STIMULATION OF GROWTH OF LIMB BONES OF MOUSE EMBRYOS OF MUTANT LINE BRACHYPODISM-H BY SOMATOTROPIC HORMONE

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UDC 612.646'753-063:612.433'65

Injection of various doses of somatotropic hormone into female bp^H/bp^H mice during 11-14 days of pregnancy stimulated growth and differentiation of the affected hind-limb bones in 17-day embryos. The greatest increase in length of the bones in the bp^H/bp^H embryos was observed after injection of hormone in a dose of 1 μg/g body weight.

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Injection of growth hormone (STH) or extracts of the anterior lobe of the pituitary into pregnant female rats has been shown to cause an increase in the weight and size of embryos and newborn animals [5, 8, 10, 12-14], and STH has also been shown to stimulate growth of anlagen of the long bones of the limbs in chick and rat embryos in vitro [7, 9]. Under the influence of STH a marked increase is observed in the weight, length, and thickness of the tibia in chick embryos, and the number of mitoses in growth zones of the bone and in the periosteum is increased [6]. In mice embryos homozygotic relative to the gene brachypodism-H (symbol bp^H), growth and differentiation of the long bones of the limbs, especially the fibula, are retarded [2, 4]. Triiodothyronine (T_3) in vitro stimulates growth of the fibula of 13-day bp^H/bp^H embryos [1], while injection of thyroxine (T_4) into pregnant females stimulates growth of the affected bones of bp^H/bp^H embryos [3]. In this investigation the effect of STH on growth of the hind-limb bones was studied in situ in bp^H/bp^H embryos.

EXPERIMENTAL METHOD

Experiments were carried out on mice of the mutant line brachypodism-H. Females homozygotic relative to the gene brachypodism-H (bp^H/bp^H) were crossed with bp^H/bp^H males. Pregnancy was counted from the day of discovery of a vaginal plug. Daily during the 11th-14th days of pregnancy the females were given intraperitoneal injections of STH obtained from the Institute of Experimental Endocrinology and Hormone Chemistry, AMN SSSR (the hormone was generously provided by E. A. Kolli), The STH was prepared by Raben's method [11]. This method rules out the possibility of contamination with biologically active thyrotropic hormone. The activity of the hormone was 1.2 unit/mg. The following doses of STH were used: 1, 2, 4, and 16 μg/g body weight. The volume of hormone injected was 0.1 ml. The same volume of solvent was injected into control pregnant bp^H/bp^H rats. After the 17th day of pregnancy the females were autopsied and the embryos investigated by the method described previously [3].

TABLE 1. Length of Hind-limb Bones of 17-day bp^H/bp^H Embryos after Injection of STH during 11th-14th Days of Pregnancy

Series of experiments	Dose of STH (in μg/g body wt.	Number of limbs	Femur		Tibia		Fibula		Foot	
			In mm	Percent of control	In mm	Percent of control	In mm	Percent of control	In mm	Percent of control
I	1	207	2,65±0,02	107,0	3,22±0,02	109,0	1,86±0,02	117,0	3,94±0,02	115,5
II	2	186	2,70±0,02	109,0	3,16±0,02	107,0	1,75±0,02	110,0	3,86±0,02	113,2
III	4	233	2,72±0,01	109,8	3,11±0,01	105,4	1,68±0,02	105,6	3,70±0,02	108,5
IV	16	133	2,59±0,02	104,4	3,07±0,02	104,0	1,79±0,03	112,5	3,57±0,02	104,7
Control	0	167	2,48±0,02	100,0	2,95±0,02	100,0	1,59±0,02	100,0	3,41±0,02	100,0

Laboratory of Simulation of Hereditary Diseases, Institute of Experimental Biology, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR V. V. Zakusov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 1, pp. 70-71, January, 1969. Original article submitted January 16, 1968.

EXPERIMENTAL RESULTS

Injection of STH in doses of 2, 4, and 16 μg/g body weight led to a marked increase in length of the embryos, but differences between the series themselves were not statistically significant. A dose of STH of 1 μg/g body weight did not increase the length of the embryos. In none of the series of the experiments was injection of the hormone accompanied by a marked increase in the number of resorbed or dead embryos.

The results given in Table 1 show that the effect of STH was to increase the length of all bones of the hind limb in all four series of experiments ($P < 0.001$). In the experiments of series I the greatest increase in length was observed for the fibula (17%). The optimal dose of hormone for the femur was 4 μg/g body weight (experiments of series III; the difference in length of the femur in series I and III is statistically significant, $P < 0.01$). In the experiments of series IV the increase in length of all the bones was less than in series I. Consequently, injection of large doses of STH did not give rise to greater stimulation of bone growth than a dose of 1 μg/g body weight.

Injection of STH slightly increased the rate of ossification of the bones.

Injection of STH into bp^H/bp^H mice during the 11th-14th days of pregnancy thus increased the length of the embryos (except in the experiments of series I) and also the length of all bones of the hind limb. In the experiments of series II, III, and IV the increase in length of the bones (as a percentage of the control) was roughly 2-3 times greater than the increase in length of the embryo. These results, and especially the results of the experiments of series I, show that STH stimulates growth of the affected limb bones more than growth of the axial skeleton, development of which is undisturbed in bp^H/bp^H mice.

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