower, and in solid or fleshy polyps it was higher. The freshly formed serotonin was almost entirely liberated into the polyp fluid, the percentage liberated ranging from 93.5 to 99. The nasal polyp can thus convert tryptophan into serotonin, and the degree of conversion of exogenous tryptophan into serotonin was a little higher than normal.

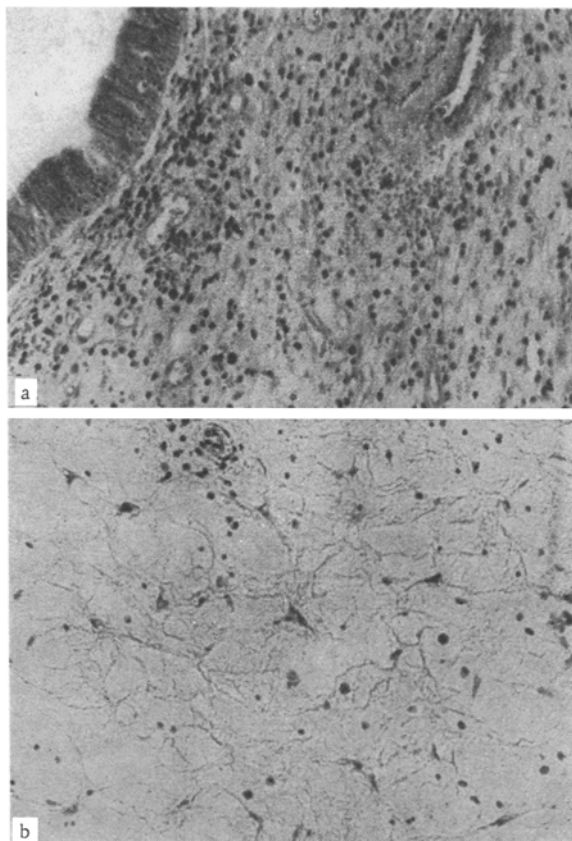


Fig. 3. Disappearance of polyp cells from tissue: a) polyp before incubation; b) same polyp after incubation for 2 h with 25 mg/ml reserpine. Toluidine blue, 100x.

Hence, during incubation of nasal polyps with chemical serotonin liberators (reserpine and compound 48/80), two special features were noted in the action of these substances: 1) marked ability of serotonin synthesis in polyps during their incubation at 37° with compound 48/80 and, in particular, with reserpine; 2) the extremely weak bond between the newly formed serotonin and the tissue, so that it is immediately liberated into the surrounding medium (into the polyp fluid). This depletory action is particularly characteristic of reserpine, during incubation with which virtually all the freshly formed serotonin was liberated into the surrounding medium. It can be considered that both reserpine and compound 48/80 are not only serotonin liberators, but also active stimulators of serotonin synthesis in nasal polyps. Simultaneous morphological studies showed that serotonin biosynthesis in nasal polyps was accompanied by marked accumulation of a metachromatic substance in the undifferentiated cells of the polyp, with the formation of serotonin cells, resembling mast cells (Fig. 1), the liberation of serotonin into the polyp fluid was accompanied by degranulation of these cells (Fig. 2) and by lysis of the granules and also of the cells themselves, and as a result, the tissues of the polyps became denuded of cells (Fig. 3a, b).

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