METABOLIC COOPERATION STUDIED BY A QUANTITATIVE ENZYME ASSAY OF SINGLE CELLS

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SUMMARY

A new method making use of a radiochemical enzyme assay at the single cell level is presented to investigate metabolic cooperation, a widely studied form of cellular communication. In this case metabolic cooperation between normal human fibroblasts and fibroblasts derived from a patient deficient for the enzyme hypoxanthine-guanine phosphoribosyl transferase has been studied.

A mixture of an equal number of both cell types was cultured in close physical contact and after trypsinisation, replating and culturing the cells for several hours in a high dilution, quantitative enzyme measurements with individual cells isolated from the mixture were carried out. From the distribution curve of the enzyme activities of the individual cells the conclusion could be drawn that a macromolecule, either the enzyme itself or DNA or mRNA, coding for that enzyme, is transferred from normal to mutant cells.

Cultured skin fibroblasts from Lesch-Nyhan patients, deficient for the enzyme hypoxanthine-guanine phosphoribosyl transferase (HG-PRT; EC 2.4.2.8.), that have been grown in close contact with fibroblasts from normal individuals, are able to incorporate radioactive label added to the medium as ^{3}H-hypoxanthine into acid precipitable macromolecules, while in the absence of the HG-PRT positive cells no label is incorporated in the mutant cells (1). This phenomenon, called metabolic cooperation, has been studied by several groups, resulting essentially in two explanations: transfer from HG-PRT^{+} to HG-PRT^{-} cells of the enzyme product inosine monophosphate (IMP) (2,3) or transfer of macromolecules (HG-PRT itself, or DNA or mRNA containing the information for that enzyme) (4,5).

Indirect evidence for both hypotheses has been obtained by most of the groups employing autoradiographic demonstration of incorporation of radioactive label in the HG-PRT^{-} cells and by turnover studies of the metabolic cooperation effect (2, 4,5). A more straightforward approach was reported by Oei and de Bruyn (3), who studied the transfer of labeled material from erythrocytes preloaded with ^{3}H-IMP to HG-PRT^{-} lymphocytes or fibroblasts.

In the present communication the possibility of the transfer of enzyme or an informational macromolecule (DNA or mRNA) has been investigated by assaying HG-PRT activities of individual fibroblasts isolated from a mixture of HG-PRT$^+$ and HG-PRT$^-$ cells previously grown in close physical contact.

MATERIALS AND METHODS

All chemicals used were obtained from Merck (analytical grade) except PRPP A grade, Calbiochem; bovine serum albumine, unlabeled hypoxanthine, inosine, IMP, Sigma and ^{14}C-labeled hypoxanthine, Radiochemical Centre Ltd. Amersham. The experimental procedure was as follows: HG-PRT$^+$ and HG-PRT$^-$ cultures of human fibroblasts were seeded in a 1:1 ratio, grown for 1 day to confluency and kept in confluency for three further days in HAM F10 medium containing 15% fetal calf serum and 100 I.U./ml penicillin and 100 µg/ml streptomycin. Subsequently, the cells were trypsinised, washed three times, resuspended in culture medium and seeded into a Plastic Film Dish (PFD;6) in such a way that there was no more contact between the cells, i.e. $1x10^5$ cells per 20 cm^2 of culture surface. Eight hours later the cells were washed and lyophilized in situ. About 100 individual cells were cut out from the plastic film bottom of the PFD under a stereomicroscope and tested for HG-PRT activity. As controls the HG-PRT$^+$ and the HG-PRT$^-$ cultures were grown in separate PFD's and treated the same way. The techniques to measure enzyme activities at the single cell level (7,8) and their adaption to radiochemical assays of purine phosphoribosyl transferase activities (9,10) have been developed and described previously.

The final assay mixture contained 0.17 M Tris-HCl buffer (pH 7.4), 17 mM $MgCl_2$, 1.7 mM PRPP, 0.13 mM ^{14}C-hypoxanthine (spec.act.52 mCi/mmole), 1.6 mg/ml streptomycin and 0.5 °/oo BSA. The incubation volume was 0.3µl. Except for the blanks, in each reaction mixture a plastic film leaflet, carrying the single cell to be assayed, was included.

After incubation for six to seven hours at 37°C in Parafilm Micro Cuvettes (PMC;6) the reaction was stopped by pressing the PMC's onto Whatmann 3MM paper strips. Separation of substrate and product was carried out with a 0.5 N ammonia, 0.05 N EDTA solution as eluent. Quantification of radioactivity was performed as described previously (10).

RESULTS AND DISCUSSION

The distribution of the HG-PRT activities in both the normal positive and the mutant negative control fibroblasts are shown in figs.1a. and 1b. The mean value of the HG-PRT activities in the individual deficient control cells was $0.07x10^{-13}$ moles/hour. The corresponding value for the normal control cells was $1.22x10^{-13}$ moles/hour. The mean value of the blank measurements (n=19) was defined as $0x10^{-13}$ moles/hour. The range was between -0.40 and $+0.56x10^{-13}$ moles/hour as is indicated in fig.1a. (bar on top). As can be seen the activity range of the HG-PRT deficient cells is comparable.

If no transfer of enzyme- or informational molecules from HG-PRT$^+$ to HG-PRT$^-$ cells would occur during cellular contact in a mixed cell population, the enzyme activity distribution of the individual cells would have been a combination of figs.1a. and 1b.

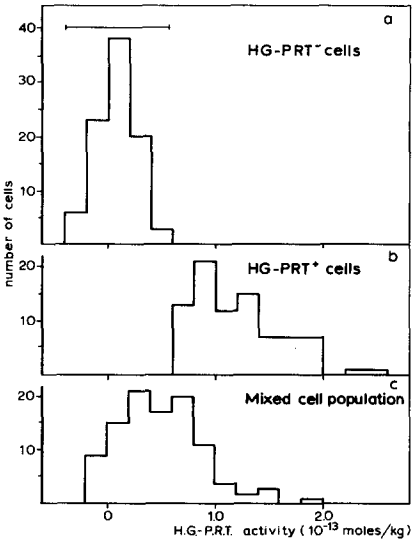

Figure 1.

a. Frequency distribution of enzyme activities of individual HG-PRT$^-$ fibroblasts (n=90). In addition, the range of the blank measurements is indicated (bar on top).
b. Frequency distribution of enzyme activities of individual HG-PRT$^+$ fibroblasts (n=84).
c. Frequency distribution of enzyme activities of individual fibroblasts (n=103), isolated from a mixture of HG-PRT$^+$ and HG-PRT$^-$ cells grown in close physical contact in a 1:1 ratio.

As can be seen from fig.1c. the observed distribution in the cell mixture was fundamentally different. Instead of two peaks to be expected for both cell types in the mixture, only one peak with intermediate HG-PRT activities was found (average activity 0.51×10^{-13} moles/hour).

These findings indicate that HG-PRT$^+$ and HG-PRT$^-$ fibroblasts grown to confluency constitute a communicating system, i.e. during cellular contact either HG-PRT or an informational macromolecule, coding for the enzyme, is transferred from cell to cell. The experiment has been repeated several times with different combinations of the HG-PRT$^+$ and HG-PRT$^-$ human cell cultures, always leading to the same conclusion.

Increase of enzyme activity in the HG-PRT$^-$ cells and decrease of enzyme activity in the HG-PRT$^+$ cells, as suggested by the data in fig.1, could be demonstrated more quantitatively if the distribution of the enzyme activities of the individual cells would be less affected by methodological errors. This could be achieved by prolonging the liquid scintillation counting time and by improving the micro-pipetting accuracy.

The method used here (i.e. the quantitative HG-PRT measurement in individual normal and mutant cells after cellular interaction) differs from the methods used by other groups with respect to the following points:

1. In this study both the HG-PRT$^+$ and HG-PRT$^-$ cells had been interacting under relatively physiological conditions, in contrast to for example the experiments of Ashkenazy and Gartler (5), who studied metabolic cooperation by adding a lysate of HG-PRT$^+$ fibroblasts to a culture of HG-PRT$^-$ cells.
2. The method used here offers an opportunity to specifically study a possible transfer of enzyme- or informational molecules, since the HG-PRT activity is radiochemically measured and the radioactive substrate is added to the individual cells after they have been isolated; a possible transfer of metabolites during cell to cell contact cannot interfere with the enzyme assay. A complementary approach to specifically study transfer or metabolites from cell to cell was reported previously by our group (3). The methods used by other groups which are based on autoradiographic demonstration of incorporation of labeled hypoxanthine can, on the other hand, not discriminate between these two possibilities.

Whereas previously the transfer of metabolite from cell to cell was demonstrated (2,3,11), the present study provides evidence for an additional mechanism of metabolic cooperation between HG-PRT$^+$ and HG-PRT$^-$ cells. Further studies should elucidate if both, none, or only one of these mechanisms have any physiological significance.

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