RECEPTION OF ERYTHROCYTIC CHALONE BY BONE MARROW CELLS OF THE ERYTHROID SERIES

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The results of several investigations suggest that chalones exert their influence on cells through a receptor mechanism [2, 6]. By analogy with the action of hormones it can be postulated that the biological effect of chalones is proportional to the number of receptor sites occupied on target cells.

The object of this investigation was to determine if there are differences in the binding of chalones to cells in different stages of differentiation.

EXPERIMENTAL METHODS

Experiments were carried out on 60 noninbred albino mice weighing 20-22 g. Extracts containing chalones (fraction 1) were obtained from rat erthrocytes after electrophoresis [1]. Areas from the gels with fraction 1 were cut out and the fraction was eluted and concentrated on Sephadex G-25 at the rate of 11 mg/ml. The isolated fraction was conjugated with fluorescein isothiocyanate (FITC, from Serva, West Germany), using 0.5 M carbonate buffer, pH 9.5, for this purpose [3]. FITC not reacting with protein was separated by gel filtration through a Sephadex G-25 column with a volume of 19 ml (1.2 × 30 cm), equilibrated with 0.67 M salt-phosphate buffer, pH 7.4. Elution with the same buffer was carried out at the rate of 24 ml/h. The protein content in the samples was determined spectrophotometrically (on the SF-16 apparatus at 280 nm). The fractions collected were pooled and concentrated again with Sephadex G-25 to the original volume. The resulting conjugate was added to mouse bone marrow and thymus cells. Myelokaryocytes were obtained from the femora and thymocytes after mincing of the thymus. The content of dead cells, determined with the aid of methylene blue, was 5% of the myelokaryocytes and 9% of the thymocytes.

The suspension of bone marrow and thymus cells was washed with Hanks' solution at 1000 rpm for 15 min. The number of cells was counted in a Goryaev's chamber and the two suspensions equalized for the number of cells in 1 ml with Hanks' solution. Labeled protein was added at the rate of 0.1 ml to 2 ml of cell suspension, the mixture was shaken for 1 min, and the cells were washed 3 times with Hanks' solution to remove unbound protein. The resulting cell suspension was investigated with the ML-2 luminescence microscope and double monochromatic spectrofluorometer with excitation wavelength of 435 nm and fluorescence wavelength of

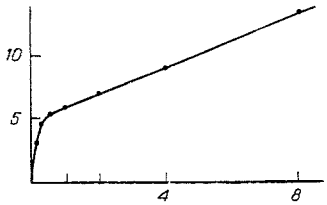

Fig. 1

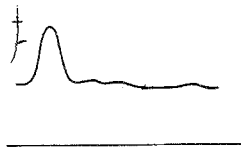

Fig. 2

Fig. 1. Calibration curve for FITC. Abscissa, dose of fluorochrome (in μg/ml); ordinate, deflection of microammeter needle (in conventional units).

Fig. 2. Densitogram of fraction 1 of erythrocyte extracts, conjugated with FITC, obtained by disc electrophoresis.

KEY WORDS: erythrocytic chalone; myelokaryocytes.

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TABLE 1. Intensity of Fluorescence of Bone Marrow and Thymus Cells after Incubation with Labeled Protein

Expt.No.	Intensity of fluorescence of cells (in conventional units)			Quantity of labeled protein bound by cells, μg		
	M	M+A	T	M	M+A	T
1	10, 10, 10, 10, 10 / 10	10, 10, 10, 10, 10 / 10	3, 4, 3, 4, 3 / 3,5	5,1	5,1	0,125
2	9, 9, 9, 9, 9 / 9	9, 9, 9, 9, 9 / 9	5, 5, 5, 5, 5 / 5	4	4	0,375

Legend. M) Myelokaryocytes; A) adrenalin; T) thymocytes. Here and in Table 3 numerator gives individual values of deflection of microammeter needle on changing the sample; denominator gives mean value. Protein content found from calibration curve (Fig. 1).

522 nm. To estimate the quantity of labeled protein bound with the cells a calibration curve was used (Fig. 1). The distilled water, Hanks' solution, and bone marrow and thymus cells, with the above parameters, did not exhibit fluorescence.

The number of luminescent cells was determined in the ML-2 microscope (NS 10-2, FS 1-2, and SZS 14-4 entrance filters, ZhS-18 and ZhZS-19 cutoff filters) in 17 fields of vision under a magnification of 80.

In some experiments binding of labeled protein by myelokaryocytes was observed against the background of addition of adrenalin to the medium in a dose of 33 μg/ml for 1 and 20 min.

To study interaction between labeled chalone and bone marrow cells at different stages of differentiation, the cells were subject to electrophoresis in a Ficoll density gradient and separated into five fractions: 1) cathodal, 2-4) intermediate, 5) anodal [2]. Labeled protein was added to the resulting fractions, containing equal numbers of cells, after which fluorescence was studied as described above.

In some experiments mice were given an injection of an unpurified preparation of erythrocytic chalone (an extract of erythrocytes) in a dose of 1 ml intraperitoneally for a period of 4 h and mitotic activity of the thymocytes and bone marrow cells was determined [1]. Physiological saline was injected into the control animals. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

A densitogram of fraction 1 of extracts of rat erythrocytes, conjugated with FITC and added to the test cells, is shown in Fig. 2. Comparison of fluorescence of myelokaryocytes and thymocytes after addition of the labeled protein showed that the label was bound mainly by bone marrow cells. The slight fluorescence observed in the thymocytes could be due to nonspecific adsorption of protein on their surface (Table 1).

The results of luminescence microscopy showed that only 8% of bone marrow cells was bound with fraction 1 of the erythrocyte extracts, and they were evidently bone marrow cells of the erythroid series, for injection of erythrocytic chalone inhibited mainly the mitotic activity of the cells of this type (Table 2). However, 23% of the cells in the myelogram were of the erythroid series, whereas only 8% were luminescent. Hence it follows that not all erythroid cells bind labeled chalone to the same extent.

This conclusion was confirmed by the results of spectrofluorometric analysis of individual fractions of bone marrow cells. It will be clear from Table 3 that bone marrow cells from electrophoretic fraction 1, rich in blast cells, bound most actively with labeled protein, whereas differentiated forms predominated in fraction 5.

To sum up, it can be concluded that the less mature erythroid precursors have the greatest ability to bind with labeled chalone, whereas differentiated cells bind much less labeled protein and are evidently less sensitive to the action of chalone.

Considering that contact between labeled protein and bone marrow cells *in vitro* lasted only 1 min, it can be assumed that the fluorescence recorded was due to binding of the chalone with surface receptors of the erythroid cells.

TABLE 2. Mitotic Activity (C-mitoses) of Thymocytes and Bone Marrow Cells of the Erythroid and Granulocytic Series 4 h after Injection of Erythrocytic Chalone (EC) (M ± m)

Cells	Number of C-mitoses after injection of		Reduction, %	P
	physiological saline	EC		
Thymus	11±1,05	9±0,84	—18	>0,05
Myeloid series	13±1,5	11±1,1	—15	>0,05
Erythroid series	10±1,26	6±0,63	—40	<0,05

TABLE 3. Intensity of Fluorescence of Bone Marrow Cells after Electrophoresis and Incubation with Labeled Protein

No. of fraction of bone marrow cells	Ratio between blast and differentiated cells, in percent	Intensity of fluorescence of cells (in conventional units)	Quantity of labeled protein bound with cells, μg
Expt. 1			
1 (cathodal)	33:67	$\frac{7, 7, 6, 6, 6}{6,5}$	1,625
2	14:86	$\frac{5, 5, 5, 5, 5}{5}$	0,375
3	8:92	$\frac{4, 3, 4, 4, 4}{4}$	0,180
4	3:97	$\frac{4, 4, 4, 5, 4, 4}{4}$	0,185
5 (anodal)	2:98	$\frac{3, 4, 3, 4, 4}{3,5}$	0,155
Expt. 2			
1 (cathodal)	Analysis not done	$\frac{7, 7, 7}{7}$	2,0
2		$\frac{5, 5, 5}{5}$	0,375
3		$\frac{5, 5, 5}{5}$	0,375
4		$\frac{5, 5, 5}{5}$	0,375
5 (anodal)		$\frac{1, 1, 1}{1}$	0,063

Combined injection of adrenalin and chalone into animals is known to inhibit mitotic activity by a greater degree than injection of each factor separately [4]. The view that under these circumstances a chalone plus adrenalin complex is formed has not been confirmed [5]. It might be expected that adrenalin would increase the number of receptive fields on the surface of the erythroid cells and would consequently potentiate the inhibitory effect of chalone. In that case fluorescence of the cells ought to be increased. However, this was not observed in the experiments (Table 1). The problem of the mechanism of the effect of adrenalin on the action of chalones thus remains unsolved. It can be concluded from the results of the present experiments that fraction 1 of erythrocyte extracts, which possesses chalone activity, is bound mainly by undifferentiated cells.

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