The results of this investigation thus suggest that the more intensive development of the duodenal glands in herbivorous mammals may be connected with the character of their diet and, in particular, with its cellulose content. Consumption of a vegetable diet with a high cellulose content evidently requires the surface of the duodenal mucosa to be protected against the mechanical and chemical action of the coarse and acid food masses arriving from the stomach. The possible mechanism of this protection is the formation of a powerful protective layer of mucus, formed on account of secretion of the duodenal glands, which are much more highly developed in such cases.

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EFFECT OF NEONATAL CASTRATION OF MALE RATS ON PITUITARY STEROID RECEPTOR CONCENTRATION

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Estrogens and androgens participate in the regulation of gonadotropin secretion in male rats by a negative feedback method as a result of interaction with corresponding receptors in the adenohypophysis and hypothalamus [4, 5]. Intracellular receptors binding estrogens and androgens are present in these structures, and protein molecules may probably partipate in modulation of the pituitary response by steroids [9, 10]. It has also been shown that changes in the hormonal background in young male rats lead to significant changes in the concentration of receptors for sex hormones in the adult hypothalamus [3, 5, 9].

The aim of this investigation was to study receptor binding of estradiol and testerone in the pituitary gland of mature male rats castrated during the first days of life or in the adult state.

EXPERIMENTAL METHOD

Noninbred male rats were used. The testes were removed from the animals at the age of 1-3 days, and they were decapitated at the age of 90 days. Males castrated at puberty, which were killed a week after the operation, were used for comparison. Adenohypophyses from 40 to 50 animals were homogenized in buffer containing 0.01 M Tris-HCl, 0.0015 M EDTA, and 0.01 M

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TABLE 1. Receptor Binding of ^3H-E_2 and ^3H-T by Subcellular Fractions of the Pituitary Gland of Adult Intact and Castrated Male Rats

Males	Receptors for estradiol		Receptors for testosterone	
	cytosol	nuclei	cytosol	nuclei
	fmoles/mg protein	fmoles/mg DNA	fmoles/mg protein	fmoles/mg DNA
Intact	147,0±25,7	165,6±6,8	35,7±5,0	1101,0±80,5
Castrated at the age of 90 days	153,4±26,0	139,1±14,8	36,0±5,4	1208,0±115,9
at the age of 1-3 days	136,9±14,0	152,8±7,3	15,2±2,0*	615,7±55,5**

Legend. Mean results of 4-8 experiments shown. *P < 0.01, **P < 0.001 compared with intact males.

TABLE 2. Concentrations of Estradiol (E_2), Testosterone (T), and LH in the Blood Serum of Intact and Castrated Male Rats

Males	E_2, pg/ml	P, pg/ml	LH, ng/ml
Intact	14,9±0,4	420,2±51,6	72,3±5,9
Castrated at the age of 90 days	10,7±0,9	12,0±1,2	505,0±27,0
at the age of 1-3 days	16,5±2,1	14,3±2,1	546,8±36,5

2-mercaptoethanol, pH 7.4, and centrifuged at 800g for 10 min. To obtain cytosol the supernatant was centrifuged at 105,000g for 90 min on a Spinco 65 L-2 ultracentrifuge. The residue obtained by centrifugation at 800g was used to obtain the nuclear fraction, as described previously [2]. 1,2,6,7-^3H-testosterone (^3H-T) and 17β-2,4,6,7-^3H-estradiol (^3H-E_2), with specific activity of 90-122 Ci/mmole, were used as labeled hormones.

To determine estrogen receptors aliquots of the cytosol and nuclear suspension were incubated either with increasing concentrations ($0.5 \cdot 10^{-9}$-$6 \cdot 10^{-9}$ M) or with one saturating concentration ($4 \cdot 10^{-9}$ M) of ^3H-E_2. To determine androgen receptors aliquots of cytosol and nuclear suspensions were incubated with increasing concentrations ($10 \cdot 10^{-9}$-$50 \cdot 10^{-9}$ M) of ^3H-T. To allow for nonspecific binding, parallel incubation of the samples was carried out with a thousandfold molar excess of the corresponding hormone. The cytosol was incubated at 23°C for 90 min and the nuclear fraction at 37°C for 60 min. Free and bound forms of the hormones (LH) were separated as described previously [1, 2]. The receptor concentration was determined by plotting specific binding curves by the method in [11].

EXPERIMENTAL RESULTS

Concentrations of cytoplasmic and nuclear receptors for estradiol in the pituitary were identical in intact male rats and rats castrated in the adult and neonatal state (Table 1). Castration of the adult males did not affect the concentration of cytoplasmic and nuclear receptors for testosterone. However, in rats castrated at birth a significant decrease was observed in the number of testosterone binding sites in the cytosol and nuclei. The estradiol concentration in the blood serum of animals of all three groups was equally low (Table 2). Castration of the males in the neonatal and adult state reduced the blood testosterone level by 30 times and increased the LH concentration sevenfold.

It can be concluded from a comparison of the blood testosterone level and the concentration of nuclear receptors for this organ in the pituitary with the blood LH concentration of intact adult male rats that the nuclear androgen receptors of the pituitary take part in regulation of LH release in males by a negative feedback mechanism.

It has been shown in the literature that injection of androgens into adult male rats immediately after castration reduces LH secretion into the blood [8, 12]. It can accordingly be postulated that the realization of this effect of androgens at the pituitary level must imply maintenance of high sensitivity of the androgen receptor apparatus of males castrated in adult life. The results of the present investigation in fact indicate that castration of adult male rats does not affect the concentration of cytoplasmic and nuclear receptors for testosterone.

In order to determine whether the mechanisms of negative feedback, operating through the pituitary androgen receptors, depends on presence of testosterone during the first days of life, the testes were removed from newborn rats. Castration of the males in the neonatal period was found to reduce receptor binding of testosterone considerably in the cytoplasmic and nuclear fractions of the pituitary. This may evidently explain the decrease in sensitivity of the pituitary to the inhibitory action of circulating testosterone in the negative feedback chain of adult males, castrated in the first days of life. For instance, implantation of silastic capsules containing testosterone did not lower the LH level when raised in animals as a result of castration [6]. It can consequently be suggested that the mechanism of negative feedback at the pituitary level is formed in male rats in the early period of life under the influence of testicular testosterone.

We know from the literature that estrogen can inhibit LH secretion in male rats more strongly than androgen [7]. The question accordingly arises, whether this effect of estradiol may be mediated through pituitary estrogen receptors. As will be clear from Table 1, receptors for estradiol were found in the pituitary of intact adult males, but the concentration of nuclear receptors for estradiol was much lower than the concentration of nuclear receptors for testosterone. It might be supposed that testicular testosterone affects the level of estrogen receptors in the pituitary at the "critical" period of development. However, the results of the present investigation indicate that absence of androgenic influences during the first days of life does not play an essential role in maturation of the pituitary estrogen. In addition the high testosterone level observed in the serum of adult male rats likewise does not affect the number of estradiol binding sites, for castration of adult males leaves receptor binding of estradiol unchanged. This was confirmed also in experiments to study competitive replacement of labeled estradiol by unlabeled sex steroids: testosterone did not compete for estradiol binding sites.

Low concentrations of nuclear receptors for estradiol in male rats of all three groups, against the background of both low and high LH concentrations in intact and castrated rats receptively, suggest that the inhibitory action of estradiol on LH secretion is not exerted at the pituitary level.

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