UPTAKE OF TESTOSTERONE-1α,2α-^{3}H(n) AND ESTRADIOL-4^{14}C BY ANLAGEN OF THE REPRODUCTIVE TRACT OF RABBIT EMBRYOS *in vitro*

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Evidence of the presence of specific receptors both for androgens [1, 8] and for estrogens [2, 6] in the tissues of embryonic target organs has recently been obtained, and it confirms the bipotentiality of anlagen of the reproductive tract toward development according to the male or female type.

The character of selective uptake of testosterone [1] or estradiol [2] by anlagen of target organs from the 18th through the 25th day of embryonic development in rabbits is similar. Tissues of female embryos have been found to bind more hormone than tissues of male embryos.

It was also observed previously that injection of excessive doses of androgens or estrogens into the pregnant female rat leads to partial or total inversion of the somatic parts of the embryonic reproductive system, whereas combined administration of both hormones does not affect normal development [3].

The object of this investigation was to study the dynamics of selective uptake of testosterone-1α,2α-^{3}H(n) (T-^{3}H) and estradiol-4-^{14}C (E-^{14}C).

EXPERIMENTAL METHOD

The reproductive tract and pieces of the femoral muscles of rabbit embryos from the 18th through the 25th days of development (age was measured from the time of mating) were incubated in medium No. 199 containing 1.5 μCi/ml each of T-^{3}H and E-^{14}C with specific activities of 56 and 56.7 Ci/mmole and molecular weights of 288 and 274 respectively (from the Radiochemical Centre, Amersham, England). The time for dissection of the organs was 30-40 min, incubation at 38°C lasted 30 min, and subsequent washing in medium lasted 1 h. The organs were weighed on torsion scales with an accuracy of 0.05 mg and they were immersed for 24 h in hyamine. Radioactivity of the double label was determined in cpm on the SL-30 scintillation counter. The counting efficiency was 7.2% for T-^{3}H and 67% for E-^{14}C. Selective uptake of labeled hormones into the tissues was determined as a percentage of their uptake in the muscles.

EXPERIMENTAL RESULTS

On incubation of anlagen of the reproductive tract of rabbit embryos at the 18th-25th days of development in medium containing T-^{3}H and E-^{14}C in equal proportions, ability to take up both hormones was observed, evidence that the tissues contain both types of receptors (Fig. 1). The character of the curve indicates that the dynamics of selective uptake of hormone by the anlagen of the homologous organs differed.

In the genital tubercle of the female embryos a single peak of uptake of both hormones was observed on the 21st day, whereas in male embryos there were two such peaks — on the 21st and 24th days. In the urogenital sinus of the females increased uptake of T-^{3}H was observed on the 18th and 21st days, and of E-^{14}C on the 18th-21st and 25th day, whereas in male embryos increased uptake of T-^{3}H was observed on the 19th and 21st days and E-^{14}C on the 19th and 24th days.

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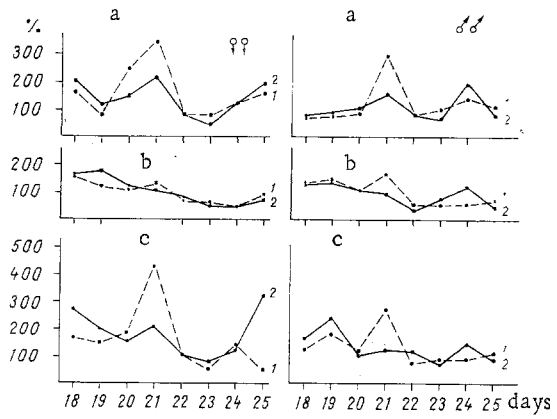

Fig. 1. Selective absorption of T-^{3}H (1) and E-^{14}C (2) by the genital tubercle (a), ducts (b) and urogenital sinus (c) in rabbit embryos (male and female). Abscissa) days of embryonic development; ordinate) radioactivity (% of muscular radioactivity; $P \leq 0.001$).

In the genital tract (Wolffian and Muellerian ducts, isolated together) no significant sex differences in hormone uptake were found. Nevertheless these organs interest the investigator because of the particular feature of their development: regression of the Muellerian ducts in male embryos and of the Wolffian ducts in female embryos. The possibility cannot be ruled out that these differences were still present but could not be detected in our experiments because the ducts could not be separated because of the delicate consistency of the tissues. In fact, in male embryos, on the 21st day of development a tendency is observed for T-^{3}H uptake to be increased, whereas in female embryos increased uptake of E-^{14}C is observed on the 19th day.

It has been shown by intrauterine castration and transplantation of the testis into the body cavity or injection of testosterone [4] that in rabbit embryos the period from the 19th through the 21st day is "critical," or a period of special sensitivity to the development of a male type of reproductive system, and it is only during this period that the direction of differentiation of the somatic parts of the reproductive tract can be changed. Accordingly it is important to note that in embryos of both sexes intensive incorporation of the androgen was observed in anlagen of the target organs precisely from the 19th to the 21st days of development (Fig. 1).

The increased uptake of T-^{3}H observed in the females is attributed to insufficiency of endogenous androgens [7] and, consequently, to the presence of unoccupied receptor combining sites; conversely, the weaker uptake observed in males is attributed to interaction between receptors and endogenous androgens [5, 7, 8].

Furthermore, more active uptake of E-^{14}C was observed in anlagen of female embryos on the 21st day than in male embryos. It is also evident (Fig. 1) that ability to bind estradiol was exhibited in female embryos as early as on the 18th day, and to a greater degree than in males, long before the appearance of morphological features of differentiation. The steroid-producing function of the gonads also has been established in earlier stages of development — before the 18th day [5]. However, the hormone level is evidently not yet sufficient for morphogenesis.

The concentration of hormones in the incubation medium was high enough to produce complete and excessive saturation of receptors, for after culture about 93% of residual androgens and 97% of estrogens were found in the medium. This all suggests that under these conditions the excess of hormones led to preferential specific binding of the hormone in accordance with the genetic sex of the embryo; consequently, the possibility of their teratogenic action can be ruled out.

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CIRCADIAN RHYTHMS OF BLOOD LEVELS OF CORTICOSTEROIDS AND THEIR PRECURSORS IN *Papio hamadryas* DEPENDING ON INITIAL FUNCTIONAL STATE OF THE HYPOPHYSEO-ADRENOCORTICAL SYSTEM

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According to much evidence in the literature adrenocortical function undergoes well-defined rhythmic changes in the course of the 24-h period [1, 2, 6-9]. However, most experimental studies of circadian rhythms of blood hormone levels have been carried out on small laboratory animals, which differ considerably from man in their biological parameters and in the spectrum of the steriods produced. Furthermore, technical difficulties until recently have prevented determination of blood levels of intermediate products of steroid biosynthesis, and this has greatly restricted the possibility of a systematic analysis of the functions of steroid-producing glands during the 24-h period. With the development and introduction of highly sensitive radioimmunologic methods, preceded by chromatographic fractionation of individual compounds, it is now possible to make a qualitative and quantitative assessment of a broad spectrum of steroids, including intermediate products of their synthesis.

Investigations have shown that monkeys and, in particular, the baboom *Papio hamadryas*, bear the closest resemblance to man in the character of steroid hormone production and metabolism [1, 2, 4]. This fact was the deciding factor in the choice of this species of baboon in order to study circadian rhythms of plasma steroid hormone levels.

The object of this investigation was to study the character of circadian rhythms of blood levels of steroid hormones (cortisol, aldosterone, and their precursors) in baboons and the dependence of blood steroid levels and manifestation of their circadian rhythms on the functional state of the hypothalamo-hypophyseo-adrenal system.

EXPERIMENTAL METHOD

Eighteen clinically healthy sexually mature male baboons aged 12-18 years and weighing 25-35 kg were used. The animals were divided into two groups. Group 1 consisted of intact baboons, kept together with a group of females without preliminary adaptation to short-term fixation and blood taking. Circadian rhythms of the blood steroid levels of the unadapted baboons were studied four times during the year at intervals of 3 months; the results were pooled. Animals of group 2 were adapted to the experimental conditions. For 15-30 days these baboons were kept in individual metabolic cages, and every day blood taking was simulated and accompanied by short-term fixation. Blood for steroid hormone assay was taken in its volume of 8-10 ml from the cubital vein into heparinized centrifuge tubes, at 3-hourly intervals for the 24. The first blood samples was taken at 9 a.m. and the last at 9 a.m. next day. Plasma was obtained by centrifuging the blood at 3000 rpm for 5 min and was kept in the frozen state in a refigerator at −20°C.

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