EFFECT OF CORTISONE ON EXPRESSION OF THE OCULAR RETARDATION GENE IN MOUSE EMBRYOGENESIS

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Injection of large doses of cortisone during the period of operation of the mutant ocular retardation (or) gene leads to increased inhibition of growth of the primitive retina in or/or mouse embryos. Small doses of the hormone, if injected during the 11th-14th day of embryogenesis, largely restore the normal development of the damaged retina and other structures of the eye. Prolonged saturation of pregnant females with various doses of cortisone, however, had no stimulant effect on growth of the retina, optic cup, or lens in or/or embryos. The adrenal glucocorticosteroid cortisone thus modifies the effects of the or gene in homozygotes, stimulating growth of the retina or inhibiting it still more in the embryonic period of development of mice.

Steroid hormones of vertebrates affect various tissues and organs and produce changes in intracellular metabolism. In particular, steroids such as cortisone and hydrocortisone control the functional activity of the genome at the levels of transcription and translation of genetic information [6-12]. The role of these hormones as regulators of gene activity in mammalian ontogeny has been inadequately studied.

This paper describes a study of the effect of cortisone on expression of the mutant ocular retardation gene in the embryonic period of development of mice.

EXPERIMENTAL METHOD

Experiments were carried out on mice of the mutant ocular retardation line. The autosomal recessive ocular retardation (or) gene inhibits proliferation of the retinal cells of the eye in mouse embryos [2] and prolongs the G_1 period of the mitotic cycle [3]. For this reason the mitotic index of the developing retina was used as the basic test of changes in the effects of the or gene. Homozygous (or/or) females weighing 25-27 g were crossed with or/or males; the day of discovery of a vaginal plug was taken as the zero day of pregnancy. Cortisone (cortisone acetate; N. V. Organon-OSS, Holland) was diluted to the required concentration with 0.85% NaCl solution. The hormone was injected into pregnant females in doses of 0.25, 1.0, or 2.5 mg per mouse. The suspension of cortisone acetate was injected intraperitoneally in a volume of 0.2 ml during the 8th-10th, 11th-14th, or 9th-14th days of pregnancy. The control (intact) animals received injections of physiological saline at the same times. No fewer than ten embryos (from three mothers), aged 15 and 18 days, were taken from the experimental and control groups for histological examination. Material was fixed in Carnoy's fluid. Serial transverse sections 5 μ in thickness were stained with Carazzi's hematoxylin. The mitotic index (in percent) was determined by counting 4500-5000 cells in the retina of 15-day embryos or 7000-9000 cells in the same tissue of 18-day embryos, as well as the number of cells (nuclei) in three or four sections through the center of the retina. The size of the optic cup and the lens and the thickness of the retina were measured. The numerical results were analyzed by the Fisher−Student method.

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TABLE 1. Effect of Cortisone on Proliferative Activity of the Retina and Dimensions of the Eye in 15-day or/or Mouse Embryos ($M \pm m$)

Injection of cortisone		Diameter of eye (in μ)		Diameter of lens (in μ)		Retina		
days of pregnancy	dose (in mg/mouse)	longitudinal (along optical axis)	transverse	longitudinal (along optical axis)	transverse	thickness (in μ)	No. of cells per section	mitotic index (in %)
8—10	2,5	453,9±23,4	406,6±19,9	340,2±12,8	263,3±15,7	50,8±2,1	870,0±62,7	2,8 ($P<0,001$)
9—10	2,5	464,5±16,4	473,7±13,4	288,0±10,4	352,6±16,4	67,7±5,9	1280,0±74,2	2,3 ($P<0,001$)
9—10	0,25	480,4±9,6	491,2±26,2	292,6±2,7	340,3±13,4	70,8±3,2	1120,0±82,2	2,2 ($P<0,001$)
11—14	2,5	454,3±21,4	432,7±54,3	254,1±13,0	309,5±20,9	58,5±2,1	650,0±50,0	2,0 ($P<0,02$)
11—14	0,25	511,2±13,7	501,2±18,4	304,3±16,0	362,3±18,0	77,0±0,0	1460,0±75,8	2,2 ($P<0,001$)
9—14	1,0	457,3±26,9	470,2±20,1	291,0±15,0	348,1±16,0	75,4±1,7	1180,0±80,2	2,1 ($P<0,01$)
9—10 *	2,5	481,5±18,9	475,8±17,3	271,0±17,3	337,3±9,2	67,7±1,7	800,0±115,9	2,2 ($P<0,001$)
11—12	1,0							
13—14	0,5							
Control		532,8±22,5	474,2±17,4	291,3±5,6	317,3±17,7	67,7±1,7	1040,0±41,1	1,9

*Animal received injections of 2.5 mg of the hormone on 9th and 10th days, 1 mg on 11th and 12th days, and 0.5 mg on 13th and 14th days of pregnancy.

EXPERIMENTAL RESULTS

Cortisone if injected in a dose of 2.5 mg into females on the 8th, 9th, and 10th days of pregnancy inhibited growth of all structures of the eye of the 15-day or/or embryos. In these embryos there was a decrease not only in the thickness of the retina (by 27.5%) but also in the number of cells (by 18.1%) in it, although the mitotic index was 47.3% higher than in the control. The reason for the high mitotic index observed in this series of experiments was evidently the compensatory growth taking place at the end of the inhibitory action of this dose of the steroid hormone (Table 1). Injection of cortisone in a dose of 2.5 mg stimulated growth of the primitive retina only on the 9th and 10th days of development. For instance, the number of cells in the retina of the 15-day embryos was increased by 19% while mitotic activity rose by 20% (Table 1). In doses of 2.5 mg or more, and if injected during the 8th, 9th, and 10th days of pregnancy into the mice, the hormone evidently potentiated the effects of the or gene. This is confirmed by investigations [6, 12, 13] showing that injection of the adrenal steroid cortisone or of hydrocortisone induces premature synthesis and activation of the enzyme glutamine synthetase, which is accompanied by acceleration of retinal differentiation in chick embryos. Injections of cortisone in a dose of 0.25 mg during the 9th and 10th days of pregnancy had a stimulant effect on proliferative processes in the retina. However, the stimulation of growth produced in this case by the steroid was rather less than after injection of 2.5 mg of the hormone at the same times of pregnancy (Table 1).

If injected in a dose of 2.5 mg during the 11th, 12th, 13th, and 14th days of embryogenesis cortisone inhibited retinal development. The number of cells in the retina of the 15-day embryos was reduced by 38.5% compared with the control. The dimensions of the optic cup and lens and the thickness of the retina also were less than in intact embryos (Table 1). Injection of the steroid in a dose of 2.5 mg both on the 8th, 9th, and 10th and on the 11th, 12th, 13th, and 14th days of pregnancy intensified the inhibition of growth of the retina in the or/or embryos. This similarity between inhibition of growth of the primitive retina was probably due to the specific character of action of cortisone at individual periods of the cell cycle. According to the literature [1, 4-5], high concentrations of cortisone or hydrocortisone in the mammal increase the duration of the phase of DNA synthesis. It has also been shown [11] that steroid hormones change the duration not only of the S-period but also of the preceding G_1 period, although they have no significant effect on the remaining phases of the mitotic cycle.

Injection of cortisone in a dose of 0.25 mg during the 11th, 12th, 13th, and 14th days of pregnancy led to stimulation of growth not only of the affected retina, but also of the other components of the eye. For instance, the number of cells and mitotic index in the retina of the 15-day embryos were increased by 43.0 and 15.7% respectively (Table 1). The dimensions of the optic cup, lens, and retina were considerably increased. On the whole the structures of the eye were better developed than in the control embryos.

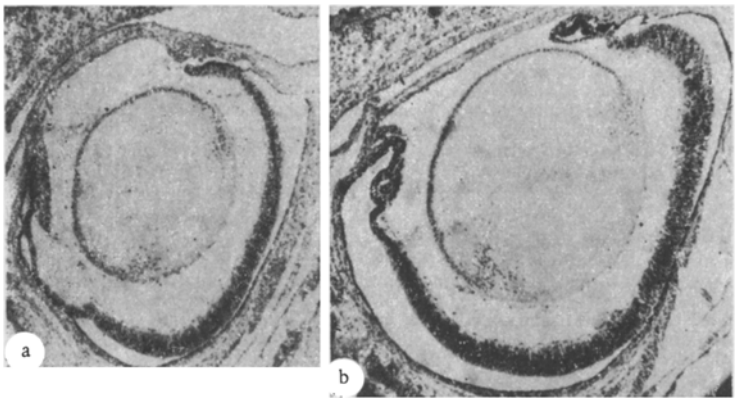

Fig. 1. Sections through the eye of 18-day or/or mouse embryos: a) control; b) after injection of cortisone acetate in a dose of 0.25 mg during 11th-14th days of pregnancy. Carazzi's hematoxylin, 70 ×.

If injected initially in a dose of 2.5 mg on the 9th and 10th days, followed by a dose of 1.0 mg during the 11th and 12th days, and, finally, by a dose of 0.5 mg on the subsequent 13th and 14th days of embryo genesis, cortisone inhibited growth of the primitive retina and the other structures of the eye. The number of cells in the retina of the 15-day embryos fell by 27.7% after hormone treatment in this way. Injection of the steroid in a dose of 1.0 mg during the 9th, 10th, 11th, 12th, 13th, and 14th days of pregnancy led to some activation of retinal growth. For instance, a small increase in the number of its cells (by 13.4%) and in its thickness (by 11.3%) was observed in this tissue of the 15-day embryos. The dimensions of the optic cup and lens were indistinguishable from the control (Table 1). Consequently, the prolonged administration of cortisone had no stimulant effect on the growth of the main components of the eye. Large doses of the hormone, injected in the early stages (8th-10th day) of development, intensified the inhibition of growth of the affected retina in the or/or mouse embryos.

The growth-stimulating effect of cortisone on the primitive retina became significantly more marked with the increasing age of the embryos. Restoration of normal development of the retina in the 15-day embryos, observed as the result of injection of the steroid in a dose of 0.25 mg on the 11th, 12th, 13th, and 14th days of pregnancy was more marked on the 18th day of embryogenesis. The dimensions of the optic cup, lens, and retina of the 18-day embryos were much greater than the corresponding dimensions in the control (Fig. 1).

Small doses of cortisone, if injected during the 11th-14th days of pregnancy, thus had the most normalizing effect on growth of the affected retina in or/or mouse embryos. Injection of large doses of the steroid hormone, whether in the early (8th-10th days) or late (11th-14th days) of development of the primitive retina, led to marked potentiation of the effects of the mutant or gene.

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