EFFECT OF THE BRACHYPODISM-H GENE ON PARAMETERS OF THE CELL CYCLE IN CARTILAGE CELLS OF THE PRIMITIVE MOUSE EMBRYONIC FIBULA

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The duration of periods of the cell cycle and the size of the proliferative pool were studied by autoradiography with thymidine-H^3 in cartilage cells of the fibula in 12-day mouse embryos homozygous for the mutant gene brachypodism-H (bp^H). The total duration of the cycle in cartilage cells of bp^H/bp^H embryos was 4 h longer than normally, largely on account of an increase in the combined duration of the G_1- and M-phases. The S-phase also was increased in the mutants, but not significantly (by 45 min). The proliferative pool of cartilage cells was greater in bp^H/bp^H than in $+/+$ embryos.

KEY WORDS: autoradiography; cell cycle; cartilage cells; proliferative pool.

Proliferation and differentiation of cells are closely interconnected processes. Recently, great attention has been paid to the study of the link between them [1, 3, 4, 7]. Cartilage tissue, in which the transition of the cells to specific synthesis is comparatively easily detectable by a number of morphological or histochemical features, are a convenient object for studying the mutual dependence of proliferation and differentiation. In mouse embryos homozygous for the brachypodism-H (bp^H) gene the anlage of the fibula at the end of the 12th day of embryogenesis is reduced in size [6]. Considerable delay in the differentiation of the cartilage cells in this bone, specifically in their hypertrophy, is observed by the 13th day of embryonic development. The fibula of 13-day bp^H/bp^H embryos differs from normal in the absence of metachromasia of the ground substance; this is evidence of absence or a very small quantity of acid mucopolysaccharides, the specific products of cartilage cells [5].

The object of this investigation was to study the proliferative activity of the cartilage cells in the fibula of bp^H/bp^H mouse embryos.

EXPERIMENTAL METHOD

The duration of periods of the cell cycle and the size of the proliferative pool in the fibula of 12-day bp^H/bp^H mouse embryos were investigated by autoradiography with thymidine-H^3. Embryos at the same times of development from normal ($+/+$) mice were used as the control. Embryos of both genotypes of equal sizes were taken. To determine the duration of the periods of the cell cycle graphs of the change in percentage of labeled mitoses at various times after a single injection of thymidine-H^3 were plotted by the method of Quastler and Sherman [8]. The duration of the periods of the cell cycle were determined in the usual way [2, 4]. Thymidine was injected into pregnant (12 days) bp^H/bp^H and $+/+$ mice intraperitoneally in a dose of 3 μCi/g. The animals were killed 1 h, 1 h 15 min, 1 h 30 min, 2 h, 2 h 30 min, and 3 h after the injection and thereafter hourly for 30 h after the single injection of thymidine. No fewer than five embryos of each genotype were taken at each time.

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To determine the proliferative pool, pregnant +/+ and bp^H/bp^H mice were given 8 injections of thymidine-H^3 in a dose of 3 μCi/g at intervals of 5 h. The animals were killed 1 h after the last injection. The percentage of labeled nuclei was determined in a section through the whole thickness of the bone in the developing zones of the epiphysis and diaphysis separately. Sagittal sections through the limb bud were coated with type M liquid emulsion (Research Institute of Photographic Chemistry, Moscow) and exposed for 3 weeks at 4°C. Nuclei containing at least 5 grains were regarded as labeled. Statistical analysis of the data was carried out by the method of Fisher and Student.

EXPERIMENTAL RESULTS

The curves of labeled mitoses for embryos of the same genotype (+/+ or bp^H/bp^H) were identical for the zones of the epiphysis and diaphysis. This shows that the cell cycle and its individual phases are identical in these zones in the embryos of the same genotype. The first labeled mitoses were observed 1 h 15 min after injection of the isotope in the epiphyses of both the bp^H/bp^H and the +/+ embryos. The number of labeled mitoses reached the first maximum (100%) in embryos of both genotypes after 2 h 30 min. The number of labeled mitoses was lower 7 h after injection of thymidine-H^3 and reached a minimum in the bp^H/bp^H embryos (5.8%) after 15 h and in the +/+ embryos after 11 h (19.1%). A second maximum of labeled mitoses was observed in the bp^H/bp^H embryos (58.7%) 24 h after, and in the +/+ embryos (58.7%) 20 h after the injection of thymidine-H^3.

Analysis of the curves of labeled mitoses showed that the duration of the mitotic cycle (T) in cartilage cells of the primitive fibula of the bp^H/bp^H embryos was 21 h 30 min, compared with 17 h 30 min for the +/+ embryos. The postsynthetic period (G_2) was the same (1 h 15 min) for cells of the fibular anlagen of the bp^H/bp^H and +/+ embryos, the period of DNA synthesis (S) was 7 h 40 min for the bp^H/bp^H embryos and 6 h 55 min for the +/+ embryos, whereas the combined duration of the presynthetic period and of mitosis (G_1 + M) was 12 h 35 min for the bp^H/bp^H and 9 h 20 min for the +/+ embryos.

Experiments with repeated thymidine-H^3 injections showed that a higher percentage of cells of the bp^H/bp^H embryos, especially in the diaphysis, than in the +/+ embryos participate in division. For instance, the percentage of labeled nuclei in the fibular epiphysis of the +/+ embryos was 75.99 ± 0.58 and in the bp^H/bp^H embryos it was 87.08 ± 1.73 ($P < 0.001$). The index of labeled nuclei in the fibular diaphysis of the +/+ embryos was 54.86 ± 2.62 and in the bp^H/bp^H embryos 82.22 ± 0.95 ($P < 0.001$).

This investigation thus showed that a change in the duration of the periods of the mitotic cycle is observed in 12-13-day bp^H/bp^H mouse embryos, characterized by inhibition of differentiation of the cartilage cells in the fibula and delay in the synthesis of acid mucopolysaccharides. This change is expressed as lengthening of the total cycle of the cartilage cells by 4 h, accounted for chiefly by an increase in the combined duration of the G_1- and M-phases (3 h 15 min). The increase in the duration of the S-phase (45 min) was not significant. Meanwhile the proliferative pool of cartilage cells was higher in the mutant embryos then normally. The results show that the mutant bp^H gene not only disturbs the differentiation of cartilage cells in some long bones, particularly the fibula, but it also affects the proliferative activity of these cells.

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