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Hexokinase Activity and Isozyme Pattern in Human Placenta: A Population Study

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Placental hexokinase plays an important role in fetal metabolism, acting either on the placental tissue itself or on the fetus (1, 2). From biochemical studies there is evidence that placental hexokinase type I increases and type III decreases as gestation progresses to term, while hexokinase types II and IV remain unchanged and very low (2). Nevertheless, the specific function of the different hexokinase types are unknown in relation to human differentiation, to placenta metabolism, or to human tissue specificity. On the other hand, for red blood cells, the main physiological function of hexokinase in humans was only understood after the finding of genetic mutants causing enzyme deficiency and hemolytic anemia (3–6).

Consequently, a search for a hexokinase genetic mutant in human placenta from different populations could be a source of information on the physiological role of this enzyme during gestation.

In the present paper we report the results of a multiple population study of placental hexokinase.

MATERIAL AND METHODS

Samples. The material studied is made up of placenta samples drawn from two populations in Brazil. Sample I was obtained in Salvador, State of Bahia, and Sample II was collected in Aracaju, capital of the state of Sergipe. The placenta samples were refrigerated after collection in the delivery room, and subsequently frozen during a temporary storage (nearly 30 days) before study.

Populations. The population of both states is a triracial mixture of blacks, whites, and Indians with different levels of racial intercrosses within the population (7). For geographical reasons during the slave trade, the State of Bahia received a more negroid population than did the State

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of Aracaju. To assess the proportion of black admixture on the genetic makeup of the placentas, the newborns were classified for race in light, medium, and dark according to methods previously described (8, 9). Information on the newborn sex was also obtained.

Enzyme assay. Fragments of about 2 g of placenta were cut and homogenized, without dilution, in a Silverson homogenizer and spun at 4000 rpm for 20 min in a refrigerated centrifuge. The supernatant was used for enzyme assay and for electrophoresis.

Hexokinase activity was measured at pH 7.4 in a constant temperature of 37°C. The reaction mixture was made of glucose, 25 mM; NADP,[1] 0.55 mM; ATP, 3.7 mM; $MgCl_2$, 7.4 mM; ME, 5.0 mM; G6PD, 0.3 IU in a Tris–HCl buffer 7.4×10^{-2} M. The enzymatic reaction was followed in a Gilford II spectrophotometric recorder at 340 nm wavelength.

Protein concentration was measured in the homogenizer's supernatant by the method of Bücher (10). Hexokinase specific activity was estimated as previously described (11).

Electrophoresis. The separation of hexokinase isozymes was performed on starch gel electrophoresis at pH 8.6 (12, 13). Electrophoresis was performed at 4°C for 20 hr at 4 V/cm. The identification of hexokinase activity was made by pouring on the sliced gel a 0.5% agar overlay made on Tris–HCl, pH 7.5, buffer and glucose, 1 mM; ATP, 1.3 mM; NADP, 7.5 mM; MTT, 5 mg/ml; PMS, 5 mg/ml; G6PD, 0.4 IU; and $MgCl_2$, 0.02 M. The zone of hexokinase activity appeared after a 2-hr incubation at 37°C.

In every electrophoresis set a control isozyme pattern of hexokinase types I and II from rat heart was run.

RESULTS

Two hundred and four placentas were studied: 100 from Sample I and 104 from Sample II. There was no case of full enzyme deficiency. The average of hexokinase activity expressed in units per milligram of protein was 0.017 ± 0.006 for Sample I and 0.013 ± 0.007 for Sample II. There was no effect of the fetus sex and race on the placental enzyme levels. In Sample I there were 59 placentas from the male fetus and 45 from the female. The average enzyme activity was 0.018 ± 0.006 U/mg of protein for the placentas of the male fetus, and 0.017 ± 0.006 U/mg of protein for those of the female. In Sample II the enzyme activity mean was 0.013 ± 0.007 U/mg of protein for placenta from both male and female fetus.

In relation to the proportion of black admixture there were 29 light, 50 medium, and 25 dark fetus in Sample I. The average hexokinase activity in the corresponding placentas was 0.018 ± 0.007 for the light, 0.018 ± 0.006 for the medium, and 0.017 ± 0.005 U/mg of protein for the dark.

[1] Abbreviations used: NADP, nicotinamide adenine dinucleotide phosphate; ATP, adenosine triphosphate; ME, mercaptoethanol; G6PD, glucose-6-phosphate dehydrogenase. MTT, methyl thiazolyl tetrazolium; PMS, phenazine methosulfate.

There was no dark fetus in Sample II. However, in 89 placentas of light fetus, hexokinase activity was 0.014 ± 0.007 U/mg of protein, while in 11 other placentas from medium fetus the average enzyme level was 0.012 ± 0.006 U/mg of protein.

All the electrophoresis results showed a single zone of hexokinase activity which had the same electrophoretic mobility as the hexokinase type I control from rat heart (Fig. 1). There was no observation of an isozyme pattern which could suggest new mutation on the hexokinase structural locus. Also, there was no minor eletrophoretic expressivity of isozymes II, III, and IV.

DISCUSSION

The results shown in the present paper suggest that the total level of hexokinase activity in human placenta varies slightly among individuals, and is not influenced by either sex or race of the fetus. This may suggest that the metabolic role of hexokinase in the human placenta is such that its levels have to be maintained within a narrow range of variation. Undoubtedly, this is a quite different situation from what is described for hexokinase type IV (14).

The finding of hexokinase type I as the only hexokinase isozyme detectable in placenta by electrophoresis has been questioned (15). On the other hand, more recent findings suggest that hexokinase type I is the main isozyme of human placenta but the other isozymes are also present even if in small amounts (2, 16). It is possible that hexokinase types II, III, and IV account for such a small amount of the total hexokinase activity that fails to have electrophoretic expressivity. In addition, the results

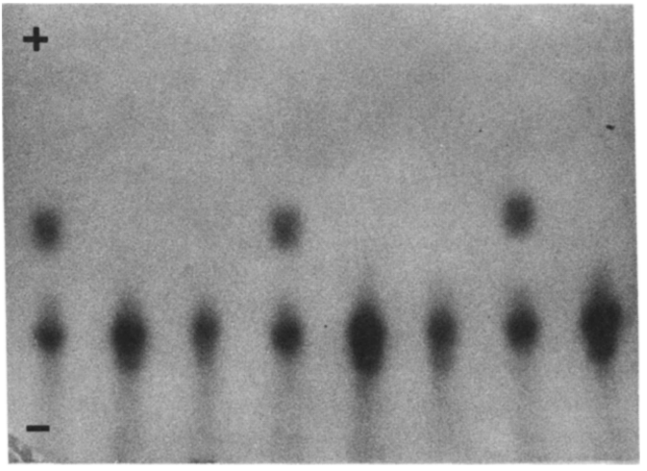

FIG. 1. Starch gel electrophoresis at pH 8.6 for hexokinase isozyme pattern from rat heart (slots 1, 4, and 7) showing hexokinase types I and II, and from human placenta (slots 2, 3, 5, 6, and 8) showing hexokinase type I.

from the present study are not limited to a few samples but represent two human population surveys. Consequently, the general finding of hexokinase type I as the only isozyme in the overall results suggests no individual variation on the expressivity of the hexokinase isozyme pattern of human placenta.

SUMMARY

Two population samples of human placenta with a different degree of black admixture were studied for hexokinase activity and electrophoretic isozyme pattern. Out of a total of 204 placentas there was no case of either full enzyme deficiency or variation on the electrophoretic pattern. All human placenta exhibited hexokinase type I as compared to a control of hexokinase types I and II from rat heart. The hexokinase activity levels on human placenta showed no effect of the newborn sex or black admixture.

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