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# Effects of Gonadectomy and Hormone Replacement on Brain Monoamine Synthesis in Male Rats

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ENGEL, J., S. AHLENIUS, O. ALMGREN, A. CARLSSON, K. LARSSON AND P. SÖDERSTEN. *Effects of gonadectomy and hormone replacement on brain monoamine synthesis in male rats.* PHARMAC. BIOCHEM. BEHAV. **10**(1) 149–154, 1979.—The synthesis of catecholamines and serotonin in the brains of castrated male rats was analyzed at either various times after castration or at various ages. It was found that (a) castration of rats at 50 days or later causes an increase in brain monoamine synthesis, and (b) this phenomenon was not observed until 20 days after castration. The increase in brain monoamine synthesis following castration was counteracted by treatment with testosterone, thus relating the biochemical consequences of castration with changed hormonal conditions of the animal. It is suggested that testosterone exerts an inhibitory influence on monoamine synthesis.

Castration Testosterone Catecholamine synthesis Serotonin synthesis Central nervous system

ACCUMULATING evidence suggests that monoamines may be involved in the mediation of masculine sexual behavior [31]. Thus, treatment of intact male rats with parachlorophenylalanine (PCPA), a drug that blocks the synthesis of 5-hydroxytryptamine (5-HT) facilitates masculine sexual behavior [1] and treatment of castrated male rats with PCPA facilitates testosterone-induced masculine sexual behavior [14, 28, 35]. Treatment with PCPA may even induce masculine activity in castrated male and female rats even without concomitant treatment with testosterone [35,36]. Similarly, 5,7-dihydroxtryptamine, a neurotoxin causing a permanent destruction of serotonergic neurons, may facilitate testosterone-induced masculine sexual behavior in castrated rats [24]. Also catecholamines (CA) have been implicated in the regulation of masculine sexual behavior, having a stimulatory [8, 27, 33, 37] rather than an inhibitory role as has been suggested for serotonin.

Studies undertaken to investigate effects of castration and subsequent hormone treatment on brain monoamine synthesis and turnover have given divergent results depending on the step of the amine synthesis analyzed, the interval of time elapsing between castration and amine analyses, and the particular structure of the brain analyzed. Kizer *et al.* [20] did not find any alterations in the tyrosine hydroxylase activity 9 days after castration in any of the hypothalamic nuclei analyzed except for the median eminence which showed an increased tyrosine hydroxylase activity after castration. Kizer *et al.* [19] analyzed the tryptophan hydroxylase activity in the limbic system, the hypothalamus and the midbrain without finding any alterations 9 days after castration. Donoso *et al.* [13] found increased noradrenaline (NA) levels in the hypothalamus 20–30 days after castration of male rats and Bernard and Paolino [4] found a slight increase in the turnover of NA, dopamine (DA) and 5-HT at 3 but not 6 weeks after castration.

The purpose of the present experiment was to study effects of castration on the synthesis of CA and 5-HT in the brain. Since the effects of castration on the monoamine synthesis may vary according to the time interval between castration and analyses, this interval was systematically varied. Furthermore, the importance of hormonal maturation for possible biochemical effects observed was assayed by studying effects of castration before and after onset of puberty.

## METHOD

### *Animals*

The animals were male Wistar rats (Möllegaard Breeding Laboratories, Skensved, Denmark) of various ages. The rats were maintained in cages containing 6 rats under a regulated dark-light cycle (dark 0700 a.m.–0700 p.m.). Food (Astra-Ewos laboratory food, MR 3) and water were available ad lib. The rats arrived from the breeding laboratories 10 days prior to surgery.

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*Surgery*

The rats were castrated under light ether anesthesia. Sham-operated rats were exposed to scrotal incisions similar to those of the castrated rats. In each rat of the various treatment groups a silastic capsule was implanted under the skin in the flank.

*Hormone Treatment*

Silastic capsules (inner dia. 1.57 mm, outer dia. 3.18 mm, Dow Corning Corp.) were filled with testosterone as described by Legan *et al.* [25]. All capsules were incubated in 0.9% NaCl at least one day before use and the saline was exchanged 30 min before implantation. The capsules were 15 mm long and implants of this size will produce plasma testosterone concentrations of about 1.0 ng/ml [11,34].

*Biochemistry*

*Monoamine synthesis.* Tyrosine and tryptophan hydroxylase activity was estimated in vivo by measuring the accumulation of dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP), respectively, after inhibition of aromatic L-amino acid decarboxylase with NSD 1015 [9]. The rats were killed by decapitation 30 min after NSD 1015 (100 mg/kg IP) and the brains were quickly removed and placed on a glass plate over ice. The following parts of the brain were taken for analysis: (1) corpus striatum, (2) limbic forebrain including the olfactory tubercle, nucleus accumbens (medial part) septum, the precommisural part of striae terminalis, nucleus amygdaloideus centralis and parts of the paleocortex, (3) the remainder of the hemispheres and (4) diencephalon (thalamus and hypothalamus) (for dissection procedures see Carlsson and Lindqvist [10]). The animals were killed between 8 and 10 a.m.

The parts of two brains were pooled and weighed. The brain parts were homogenized in 10 ml 0.4 N perchloric acid containing 5 mg $Na_2S_2O_5$ and 20 mg EDTA. The extract was purified on a strong cation exchange column (Dowex 50) [18]. The following spectrophotofluorimetric analyses were performed: tyrosine [38], DOPA [18], tryptophan [3] and 5-HTP [2]. At the time of sacrifice the seminal vesicles and ventral prostate were dissected out, blotted dry on filter paper and weighed.

*Tyrosine Hydroxylase Activity In Vitro*

The in vitro tyrosine hydroxylase activity was also analyzed in brain parts of rats in Experiment 1 (see below). Two rats of each group were used for this part of the study. After decapitation of the rats the brains were immediately removed and dissected as described above. For comparative purposes the adrenals were also taken out for analysis. After weighing the brain parts and the adrenals were frozen on dry ice and stored at −70°C for later analysis. For the tyrosine hydroxylase assay the frozen brain parts or adrenals were thawed and homogenized by a glass homogenizer in small volumes of ice cold DβH-buffer (8.5% sucrose added with 9 ml/450 ml sucrose of 1M $KPO_4$ pH 6.5). The assays were run in duplicate, following in essence the method described by Nagatsu *et al.* [29], and the mean value of the two determinations were used. Tritiated l-tyrosine (ring 3,5-$^3$H) was obtained from New England Nuclear Chemicals and further purified by cation exchange chromatography.

*Experimental Design*

The rats were randomly allocated to three groups:
(1) Castrated rats implanted with an empty capsule.
(2) Castrated rats implanted with a silastic capsule containing testosterone.
(3) Sham-operated rats implanted with an empty capsule.

*Experiment 1*

The animals were operated upon at the age of 70 days and then killed for biochemical analyses at 12 and 24 hr; 10, 20, 30 and 40 days thereafter.

*Experiment 2*

The purpose of this experiment was to investigate possible changes in monoamine synthesis after castration as influenced by the age of the animals in relation to puberty.

Since the results obtained in Experiment 1 did not reveal any significant biochemical changes before 20 days after castration, this time interval was chosen for Experiment 2. The animals were operated upon at the age of 30, 40, 50, 60 and 70 days.

*Statistics*

The data were subject to a two-way analysis of variance (ANOVA) followed by *t*-test [39].

## RESULTS

*Experiment 1*

*Tyrosine.* In the limbic forebrain and diencephalon of castrated rats, the castration was followed by statistically significant increased tyrosine levels from 20 days and 30 on, respectively (Fig. 1). This increase was counteracted by testosterone except in diencephalon at 40 days. A statistically significant increase, not counteracted by testosterone, was also seen in the limbic forebrain at 12 hr. Similar changes were found in the hemispheres and the striatum. Inspection of the graphs indicates a tendency of a peak of the castration induced increase in tyrosine levels at 20 days.

*DOPA.* In the limbic forebrain but not in the diencephalon there was an increased accumulation of DOPA after treatment with NSD 1015 in the castrated rats from 20 days on (Fig. 2). This increase was counteracted by testosterone. A significant increase in the DOPA accumulation was also found in the hemispheres and in the striatum. As was the case with the tyrosine levels, there was a tendency for a peak in the castration-induced increase in DOPA accumulation at 20 days.

*Tryptophan.* The castrated rats showed an increase in tryptophan levels in the limbic forebrain, the striatum and diencephalon (Fig. 3). No significant changes were found in the hemispheres. However, individual comparisons do not indicate any consistent pattern in the castration-induced changes in tryptophan levels.

*5-HTP.* In the limbic forebrain (20 and 30 days after castration) and the diencephalon (from 20 days on) but not in the striatum there was an increased accumulation of 5-HTP after treatment with NSD 1015 in castrated rats (Fig. 4). This increase was counteracted by testosterone in the diencephalon but not in the limbic forebrain.

*Tyrosine Hydroxylase Activity in vitro.* There were no statistically significant changes in the tyrosine hydroxylase

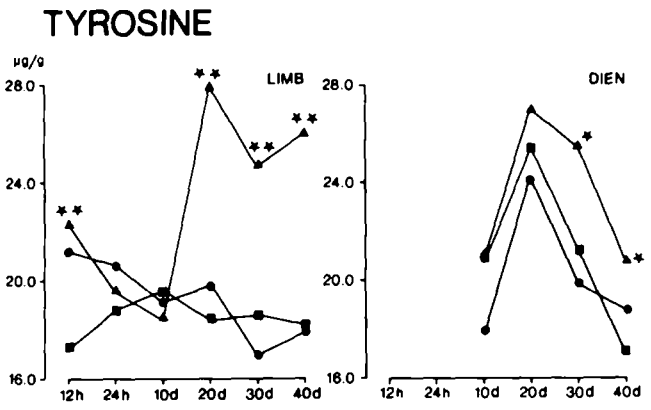


FIG. 1. Tyrosine levels at various time intervals after castration of 70-days old male Wistar rats. Rats were killed 30 min after injection of NSD 1015 (100 mg/kg IP). Shown are means of 2 experiments (brain parts from 2 rats per experiment). Statistical evaluation was performed by means of a two-way Anova followed by a *t*-test for comparisons with sham-treated controls. $*p<0.05$; $**p<0.01$. ▲——▲ castrated ■——■ sham-operated; ●——● castrated+testosterone. *Limbic forebrain.* Time: F(5,18)=1.16 NS. Treatment: F(2,18)=28.63 $p<0.01$. Time×Treatment: F(10,18)=5.36 $p<0.01$. *Diencephalon.* Time: F(3,12)=22.75 $p<0.01$, Treatment F(2,12)=8.22 $p<0.01$ Time×Treatment: F(6,12)=1.38 NS

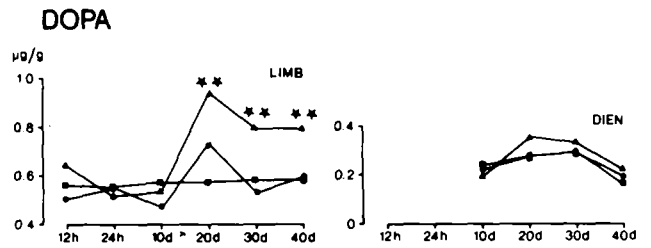


FIG. 2. Same as Fig. 1 except for DOPA. *Limbic forebrain.* Time: F(5,18)=22.82 $p<0.01$ Treatment: F(2,18)=41.26 $p<0.01$ Time× Treatment: F(10,18)=6.80 $p<0.01$. *Diencephalon.* Time: F(3,12)= 22.89 $p<0.01$ Treatment: F(2,12)=3.61 NS, Time×Treatment: F(6,12)=2.41 NS

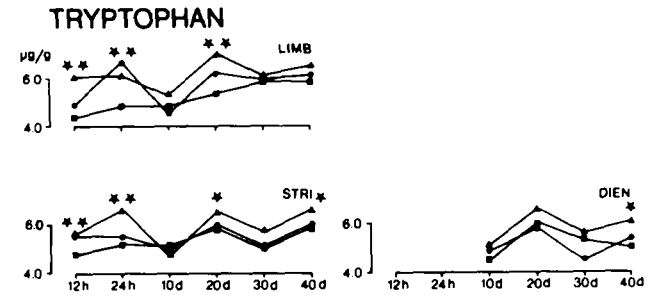


FIG. 3. Same as Fig. 1 except for tryptophan. *Limbic forebrain.* Time: F(5,18)=14.54 $p<0.01$ Treatment: F(2,18)=2.55 $p<0.01$ Time×Treatment: F(10,18)=3.30 $p<0.05$ *Striatum.* Time: F(4,15)= 18.27 $p<0.01$ Treatment: F(2,15)=17.06 $p<0.01$ Time× Treatment: F(8,15)=3.39 $p<0.05$ *Diencephalon.* Time: F(3,12)= 10.00 $p<0.01$ Treatment: F(2,12)=4.94 $p<0.05$ Time× Treatment F(6,12)=1.47 NS

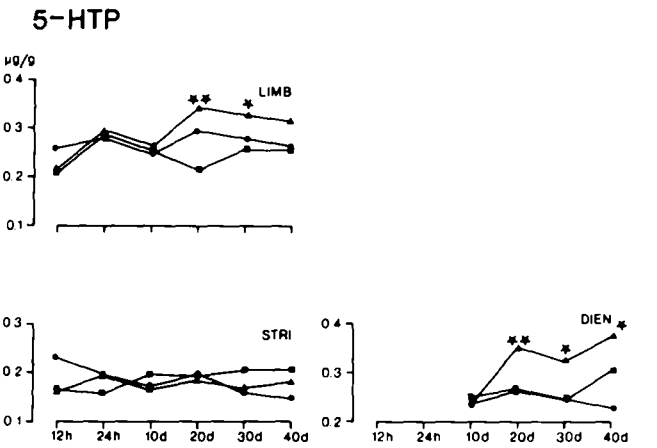


FIG. 4. Same as Fig. 1 except for 5-HTP. *Limbic forebrain.* Time: F(5,18)=2.70 NS Treatment: F(2,18)=6.31 $p<.01$ Time×Treatment F(10,18)=1.80 NS *Striatum.* Time F(4,15)=0.88 NS Treatment: F(2,15)=0.90 NS Time×Treatment F(8,15)=2.36 NS *Diencephalon.* Time: F(3,12)=7.77 $p<.01$ Treatment: F(2,18)=19.56 $p<0.01$ Time×Treatment F(6,12)=3.64 $p<0.05$

activity in any brain area studied. Neither were any significant changes observed in the tyrosine hydroxylase activity in the adrenals, indicating that the surgical trauma did not induce any long-lasting changes of the sympathetic activity.

*Weight of the Accessory Sexual Glands.* Within a week after castration there was a maximum decrease in the weight of the seminal vesicles and ventral prostate which was completely prevented by the testosterone treatment.

*Experiment 2*

*Tyrosine.* Compared to the sham-operated rats, the castrated animals showed a statistically significant increase in the tyrosine levels in the limbic forebrain but not in the diencephalon when surgery was performed at 50 days of age and later (Fig. 5). In the 60 and 70 days old animals testosterone counteracted the increase in tyrosine levels. Treatment differences were further found in the striatum and the brain stem but not in the hemispheres.

*DOPA.* There was a significant increase in the accumulation of DOPA in the limbic forebrain at all ages except at 40 days of age (Fig. 6). This increase was counteracted by testosterone. Significant treatment effects were further observed in the striatum and the hemispheres but not in the diencephalon or the brain stem.

*Tryptophan.* The tryptophan levels were slightly increased after castration in the limbic forebrain (40, 60, 70 days of age) (Fig. 7). This increase was counteracted by testosterone at 60 days. An increase in tryptophan levels following castration was observed in the striatum and the hemispheres but not in the diencephalon and the brain stem.

*5-HTP.* In the limbic forebrain, a significant increase, counteracted by testosterone, of the 5-HTP accumulation was observed from 60 days (Fig. 8). A slight but not significant increase in the 5-HTP accumulation was also observed in the diencephalon. Concomitantly there was a decrease in the 5-HTP accumulation with age in the intact controls resulting in a significant interaction. A strong negative correlation (r=0.70–0.79) was found in the various brain areas

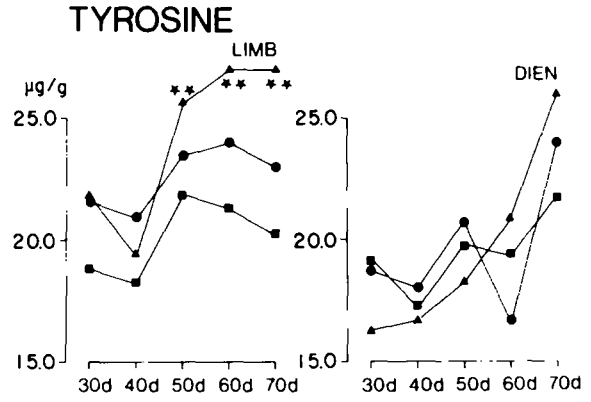


FIG. 5. Tyrosine levels at various ages, 20 days after surgery. Rats were killed 30 min after injection of NSD 1015 (100 mg/kg IP). Shown are means of 2 experiments (brain parts from 2 rats per experiment). Statistical evaluation was performed by means of a two-way ANOVA followed by a *t*-test for comparisons with sham-treated controls. $*p<0.05$; $**p<0.01$. ▲——▲ castrated ■——■ sham-operated; ●——● castrated+testosterone. *Limbic forebrain.* Time: $F(4,19)=14.53$, $p<0.01$ Treatment: $F(2,19)=26.37$ $p<0.01$ Time×Treatment $F(8,19)=2.97$ $p<0.05$ *Diencephalon.* Time: $F(4,19)=15.91$ $p<0.01$ Treatment: $F(2,19)=0.02$ NS Time×Treatment $F(8,19)=2.66$ $p<0.05$

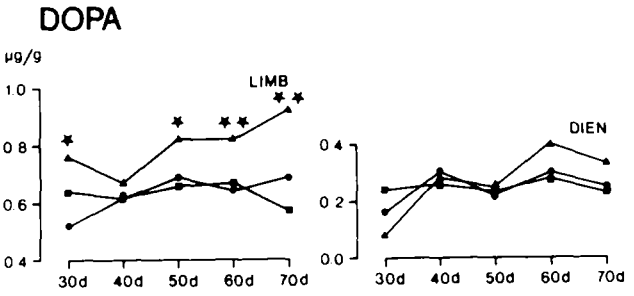


FIG. 6. Same as Fig. 5 except for DOPA. *Limbic forebrain.* Time: $F(4,19)=4.49$ $p<0.05$ Treatment: $F(2,19)=34.08$ $p<0.01$ Time×Treatment $F(8,19)=3.59$ $p<0.05$ *Diencephalon.* Time: $F(4,19)=50.49$ $p<0.01$ Treatment: $F(2,19)=3.44$ NS Time×Treatment $F(8,19)=14.38$ $p<0.01$

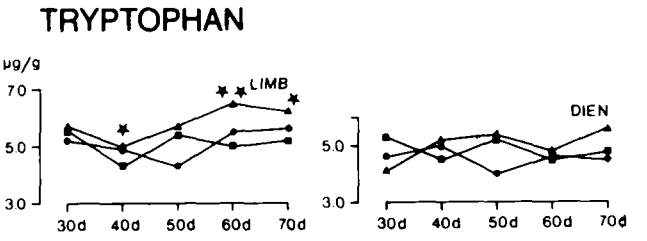


FIG. 7. Same as Fig. 5 except for typtophan. *Limbic forebrain.* Time: $F(4,19)=8.16$ $p<0.01$ Treatment: $F(8,19)=16.68$ $p<0.01$ Time×Treatment $F(8,19)=4.38$ $p<0.01$ *Diencephalon.* Time $F(4,19)=0.65$ NS Treatment: $F(2,19)=3.17$ NS Time×Treatment $F(8,19)=2.88$ $p<0.05$

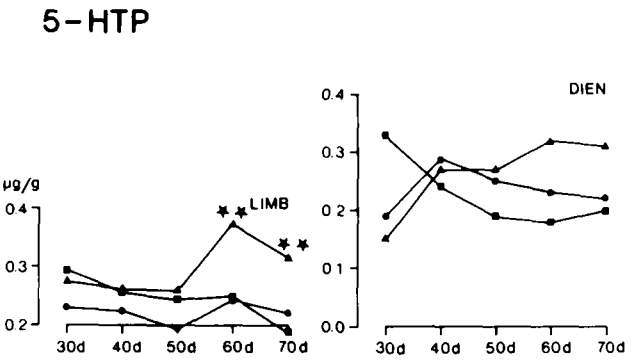


FIG. 8. Same as Fig. 5 except for 5-HTP. *Limbic forebrain.* Time: $F(4,19)=4.36$ $p<0.05$ Treatment: $F(2,19)=15.78$ $p<0.01$ Time×Treatment $F(8,19)=5.99$ $p<0.01$ *Diencephalon.* Time: $F(4,19)=1.62$ NS Treatment: $F(2,19)=3.22$ NS Time×Treatment $F(8,19)=9.53$ $p<0.01$

between age and 5-HTP accumulation in the intact animals (hemispheres $p<0.05$, all other brain regions $p<0.01$). Treatment effects were further found in the striatum and the brain stem.

## DISCUSSION

The present results show that castration of 70 days old rats causes an increase in the brain monoamine synthesis, and this increase is not observed until 20 days after castration. Considering the general pattern of castration-induced changes in the levels of the amino acids measured it appears that the most consistent changes occur in animals castrated at 50–70 days of age, i.e., after onset of puberty [23,24]. The increase in brain monoamine synthesis and decrease in the weight of the accessory sex glands following castration was counteracted by treatment with testosterone, thus relating the biochemical consequences of castration to changes in hormonal conditions of the animal.

Already within a few hours after castration plasma testosterone levels decrease to almost undetectable levels [30] and as demonstrated in the present experiments there was a rapid decrease in the weight of the accessory sexual organs. The delay in onset in the effects on monoamine synthesis remains to be explained. Interestingly a similar delay is seen in the effects of castration on sexual behavior [12,22]. Since removal of the adrenal glands, another source of androgen, in addition to gonadectomy, does not cause a further decrease in the sexual activity [6], the retention of sexual activity after castration is probably not due to presence of androgen in the circulation. No satisfactory explanation can at present be given to the delay in disappearance of sexual behavior after castration. The coincidence of changes in sexual behavior and changes in the monoamine synthesis suggests the possibility of a relationship between these phenomena, which may both be due to trophic neuronal changes.

The male rats reach puberty between 50 and 60 days of age as evidenced by onset of sexual activity [23,24] and a rise of plasma testosterone levels beginning at 40–50 days [16, 21, 34]. These facts suggest that the lack of castration-induced changes in monoamine synthesis before 50 days as observed

in the present study may be related to mechanisms involved in controlling onset of puberty.

According to preliminary data in this laboratory, injection of 25 mg/kg of testosterone propionate to male rats castrated in adulthood resulted, 24 hr later, in a marked decrease in the monoamine synthesis suggesting a direct relationship between the synthesis of monoamines and hormonal levels. The rapidity of the changes taking place after treatment with testosterone is in contrast to the slowness of the changes in the monoamine synthesis taking place after castration. The possible role of the gonadotropins in the biochemical effects obtained after castration should be considered in this context since the increase in gonadotropins following castration seems to develop only slowly [11].

The duration of the biochemical changes following castration seems to be at least 40 days. In a preliminary experiment, however, data have been obtained indicating a regress of these changes at 60 days after castration.

In the castrated rats there was an increase not only in DOPA accumulation but also in tyrosine levels, with an apparent temporal as well as regional correlation between the two phenomena. The question arises whether the increased DOPA formation could be secondary to increased levels of substrate for tyrosine hydroxylase. This enzyme is generally supposed to be saturated with its substrate under normal conditions, although this has been challenged [40]. Although more work is needed to clarify this point our data neither prove nor disprove a causal relationship between tyrosine levels and DOPA formation. Nevertheless the increase in tyrosine levels after castration is of great interest. It should be realized that the catecholamine neurons form a very minute part of the whole brain tissue, and thus the increase in tyrosine levels must be assumed to encompass also other cells. The tryptophan levels showed changes somewhat reminiscent of the tyrosine levels, although less pronounced. It should be emphasized that the changes in tyrosine and tryptophan levels observed so far are limited to rats pretreated with NSD 1015. It remains to be elucidated if similar changes occur in rats not treated with this inhibitor.

No significant changes in tyrosine hydroxylase activities, measured in vitro, were observed during the period 20 to 40 days after castration, when DOPA formation was significantly elevated. This suggests that the increased synthesis is not due to an increased number of enzyme molecules but rather to an increased enzymatic activity of existing molecules or an increased substrate availability. Kizer *et al.* [19,20] found no changes in hypothalamic tyrosine and tryptophan hydroxylase activities after castration. However, the males used in their studies were killed already 9 days after castration, when no changes were found in the monoamine synthesis in our rats.

It has been reported that, in an open field situation, castrated male rats display an increase in locomotor activity correlated with an increase in hypothalamic DA turnover [4,5]. However, in preliminary experiments, using photocell-equipped activity cages we observed no changes in locomotor activity of castrated rats at time intervals corresponding to those when changes in catecholamine and 5-HT synthesis was observed (unpublished data). It should be emphasized that the increased catecholamine and 5-HT synthesis in the brain cannot be directly interpreted in terms of increased physiological neuronal activity. This can be done only after supplementary measurements of transmitter utilization. Even if the physiological activity of the catecholamine neurons turned out to be elevated, this is not contradictory to the absence of increased motor activity. For example, an increased catecholaminergic activity could be compensatory, in analogy to the situation observed after treatment with neuroleptic drugs, or the increase could be balanced by stimulation of antagonistic neuronal systems.

At present it is not possible to interpret the castration-induced changes in monoamine synthesis in precise functional terms. Interestingly, the changes induced by castration in the synthesis of 5-HT and catecholamines were localized to different parts of the brain. The most consistent changes in the 5-HT synthesis were found in diencephalon. On the other hand, no changes in the catecholamine synthesis were found in the diencephalon, while the striatum and the limbic forebrain area showed a marked increase in the synthesis of catecholamines. The selective increase of the 5-HT synthesis in the diencephalon is of particular interest in view of the importance of this area for the control of pituitary function and sexual behavior. Thus a high concentration of testosterone binding neurons are found in the preoptic-anterior hypothalamic continuum [32]. Hormonal implants in this area may facilitate the expression of masculine sexual behavior [26] while lesions in the same region may abolish sexual behavior [15,17]. Previous finding of a faciltation of the display of masculine sexual behavior following inhibition of the synthesis of 5-HT by parachlorophenylalanine (see Introduction) together with the present findings of an increased synthesis of 5-HT after castration support the notion of a serotonergic link in the neural substrate of masculine sexual behavior including diencephalic structures. One possible explanation of the increased dopamine synthesis after castration is that testosterone enhances the sensitivity of dopamine receptors and that the castration-induced changes are feedback mediated, analogous to the stimulation of dopamine synthesis and turnover by dopamine-receptor antagonists. The antipsychotic action of these agents is thought to be mediated via an antidopaminergic action in the limbic system. Thus the effect of castration on DOPA formation in the dopamine-rich limbic forebrain regions is of considerable interest. As a point of speculation it may be recalled that the onset of schizophrenia often occurs at puberty, or shortly afterwards, and thus an involvement of sexual hormones in the pathogensis of schizophrenia cannot be excluded. Low plasma testosterone levels have been reported in schizophrenic patients [7].

The question arises why there are no more striking changes in the synthesis of brain monoamines at puberty, as might be expected from the effects of castration, apart from the moderate decrease in 5-HT synthesis mentioned above. A possible explanation might be that the action of testosterone on monoamine synthesis is balanced by the opposite action of another factor developing at puberty. Investigation of the influence of the pituitary on the brain monoamines may possibly help to throw light on this problem.

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