FLUORIMETRIC DETERMINATION OF HISTAMINE AND SEROTONIN IN MAST CELLS OF NORMAL AND GERMFREE RATS TREATED WITH RESERPINE AND CYCLIC AMP

P. N. Aleksandrov, G. I. Podoprigora, and Academician A. M. Chernukh*

UDC 612.018.2-06:547.963.32

The content of histamine (by the reaction with orthophthalic aldehyde) and serotonin (by Falk's reaction) in mesenteric mast cells of normal and germfree rats was determined microspectrometrically. Reserpine (10 mg/kg, intramuscularly) was shown not to change the histamine content, whereas the serotonin content was reduced by 50%. Injection of cyclic AMP (3.5 mg/kg, intraperitoneally) caused the serotonin content to be increased by 80% and the histamine content by 280-320%.

KEY WORDS: *Fluorimetry; mast cells; histamine; serotonin; cyclic AMP.*

In the modern view [4], hormones act on cell processes by changing the intracellular content of cyclic adenosine-3',5'-monophosphate (AMP), which behaves as a messenger of the second order. There is reason to suppose that the action of many drugs ultimately is effected through a change in the intracellular cyclic AMP content [3].

On the basis of these observations it was decided to study the effect of cyclic AMP and reserpine on the histamine and serotonin content in mast cells of the rat mesentery.

EXPERIMENTAL METHOD

Experiments were carried out on normal and germfree Wistar rats of strain F49, weighing 200-250 g, reared at the Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR. The content of histamine and serotonin per mast cell was determined fluorimetrically [1] by means of the FMÉL-IV42 luminescence photometer. The reaction with paraform was used for the fluorescence detection of serotonin in the mast cells [2]. Histamine was determined by means of orthophthalic aldehyde, which forms a fluorescent compound with histamine [5]. Reserpine (Rausedil, Gedeon Richter, Hungary) was injected intramuscularly in a single dose of 10 mg/kg. Cyclic AMP (synthetic dibutyryl-3'-5'-AMP) was given as a single intraperitoneal injection in a dose of 3.5 mg/kg. The animals were killed 24 h later and specimens prepared for photometry of the mast cells. The histamine and serotonin content was expressed in relative fluorescence units (FU). The fluorescence unit was taken to be one scale division of a type M-95 microammeter, using a probe 20 μ in diameter, and 100× immersion objective, a 20× ocular, and a voltage of 1000 V on the photoelectronic multiplier.

EXPERIMENTAL RESULTS AND DISCUSSION

The study of specimens treated with paraform and orthophthalic aldehyde showed that serotonin in the rat mesenteric mast cells gives bright greenish-gold fluorescence with a maximum of the fluorescence spectrum in the region of 525 mm. Histamine gives a duller greenish fluorescence with a maximum in the region of 515 mm. The results given in Table 1 show that the mean content of both histamine and serotonin per mast cell was the same for the rats of the two groups. Injection of reserpine caused the serotonin level in both germfree and normal rats to fall by 50-55%. The histamine content in the last cells remains unchanged

*Academy of Medical Sciences of the USSR.

Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR. Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 2, pp. 175-177, February, 1977. Original article submitted May 28, 1976.

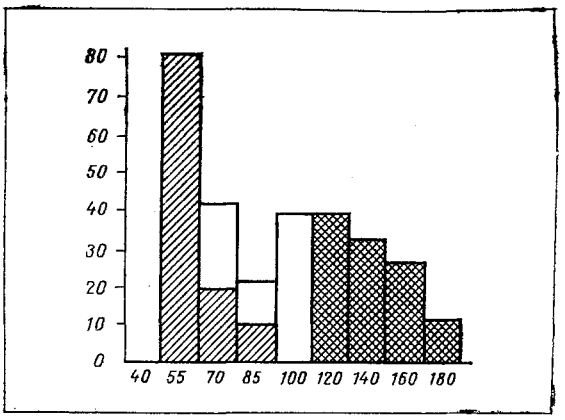

Fig. 1. Distribution of mast cells by serotonin content. Unshaded columns — control; obliquely shaded columns — after injection of reserpine; cross-hatched columns — after injection of cyclic AMP. Abscissa, relative fluorescence units; ordinate, percentage of cells.

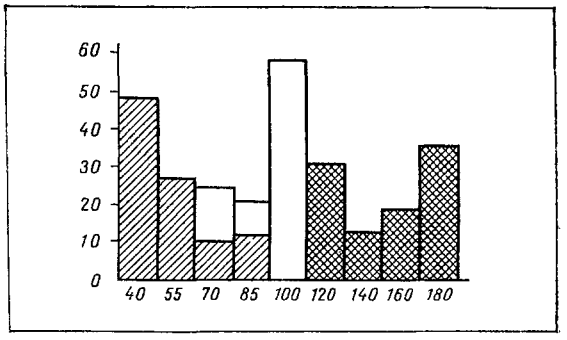

Fig. 2. Distribution of mast cells by histamine content. Legend as in Fig. 1.

TABLE 1. Mean Content of Histamine and Serotonin (in FU) per Mesenteric Mast Cell of Germfree and Normal Rats

Group of animals	Conditions	Serotonin	Histamine
Germfree rats	Control	53,17±1,94	39,85±2,05
	Reserpine	23,74±2,13	41,12±1,87
	P	<0,05	>0,05
	Cyclic AMP	96,83±5,31	110,34±4,36
	P	<0,05	<0,05
Normal rats	Control	56,36±1,78	43,50±1,70
		>0,05	>0,05
	Reserpine	25,24±1,32	40,88±1,43
	P	<0,05	<0,05
	Cyclic AMP	103,74±2,52	129,11±4,99
	P	<0,05	<0,05

in the animals of both groups under the influence of reserpine.

Injection of cyclic AMP caused a sharp increase in the serotonin (by 80-85%) and histamine (by 275-320%) content in both normal and germfree rats.

To assess the distribution of the substance in the various cells, the results were displayed as histograms.

In the control experiments the mast cells were distributed by their serotonin content

(Fig. 1) as follows: 38% of cells had 100 FU of serotonin, 20% had 85 FU, and 42% had 70 FU. Cells with the highest content were considered to be reserve cells, in a state of relative rest; cells with the lowest content were considered to be actively functioning, i.e., utilizing their contents. Cells with an intermediate content of serotonin were in an intermediate functional state.

Under the influence of reserpine the number of mast cells with the lowest serotonin content (55 FU) reached 78%. Cells with the maximal serotonin content disappeared completely. The number of cells with a content of 70 FU was reduced by half and the number with a content of 85 FU was reduced to one-seventh. Reserpine, which lowers the serotonin content of mast cells, thus caused the appearance of a shift to the left on the histogram, i.e., an increase in the number of cells with a low serotonin content. Cyclic AMP caused a shift to the right, i.e., it not only increased the serotonin content in the cells, but also increased the number of cells with a high serotonin content. In the control series there were 25% of cells with 70 FU histamine, 20% with 85 FU, and 55% with 100 FU (Fig. 2). In other words, under normal conditions half of the total number of cells was "in reserve." After injection of reserpine the mean histamine content in the cells was unchanged (Table 1). However, as a result of redistribution, under these circumstances there was a distinct shift to the left on the histogram. The number of cells with less than 70 FU histamine was increased to 87% and cells of the "reserve" had disappeared completely. Cyclic AMP caused a shift to the right. Under these conditions all the cells contained histamine in an even greater amount than the reserve cells. There were 32% of cells with 120 FU and 34% with 180 FU of histamine.

It can accordingly be concluded from the results of these investigations that under the influence of cyclic AMP the histamine content in the mesenteric mast cells of normal and germfree rats is on average trebled and their serotonin content doubled. On the other hand, injection of reserpine is not reflected in the mean histamine content in the mast cells, whereas the mean serotonin content is halved.

LITERATURE CITED

1. P. N. Aleksandrov, in: The Microcirculation. Proceedings of the First All-Union Conference on the Microcirculation [in Russian], Moscow (1972), p. 99.
2. B. Falk and C. Owman, Acta Univ. Lund., Sec. 2, No. 2, 5 (1965).
3. H. M. Goodman, Nature, 219, 1053 (1968).
4. E. W. Sutherland, J. Am. Med. Assoc., 214, 1281 (1970).
5. R. Thunberg, Exp. Cell Res., 47, 108 (1967).