IDENTIFICATION OF AN AMP-ACTIVATABLE PYRUVATE DEHYDROGENASE ISOZYME IN EMBRYOS AND TUMORS

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1. Introduction

We have found that the pyruvate dehydrogenase of ascites tumor cells is activated by AMP in what appears to be an allosteric effect not detected in any of several normal tissues examined [1]. We report now that this activation of a pyruvate dehydrogenase by AMP applies also to the enzyme in a human embryo, chicken embryos and a human tumour. These observations suggest that there is a fetal isozyme of pyruvate dehydrogenase, or at least of its decarboxylase component [2], that tends to appear in tumours. To our knowledge no information has been published on the occurrence of isozymes of pyruvate dehydrogenase.

2. Materials and methods

The human embryo, kindly supplied by Dr F. Oñorbe (Ciudad Sanitaria La Paz, Madrid) was from an abortion of 14 weeks. Chicken embryos (14) of 6 days (obtained from Avicola Grau, Madrid) were pooled; they were washed in 115 mM NaCl, 5 mM potassium phosphate (pH 7.4) and suspended at 1/4 (w/v) in 0.25 M sucrose, 2 mM EDTA, 5 mM β-mercaptoethanol, 10 mM sodium phosphate (pH 7.4) and homogenized by sonication at 12 microns × 8 times for 5 s in a MSE sonifier; the homogenates were centrifuged at 40 000 × *g* for 30 min and the supernatants were used for enzymatic assays. The suspension of cells from a case of human chronic myeloid leukemia, kindly donated by Dr L. M. Barbolla (Clínica Puerta de Hierro, Madrid), had 95% abnormal mature cells and 5% blastic cells and the Philadelphia chromosome was positive in 90% of the cells; mitochondria were isolated as in [1] and homogenized with 0.5% Triton X-100; the mitochondrial homogenate was centrifuged at 40 000 × *g* for 30 min and the supernatant used for enzymatic assay. Pyruvate decarboxylase activity was measured at 37°C in the presence of 0.5 mM [1-^{14}C]pyruvate (Radiochemical Centre, Amersham) with a final spec. act. of 15 kBq/μmol, 0.2 mM thiamine pyrophosphate and varying AMP concentrations, in the absence of CoA and NAD^+, in a system containing 50 mM TES buffer, 100 mM KCl, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol (pH 7.4). The reaction was in a tube tightly fitted with a rubber cap holding a central container with 0.5 ml of 1 M hyamine in methanol. The reaction was stopped after 3 min with 1 ml 7% $HClO_4$ and allowed to stand for 90 min to fix the $^{14}CO_2$. The hyamine solution was transferred to a vial and counted for radioactivity. A control was run without enzyme to correct for spontaneous decarboxylation of pyruvate. Protein was determined by the Coomassie blue method [3].

3. Results and discussion

Since tumours in general have considerably less mitochondria than normal tissues, the likely contamination of tumour cells with normal cells in solid tumours would hinder the possibility of detecting an activation of pyruvate dehydrogenase by AMP. For this reason we examined a case of chronic myeloid leukemia with a very high count of white cells and enrichment by leucophoresis. As shown in fig.1 this tumour proved to have an AMP-activatable enzyme. We then

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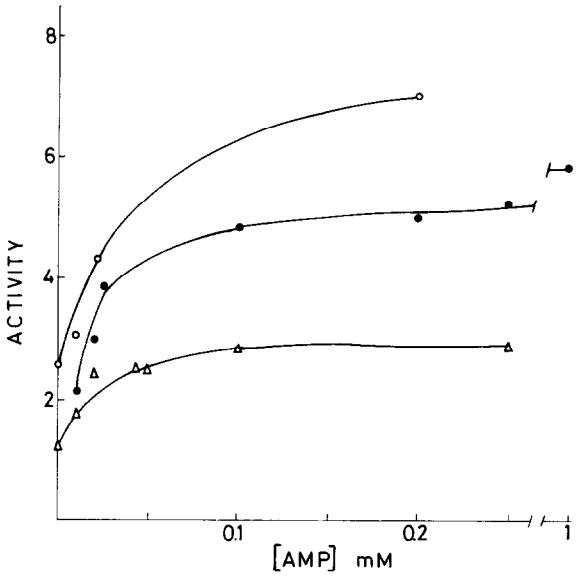

Fig.1. AMP activation of the pyruvate decarboxylase activity of the pyruvate dehydrogenase complex from human (○) and chicken (△) embryos and human chronic myeloid leukemia (●). Activities are expressed in nmol . min^{-1} . mg $protein^{-1}$, ×10 for the human embryo, and ×100 for the chicken embryo.

examined some embryos, following the clue that in several cases of enzymes for which a fetal isozyme is known, it frequently appears also in tumours [4]. Both a 3-month-old human embryo from accidental abortion and a pool of 6-day-old chicken embryos also gave activation of pyruvate dehydrogenase by AMP (fig.1).

The maximal activation by AMP achieved, calculated from the data shown in fig.1, using a double reciprocal plot of 1/AMP vs $1/v_a - v$, were 2.5-, 2.9- and 3.0-fold for chicken and human embryos and the leukemia cells respectively, with K_a values of 10, 15 and 18 μM. For the ascites tumour we had obtained a maximal activation of ~2.0 and a K_a of 40 μM [1]. On the whole it seems that maximal activation of the new isozyme by AMP is ~2–3-fold with a K_a of ~20 μM (within a factor of 2).

Some cases of congenital lactic acidosis in children have been shown to involve a deficiency in the pyruvate decarboxylase component of pyruvate dehydrogenase [5–8]. In a case of complete deficiency [6] the infant, born premature (8 months, 1.3 kg), showed rapid respiration during the first days of life and died at 6 months despite treatment to lower the elevated lactic acid in serum. It can be speculated that intrauterine development in cases of congenital deficiency of pyruvate decarboxylase could initially proceed unimpaired because of the fetal isozyme; it will be interesting to ascertain the timing of the shift from fetal to adult isozyme for pyruvate decarboxylase in humans.

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