EFFECT OF DOSE OF GENE FOR CYTOPLASMIC GLUTAMATE-OXALOACETATE TRANSAMINASE IN MOUSE EMBRYOS WITH TRISOMY FOR AUTOSOME No. 19

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KEY WORDS: trisomy for chromosome 19; mouse embryos; glutamate-oxaloacetate transaminase.

The problem of how changes in the dose of genes are reflected in synthesis of macromolecules coded by these genes has not been adequately studied [1], especially in mammalian embryogenesis. It has been shown that in the very first stages of development in female mouse embryos the genes of both X chromosomes are transcribed, and this gives rise to a double gene dosage effect for certain enzymes whose synthesis is coded by the X chromosome [8, 10]. At later stages of development, i.e., after inactivation of one of the X chromosomes, the activities of these enzymes become equal in male and female embryos [2]. Only one paper has been published in which the effect of gene dose has been described in mouse embryos with trisomy for autosome No. 1 [9]. The problem of how trisomy for other autosomes is reflected in mammalian embryos on synthesis of enzymes coded by superfluous chromosomes has not yet been investigated.

This paper describes the results of a study of the effect of trisomy for autosome No. 19 on the activity of a cytoplasmic enzyme in mouse embryos whose synthesis is coded by the locus of this autosome [7], namely glutamate-oxaloacetate transaminase (GOT-1; synonym aspartate aminotransferase; EC 2.6.1.1). For control purposes, activity of lactate dehydrogenase (LDH; EC 1.1.1.27), the structural genes of whose subunits are localized in autosomes Nos. 6 and 7 [11, 13], and of malate dehydrogenase (MDH-1; EC 1.1.1.37) also was measured in the soluble fraction obtained from the embryos.

EXPERIMENTAL METHOD

Embryos with trisomy for autosome No. 19 were obtained by the method described previously [3], using mice heterozygous for two Robertsonian translocations involving autosome No. 19 (Rb1Wh/Rb 163). On the 12th-14th day of pregnancy the embryos were removed from the uterus together with the fetal membranes, and chromosome preparations were obtained from the fetal membranes [14] in order to determine the karyotype, whereas the embryos themselves were used for biochemical analysis. Embryos in which trisomy was found were investigated separately from embryos with the normal karyotype. The embryos were homogenized in 0.25 M sucrose and 0.05 M phosphate buffer, pH 7.4, in the proportion of 1:29 (w/v). The soluble fraction was obtained by centrifuging the homogenates in the cold at 45,000 g for 60 min. GOT-1 activity was measured directly in the soluble fraction. Before activities of MDH-1 and LDH were measured the soluble fraction was diluted fivefold with physiological saline. Activities of GOT-1 and MDH-1 were determined by the use of kits from Boehringer [5, 6], and LDH activity was determined from the rate of reduction of pyruvate [4]. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

The results are shown in Figs. 1 and 2. At the period of embryogenesis studied specific activities of GOT-1, MDH-1, and LDH showed no sharp changes. Individual differences between the specific activities of these enzymes in embryos of the same day of development did not exceed 20%. In trisomy for autosome No. 19 the specific GOT-1 activity was increased by 1.4-1.7 times (mean 1.5 times); this was found in all trisomic embryos on the 12th, 13th, and 14th days of development. MDH-1 activity in embryos with trisomy was the

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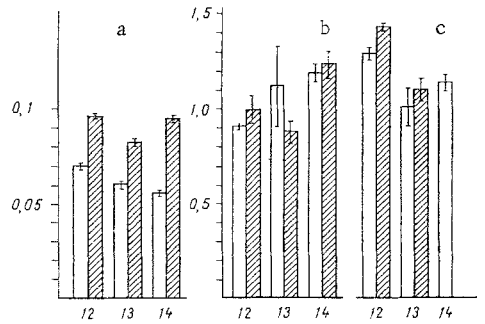

Fig. 1

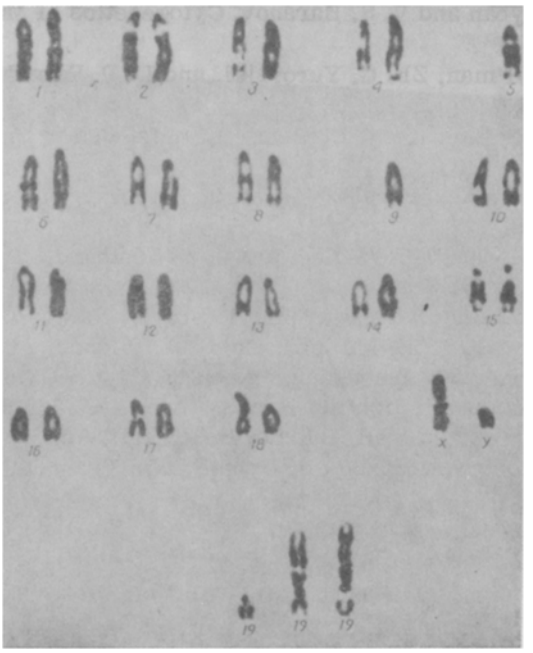

Fig. 2

Fig. 1. Specific activity of GOT-1 (a), MDH-1 (b), and LDH (c) in soluble fraction obtained from mouse embryos on 12th-14th day of development. Abscissa, day of development; ordinate, specific activity (in μmoles/min/mg protein). Arithmetic mean values and their errors shown. Unshaded columns – normal, shaded columns – trisomy for chromosome 19.

Fig. 2. Karyotype of mouse embryo with trisomy for chromosome No. 19 on 14th day of development.

same as in embryos with the normal karyotype and variations in the activity of this enzyme in individual embryos with trisomy were within the limits of variations observed in embryos with the diploid karyotype. Meanwhile LDH activity was 1.1 times higher in embryos with trisomy for autosome No. 19 than in those with the normal karyotype, and these differences were statistically significant.

A gene dose effect is thus clearly manifested in trisomy for autosome No. 19: GOT-1 activity was increased in the embryos strictly proportionally to the number of autosomes No. 19 containing the structural locus coding synthesis of this enzyme. The same gene dose effect was observed in mouse embryos with trisomy for autosome No. 1, in which the structural gene for isocitrate dehydrogenase is located [9]. These facts are evidence in support of the absence of controlling mechanisms in mammals during embryogenesis functioning at the chromosome level by the feedback principle, which could compensate for the presence of an excessive number of transcribed autosomal loci coding synthesis of particular enzymes in the cells. Further investigations are needed to determine whether the gene dose effect is manifested in the earliest stages of mammalian embryonic development when transcription of autosomal loci begins [2], or only in the later stages of embryogenesis.

The absence of effect of trisomy for chromosome No. 19 on specific activity of MDH-1 in the embryonic tissues probably indicates that the MDH-1 gene is not located in chromosome No. 19 in mice. If the increase in GOT-1 activity must be assessed as the primary result of an increase in the dose of transcribed structural loci for GOT-1, changes in LDH activity in trisomic embryos can be interpreted as a secondary consequence of general disturbances of metabolism which evidently arise in trisomy for autosomes [12]. Another possibility is that not only specific, but also nonspecific biochemical effects of trisomy play an important role in the pathogenesis of embryonic mortality associated with these disturbances of the karyotype. The writers propose to devote further investigations to this problem.

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SPONTANEOUS CHROMOSOMAL ABERRATIONS IN DIFFERENT TYPES OF CELLS FROM RHESUS MONKEYS

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Because monkeys are widely used for experimental purposes the study of the spontaneous level of chromosomal aberrations is important for the establishment of cytogenetic norms, for the assessment of induced chromosomal mutations, and also to shed light on the nature of the mutation process [1]. However, information in the literature on this subject is scanty [4, 5, 9].

The spontaneous level of chromosomal aberrations in monkeys was studied in different types of somatic cells with different rates of proliferation: bone marrow, peripheral blood lymphocytes, and kidney epithelial cells.

EXPERIMENTAL METHOD

Cytogenetic norms were studied in 25 monkeys (12 males and 13 females) in which 4290 metaphases were analyzed in 33 experiments. In 16 animals chromosomes in a single tissue were studied: bone marrow, peripheral blood lymphocytes, or kidney epithelial cells. In another eight monkeys either the first two or the last two types of cells were studied simultaneously, and in the remaining monkey cells of all three types were investigated. Because of the very low mitotic activity, bone marrow was not studied in the remaining monkeys of this group.

Data on the spontaneous level of chromosomal aberrations in monkeys were obtained for the following age groups: immature monkeys aged 2-3 years, mature monkeys aged 6-10 years, middle-aged monkeys aged 14-19 years, and old monkeys aged 21-22 years. The material was always obtained at the same time of day – in the morning.

In each animal 100-200 metaphases were analyzed to discover structural aberrations in chromosomes identifiable without karyotypic analysis. Cells with 40-43 chromosomes and polyploids were examined; the normal karyotype of Macaca rhesus is 2n-42 [6, 10]. All types of aberrations were taken into account; stained regions of chromosomes displaced relative to their axis or length were interpreted as fragments.

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