CELL DENSITY AFFECTS THE BINDING OF THE TOXIC LECTIN ABRIN TO HELA CELLS IN MONOLAYER CULTURES

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1. Introduction

The toxic lectin abrin consists of two polypeptide chains joined by a disulfide bond. The B-chain binds the toxin to cell surface receptors containing terminal galactose residues. Subsequently, by a process which is poorly understood, the toxin is brought into the cytoplasm where the A-chain, which is an enzyme, inactivates the 60 S ribosomal subunits, thus inhibiting cellular protein synthesis (reviewed [1]).

In attempts to elucidate the role of pinocytosis in the uptake of abrin we have measured the binding and toxicity of abrin to HeLa cells in monolayer cultures at different cell densities. The experiments were prompted by the report [2] that cell contact induces increased pinocytotic activity in cultured epithelial cells. The unexpected finding was made that the binding of abrin per cell decreased with increasing cell density. The data indicate that the cell density does not influence the number of available surface receptors, but affects their affinity for the lectin. The results are reported here since the binding of other ligands to cell surface receptors may possible be affected in a similar way by cell density and the phenomenon may thus be of some general interest.

2. Materials and methods

Abrin was prepared as in [3] and labelled with ^{125}I using the lactoperoxidase method [4].

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine–*N'*-2-ethane sulfonic acid

HeLa cells were maintained in shaker culture at 37°C in Gibco Minimum Essential Medium as in [4]. The cells studied in monolayer were transferred the day before the experiment to tissue culture dishes (3002 tissue culture dish, 60 X 15 mm style, Falcon, 1950 Williams Drive, Oxnard, CA 93030).

To measure binding of abrin to cells in monolayer, the cells were first washed twice with Hanks' solution and serum-free medium was added. Then increasing amounts of ^{125}I-labelled toxin were added and the cells were incubated at 37°C for 20 min if not otherwise indicated in the figure legends. Finally the cells were washed twice with cold Hank's solution, dissolved in 0.1 M KOH, and the amount of cell-bound radioactivity was measured in an Intertechnique CG 30 automatic gamma spectrometer. To calculate the number of binding sites per cell and the apparent association constants, the data were plotted according to Scatchard [5]. Studies of binding of ^{125}I-labelled abrin to HeLa cells in suspension were performed as in [4].

To measure the amount of ^{125}I-labelled abrin irreversibly bound, cells were incubated with ^{125}I-labelled abrin as above, then the medium was removed and the cells were incubated for 15 min with phosphate-buffered saline containing 0.1 M lactose. The cells were then washed 3 times with this solution and dissolved in 0.1 M KOH.

To measure inhibition of cellular protein synthesis the cells were washed as above and 3 ml Eagle's minimum essential medium containing 21 mM Hepes (pH 7.7) instead of bicarbonate, and 1/10 the usual amount of leucine, 100 μg/ml gentamycin and increasing amounts of abrin were added to each dish.

After incubation at 37°C for 3 h, 0.5 μCi [^{14}C]leucine was added to each dish and after 1 h more at 37°C the medium was removed, the cells were dissolved in 0.1 M KOH, trichloroacetic acid was added to a final concentration of 10% (w/v), and the acid precipitable radioactivity was measured.

3. Results and discussion

Earlier experiments have shown that when HeLa cells are incubated with ^{125}I-labelled abrin at 0°C, all toxin molecules are reversibly bound and can be removed by washing with lactose, whereas at 37°C there is a time-dependent increase in the amount of toxin which cannot be washed off the cells with lactose [4]. It is believed that this amount represents toxin taken into the cells by pinocytosis, and it will be referred to as the amount of irreversibly bound toxin.

When HeLa cells at 2 different cell densities were incubated with ^{125}I-labelled abrin at 37°C and the total amount of bound abrin and the amount bound irreversibly were measured, it was found that the irreversible binding as % total binding of toxin was greater at a higher cell density. This is shown in fig.1

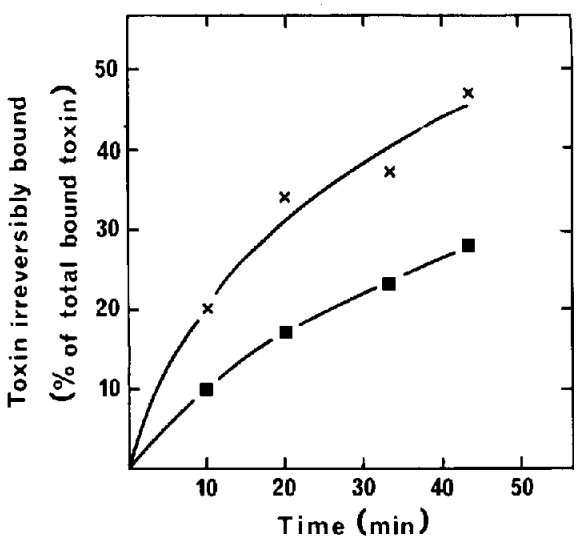

Fig.1. Irreversible binding of ^{125}I-labelled abrin to HeLa cells. ^{125}I-labelled abrin (5.8×10^{-9} M) was incubated with cells in serum-free medium at 37°C. The amount of irreversibly bound abrin was determined as in section 2. (×) 9.2×10^4 cells/cm^2; (■) 1.8×10^4 cells/cm^2.

and is consistent with the finding that cell contact induces an increase in the pinocytotic rate in cultured epithelial cells [2]. However, unexpectedly it was found that an appreciably lower amount of abrin was bound per cell at high than at low density, indicating a change in the K_a or the number of receptors for abrin. Hence, the apparent association at 37°C and the number of receptors at different cell densities were calculated from data obtained after 20 min incubation. This value was used since it was found that the amount of reversibly bound toxin was constant after about 20 min incubation, both at high and low cell density (data not shown). In fig.2 representative Scatchard plots of 'equilibrium binding' carried out at 2 different cell densities are shown. It is seen that the slopes of the lines differ considerably, but they both intercept the abscissa at the same point. In table 1 the results are summarized. It is clear that the strength of the binding of abrin to HeLa cells increases with decreasing cell density and that the number of receptors is the same at all cell densities. This is the same number of receptors found earlier for HeLa cells at 0°C [4], so the results do not seem to be much affected by the irreversible binding of toxin occurring at 37°C. Since the irreversible uptake increases with increasing cell density while the affinity constant

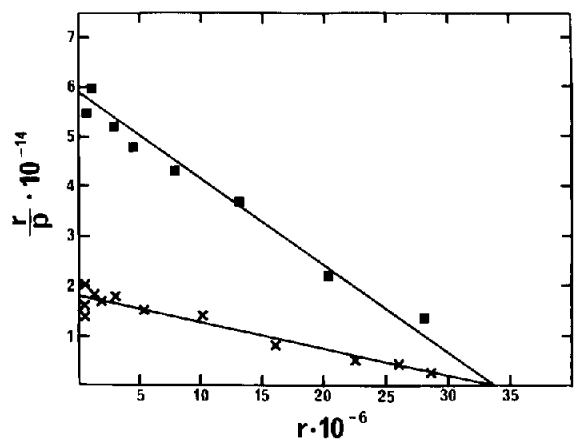

Fig.2. Scatchard plot of binding of ^{125}I-labelled abrin to HeLa cells in monolayer cultures at high and low cell densities. The cell density was 1.1×10^5 cells/cm^2 (×) and 1.9×10^4/cm^2 (■) and the experiment was carried out at 37°C. The amount of bound abrin was measured as in section 2. (r) no. toxin molecules bound/cell; (P) the molar concentration of free toxin.

Table 1
Binding of abrin to HeLa cells at different cell densities

Cell density ($\times 10^4$/cm^2)	K_a ($\times 10^6$ M^{-1})	No. binding sites/cell ($\times 10^7$)
10.6	5.3[a]	3.4
5.3	3.8[a]	3.3
1.9	17	3.4
0.5	21	3.3

[a] Difference within experimental error

Data are obtained from experiments as shown in fig.1

decreases, and since the former effect is smaller than the latter, the data do not permit conclusions as to the role of pinocytosis.

Since we have found earlier that the toxic effect is determined by the amount of toxin bound to the cells [6,7], the inhibition of protein synthesis by abrin at different cell densities was measured. In agreement with the results above, it was found (fig.3), that a 3–4-times higher concentration of abrin is necessary to give 50% inhibition of protein synthesis when the cell density is raised from $5.3 \times 10^3 - 5.3 \times 10^4$ cells/cm^2.

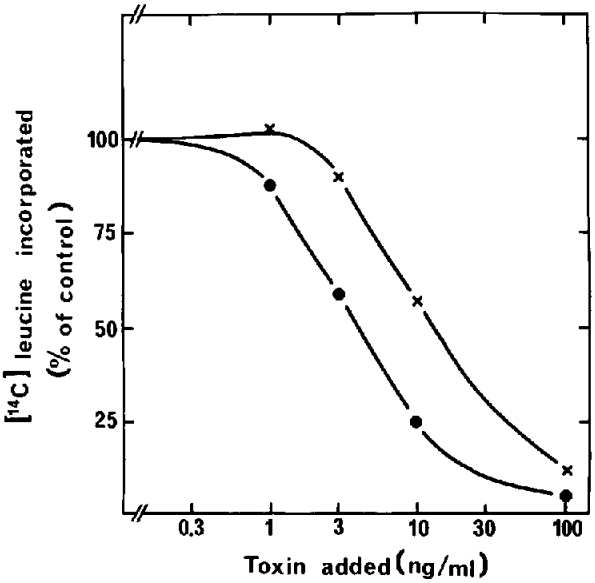

Fig.3. Inhibition of protein synthesis by abrin in HeLa cells at high and low cell density. Increasing amounts of abrin were added to cells growing in monolayer, and protein synthesis was measured after 3 h as in section 2. (•) 5.3×10^3 cells/cm^2; (×) 5.3×10^4 cells/cm^2.

Table 2
The rates of association for the binding of abrin to HeLa cells at 37°C at different cell densities

Cell density ($\times 10^4$/cm^2)	Rates of association ($\times 10^4$ M^{-1} s^{-1})
9.6	1.9
1.1	13

^{125}I-labelled abrin was added to cells in serum-free medium to a concentration of 2.9×10^{-8} M. After 30 s incubation the amount of bound abrin was measured as in section 2 and the rates of association were calculated assuming a second order reaction

The difference found in the association constants could be due to a difference in the rate of association, dissociation or both. The rates of association of abrin to HeLa cells at different cell densities were calculated after measuring the amount of ^{125}I-labelled abrin bound after 30 s, assuming a second order reaction. As shown in table 2 the rate is highest at the lowest cell density. The difference in rates of association is sufficiently large to account for the observed difference in the association constants. The rates of dissociation could not be measured due to the difficulty involved in diluting cells growing at the bottom of the tissue culture dishes.

The reason for the lower rate of association found at high cell density is not obvious. When the binding of abrin to HeLa cells in suspension was studied no difference in association constant was found at cell densities from $1.7–6.0 \times 10^5$ cells/ml. Possibly, steric factors arising from cell to cell contact may affect the rate of association.

The phenomenon here described, that the association constant for abrin decreases with increasing cell density, is of importance when the binding of toxin is to be correlated with other effects, e.g., the intoxication of cells, or when results from different experiments are being compared. Probably, the same phenomenon may occur in the interaction of other molecules with cell surface receptors.

Acknowledgement

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