DETERMINATION OF THYROXINE AND TRIIODOTHYRONINE IN THE THYROID GLAND OF ENCEPHALECTOMIZED RAT FETUSES

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Thyroxine and triiodothyronine in the thyroid gland of encephalectomized and intact fetuses of rats with normal and prolonged gestation period were determined by a radioisotope method. Encephalectomy on 19-day fetuses had no significant effect on the concentration of thyroid hormone in the gland 2 and 4 days after the operation. The results are evidence against hypothalamic control of pituitary thyrotropic function in the prenatal development of rats.

KEY WORDS: rat fetuses; encephalectomy; thyroid gland; thyroxine; triiodothyronine

In an earlier investigation using the thyroid response as a test for the presence of hypothalamic control of pituitary thyrotropic function it was shown that no such control exists in rats before birth. A negative result was obtained in 24-day fetuses of mothers with artificially prolonged gestation [1]. However, data have appeared in the literature to show that in rats toward the end of the intrauterine period thyrotropin-releasing hormone (TRH) is present in the hypothalamus and that the pituitary in fetuses at this stage of development can respond in vitro to the addition of TRH by secreting thyrotropic hormone [5]. It has also been shown that TRH, if injected into rats on the 20th day of pregnancy, appreciably increases the number of drops of colloid in the thyroid gland of the fetus [7], and this was regarded as evidence of increased thyrotropic function of the fetal pituitary under the influence of TRH. If injected into the umbilical vein or peritoneal cavity of 21-day rat fetuses, TRH caused the thyrotropic hormone level in the fetal serum to rise [8]. Injection of synthetic TRH into pregnant rats on the last day of pregnancy also stimulated the activity of the fetal pituitary and thyroid glands [4].

All these facts are evidence that the components of the hypothalamic–pituitary–thyroid system already possess functional activity during prenatal development and that connections between the hypothalamus and pituitary are perhaps also laid down before birth.

It was accordingly necessary to check the previous data by using a more modern and sensitive method of quantitative determination of thyroxine and triiodothyronine in the thyroid gland and blood serum of rat fetuses for this purpose.

EXPERIMENTAL METHOD

Fetuses of noninbred albino rats on which encephalectomy was performed at the age of 19.5 days were used [2]; intact fetuses of the same litter served as the control. To prolong gestation to 24 days progesterone was given by intramuscular injection to the mothers in a dose of 1 mg daily from the 20th day of pregnancy until the day of sacrifice.

At the stages of 21.5 and 23.5 days of development, under pentobarbital anesthesia, the fetuses were removed from the mothers and, without delay, blood (with a pipet from the incised heart) and the thyroid gland were taken from the fetuses. Blood from fetuses of the same group and belonging to the same litter was pooled.

The isolated thyroid glands were weighed separately from each fetus and placed in glass capsules with rubber stoppers. Samples of serum and the thyroid glands were kept until use at −15°C. To determine the content of thyroxine and triiodothyronine in the thyroid glands a 1-2% tissue homogenate was prepared in borate buffer by the method used for chromatography of thyroid hormone [3].

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TABLE 1. Concentrations of Triiodothyronine and Thyroxine in Thyroid Gland of Rat Fetuses after Encephalectomy

Experimental conditions	Age of fetuses, days		Number of determinations	Triiodothyronine ng/mg	Number of determinations	Thyroxine, ng/mg
	at operation	at sacrifice				
Control	19,5	21,5	11	1,86±0,30	6	43,68±13,65
Encephalectomy			13	1,95±0,33	6	49,56±8,12
Control	19,5	23,5	26	3,45±0,38	13	65,05±15,06
Encephalectomy			23	2,80±0,39	13	53,00±11,78

Legend. Glands from 2 fetuses were used for each determination.

Thyroxine was determined in the tissue homogenate and blood serum by means of the Thyopac-4 isotope kit (from the Radiochemical Centre, Amersham, England). Triiodothyronine was determined radioimmunologically by means of the Triakit from the same firm.

EXPERIMENTAL RESULTS

The main determination of thyroxine and triiodothyronine was carried out in tissue homogenates. The results of determination of thyroxine and triiodothyronine in the thyroid glands demonstrated wide individual variations in the contents of these substances in the fetuses at the two periods of investigation: 21.5 and 23.5 days. This was evidently due to differences in the level of maturation of the thyroid gland in individual fetuses.

Examination of the mean values obtained (Table 1) shows some decrease in the thyroxine and triiodothyronine concentrations in the glands of the anencephalic fetuses compared with the intact fetuses or after prolongation of gestation to 23.5 days. In the anencephalics at this stage of development, for instance, the triiodothyronine concentration was 2.8 ng/ml and the thyroxine concentration 53.0 ng/ml, whereas in intact fetuses the corresponding values were 3.45 and 65.05 ng/mg. However, this difference is not statistically significant and it cannot therefore be concluded that the hypothalamus influences pituitary thyrotropic function in rat fetuses. The results of the study of the blood thyroxine and triiodothyronine levels agreed with data in the literature on the concentrations of these substances in the blood plasma of young rats during the first days after birth. For instance, Dussault et al. [6] showed that the blood plasma triiodothyronine concentration in rats aged 2-4 days is very low and the thyroxine concentration is 6-10 ng/ml. According to the present experiments, in fetuses obtained after prolongation of gestation to 23.5 days the triiodothyronine concentration in the blood plasma was very low, so that often it could not be determined, and the thyroxine concentration was 7.5-8.7 ng/ml. The results of the present investigation thus clearly showed absence of hypothalamic control over pituitary thyrotropic function during prenatal development of rats, concerning the data published previously based on the goiter effect [1].

Hypothalamic control over pituitary thyrotropic function in rats is evidently established only after birth, in agreement with recent findings by other workers [6] who used radioimmunological methods to determine TRH in the hypothalamus, the thyrotropic hormone (TTH) of the pituitary, and thyroxine and triiodothyronine in the blood serum of newborn rats aged from 1 to 50 days. The concentrations of these hormones, although very low at birth, quickly reached their maximum: TRH by the 16th day, TTH by the end of the 1st week, thyroxine between the 4th and 16th days, and triiodothyronine between the 4th and 28th days.

Since functional activity of the pituitary–thyroid axis has repeatedly been demonstrated previously during the prenatal period in rats, it can be concluded that the rapid rise of this activity observed during the first week after birth is due to establishment of connections with the hypothalamus.

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