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# Changes of Norepinephrine Levels, Tyrosine Hydroxylase and Dopamine-Beta-Hydroxylase Activities after Castration and Testosterone Treatment in Vas Deferens of Adult Rats[1]

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## ABSTRACT

*Norepinephrine levels and tyrosine hydroxylase and dopamine-β-hydroxylase activities have been used to evaluate the effect of castration and testosterone treatment on the sympathetic innervation of the adult vas deferens. Castration was followed by a decrease in both norepinephrine content and tyrosine hydroxylase activity, even though the changes were not concomitant. Treatment of castrated animals with testosterone reversed the effect of castration on organ weight and norepinephrine content, but only a short-lasting increase in tyrosine hydroxylase activity occurred at the beginning of testosterone treatment. In contrast, the testosterone-induced recovery of norepinephrine content observed at this time was accompanied by a marked increase in dopamine-β-hydroxylase activity.*

*The results suggest that in rat vas deferens, norepinephrine levels are under androgenic control and that this regulation mainly involves changes in dopamine-β-hydroxylase activity rather than a modulation of tyrosine hydroxylase.*

## INTRODUCTION

Norepinephrine (NE) levels in sympathetic innervated tissues are regulated by factors affecting its biosynthesis, storage, and release. The biosynthetic process has been the subject of considerable interest to many workers mainly because of the multiple enzymatic steps involved. One of these steps, the transformation of tyrosine into dihydroxyphenylalanine, is catalyzed to tyrosine hydroxylase (TH), an enzyme postulated to be the rate-limiting step in NE biosynthesis. TH is subjected to both short-term and long-term regulation. The former is related to affinity changes ($K_m$) of the cofactor pteridine induced by pH, phosphorylation, impulse flow, intracellular calcium, and catecholestrogen, among others (Ames et al., 1978; Bustos, 1978; Hiemke and Ghraff, 1982; Marm and Hill, 1983; Iuvone, 1984). Long-term regulation chiefly involves changes in apparent maximal velocity ($V_{max}$) and is probably related to the number of enzyme molecules. These quantitative changes of the enzyme may result from trophic influences and hormonal regulation of the target tissues (Fillenz, 1977).

Evidence exists that TH activity is regulated by hormonal influences. Testosterone (T) is one of the hormones affecting TH. In neonatal and adult rats, it regulates maturation of sympathetic ganglia and the levels of TH activity (Dibner and Black, 1976, 1978). Castration decreases the enzyme activity in the hypogastric ganglion, and T reverses this effect (Hamill et al., 1984). Some authors have correlated these effects with changes in the mass of tissue in relation to the innervation of the organs. Thus, castration not only results in marked atrophy of the ventral prostate, but also decreases the NE content and TH activity of the gland. T reverses this effect (Rastogi et al., 1977). Oviducts obtained from ovariectomized rabbits exhibit decreased levels of NE and TH activity, and these effects are also reversed by estradiol and/or

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progesterone (Marshall, 1981). Since these results show a correlation between TH activity and NE levels, TH activity has been considered the principal factor governing NE availability for synaptic functions. However, experimental and physiological circumstances exist in which dopamine-β-hydroxylase (DβH) activity correlates better with NE levels (Belmar et al., 1983; Cooper et al., 1986), suggesting that DβH, which catalyzes the last step of NE biosynthesis, may represent, under some conditions, a regulatory step. DβH is also used as a biochemical marker of the vesicles that store NE (Viveros, 1976).

Together with NE levels and TH activity, DβH can be used as marker of noradrenergic function in organs that receive sympathetic innervation. However, in many organs—especially those affected by the endocrine status of the animal—the functional significance of their adrenergic innervation, as well as the mechanism underlaying hormone-induced changes in NE levels, remain unknown. We have previously found that castration not only decreases the NE content but also decreases the storage capacity and the release of the neurotransmitter in the rat vas deferens (Lara et al., 1985). These results showing that T affects two processes related to the regulation of NE tissue levels have prompted us to study the influences that this hormone exerts on TH activity and its relation with NE levels. We now present evidence that, after a long period of castration and/or T treatment, DβH rather than TH is principally involved in the mechanism by which T regulates NE levels in the rat vas deferens.

## MATERIALS AND METHODS

### *Animals and Experimental Groups*

Sprague-Dawley rats with body weights of 250 g were used to organize experimental groups, 6 animals each. Groups were maintained in a temperature-controlled room (21°C) with a 12L:12D cycle and free access to water and food. The following experimental groups were used: a) Castrated groups, which consisted of rats castrated under pentobarbital anesthesia (33 mg/kg weight/l, i.p.); these animals were killed at different times: 5, 10, 15, 20, and 40 days post-operation. b) Sham-operated rats, used as controls of castrated rats. Sham-operation was performed at the same time and under the same conditions as for the castrated group, but only the testis was exposed. Both castrated and sham-operated animals were studied in parallel. c) Rats treated with testosterone: starting 10 days after the operation, castrated and sham-operated animals were treated, in parallel, with a dose of testosterone propionate (8 mg per kg weight) according to Lara et al. (1985). Animals were treated during 2, 5, 10, and 20 days before they were killed. d) Impact rats, not subjected to any surgical manipulation, to characterize the initial values for noradrenergic marker activities or levels and the reproducibility of enzymatic activity measurements.

### *Tissue Extracts*

Vas deferens were rapidly removed, cleaned from adherent tissue, weighed, and then homogenized in ten volumes of acetate buffer 0.1 M, pH 6.1, containing Triton X-100, 0.2% v/v, in a glass-glass homogenizer at ice-water temperature. The tissue extract was centrifuged (20,000 × *g*, 10 min) and the supernatant was used for the biochemical determinations.

### *Assays*

TH was assayed according to Waymire et al. (1971) by measuring the $^{14}CO_2$ delivered from L-1-$^{14}$C-tyrosine after hydroxylation by TH and subsequent decarboxylation obtained by addition of an extract of dopa decarboxylase (DDC). This enzyme was purified from the pig kidney cortex (Waymire et al., 1971). A 50-μl aliquot of tissue supernatant was brought to a final volume of 0.5 ml with a solution containing, in final concentration: 0.1 M sodium acetate buffer, (pH 6.1), 0.1 M ferrous sulphate, 40 mM 2-mercaptoethanol, 2 mM sodium phosphate; 10 μM pyridoxal phosphate, 25 μl DDC purified extract, and 0.25–1.0 mM of 6-methyltetrahydrobiopterine (6-MPH$_4$). The reaction at 37°C was stopped after 15 min by adding 0.5 ml of 10% trichloroacetic acid. The $^{14}CO_2$ liberated was absorbed in Whatman No. 1 filter paper (Whatman, Clifton, NJ), containing NCS solubilizer (Amersham/Searle, Arlington Heights, IL), which was added to a tube with scintillation mixture PPO (2, 5-diphenyloxazole, New England Nuclear, Boston, MA) 4 g/l and POPOP (1,4-bis [5-phenyl-2-oxazolyl] benzene, New England Nuclear) 0.5 g/l, both dissolved in toluene. Counting efficiency was 85% in a Nuclear Chicago Scintillation Counter, Mark II (Nuclear Chicago Co., Des Plaines, IL); 50 μl homogenization buffer was used as blank. Enzymatic activity was expressed

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as pmoles of $CO_2$ formed per minute. Kinetic constants of TH were obtained by linear adjustment according to the method of Lineweaver and Burk (Cantarow and Schepartz, 1962). Recovery of the assay (70%) was controlled with a crude TH preparation from bovine adrenal medulla. Duplicated samples were always used for the assay; the inter- and intraassay variations were evaluated according to Snedecor (1966). Intraassay variation was 9.8% and interassay variation was 12%.

DβH activity was assayed according to the method of Kirshner et al. (1972) modified by Lara et al. (1985). $^3$H-Tyramine (sp. act., 6.7 Ci/mmol) was used as substrate and $^3$H-octopamine formed by hydroxylation was oxidized to p-hydroxybenzaldehyde, which was extracted by toluene and added to a scintillation mixture. During the assay, optimal concentrations of $Cu^{2+}$ (0–5 μM) and p-chloromercurybenzoate (50 μM) were used. The scintillation mixture consisted of 10 ml toluene containing 4 g/l PPO and 0.1 g/l POPOP. Counting efficiency was 35%. Recovery of DβH activity from the assay averaged 98% and was determined by using a purified preparation of the enzyme (Sigma D-1983, Sigma Chemical Co., St. Louis, MO). Duplicate samples were always used for the assay. Assay precision was evaluated by calculation of intra- and interassay variance: they were 4.1% and 5.1%, respectively. All results were corrected for efficiency and quenching. Boiled samples were used as blanks. DβH activity was expressed as pmoles of product formed per min.

NE was determined according to Campuzano et al. (1975) after adsorption on alumina of the acid supernatant fraction that resulted from protein precipitation with 0.4 N perchloric acid. NE values were expressed as micrograms (μg) and results were corrected for recovery as determined by NE used as internal standard.

Proteins were determined according to Lowry et al. (1951), using serum albumin as standard.

### *Statistical Analysis*

Results were expressed as mean value ± standard error. The significance of differences between two means was evaluated by using the two-tailed Student's *t*-test. Multiple data were analyzed with the one-way analysis of variance (ANOVA) followed by the Duncan multiple range test. A value of $p<0.05$ was considered significant.

## RESULTS

### *Effects of Castration and T Treatment on the Weight, NE Content, and TH Activity Per Pair of Rat Vas Deferens*

After castration, the weight of the vas deferens declined steadily (Fig. 1A). At 40 days post-castration, the mean value of the organ weight was 33% of that of controls. NE content decreased rapidly; lowest values were found on Day 5 after castration (43%). TH activity, however, decreased steadily throughout the whole period studied after castration and at 40 days was 25% of that of controls. Treatment of castrated rats with T reversed the effect of castration on the weight of the vas deferens, which after 20 days of treatment was similar to that of sham-operated animals (Fig. 1B). NE content changed in a bimodal way. After 2 days of T administration, it increased rapidly to decrease until Day 10 and increase after 20 days of T treatment. TH activity showed a slight increase after Day 2. However, no further significant change in enzyme activity was observed.

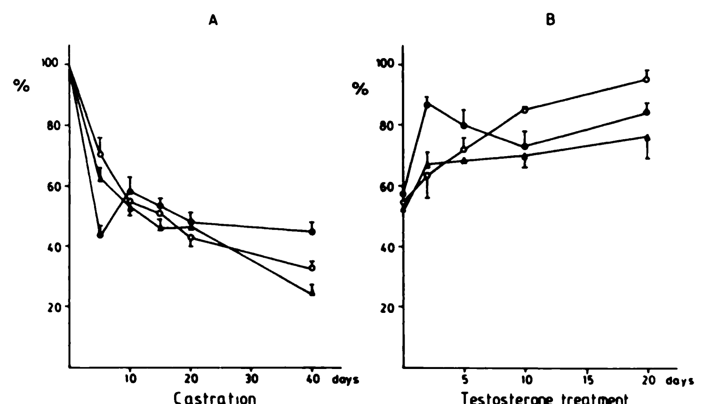


FIG. 1. Effect of castration and testosterone treatment on the rat vas deferens weight (○), norepinephrine content (●), and tyrosine hydroxylase activity (△). *A)* Effects of castration. *B)* Effects of testosterone treatment on castrated animals 10 days post-operation (weight/pair vas deferens, 62 ± 1 mg; norepinephrine 0.68 ± 0.06 μg/pair vas deferens; tyrosine hydroxylase activity 177 ± 8 pmoles $CO_2$/min/pair vas deferens). Results are expressed as percentage of the values found in sham-operated rats. The values of these parameters in intact controls at the beginning of experimental treatment were weight/pair vas deferens, 107 ± 3 mg; norepinephrine 1.30 ± 0.08 μg/pair vas deferens; tyrosine hydroxylase activity 271 ± 14 pmoles $CO_2$/min/pair vas deferens. Data represent mean ± SEM of 4–6 experiments.

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*Effect of Castration and T Treatment on TH Activity and NE Level Per Gram of Tissue of Vas Deferens and Per Pair of Organs*

As a consequence of aging, the weight of the vas deferens from sham-operated animals was significantly increased at the end of the observation period. Conversely, at the end of a similar period, the organ weight of castrated animals was smaller than that of sham-operated or intact controls rats. Therefore, both NE and TH activity might be under general trophic effects of T, which might affect the mass of tissue rather than the nervous system. To study the relationship between NE content and TH activity with the total mass of the tissue, we also expressed our results per gram wet tissue and per pair of organs.

NE concentration (μg/g tissue) in sham-operated animals remained more or less constant during this period; at Day 40, it was significantly lower than in castrated animals (Fig. 2A). When 10-day-castrated rats were treated with T, both NE concentration and NE content (μg per pair of organs) stabilized near the values of the sham controls—also treated with T (Fig. 3A).

TH activity (nmol/g tissue) of sham-operated animals increased throughout the observation period; at Day 40, it was significantly higher than initial values (Fig. 2B). In castrated animals, a similar increase of the enzyme activity per gram of tissue was also observed up to Day 20. TH activity per gram of tissue decreased in the sham-operated animals up to Day 5 of T treatment and then returned to the initial values. In 10-day-castrated animals, the inhibitory effect was observed up to 10 days of injections. On Day 20, although the enzyme activity in both groups was slightly increased, the values observed in cas-

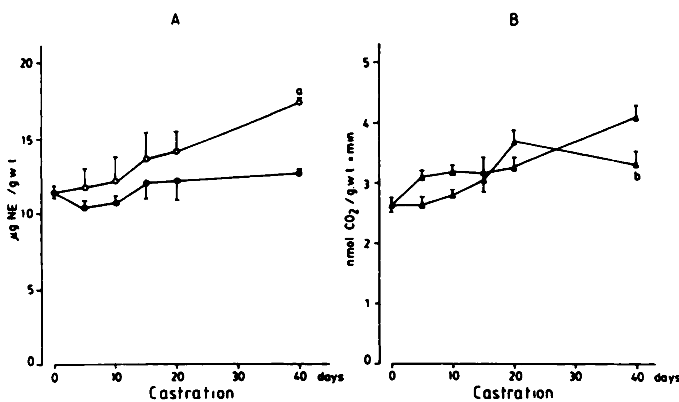


FIG. 2. Effect of castration on norepinephrine (*NE*) concentration (*A*) and on tyrosine hydroxylase activity per gram of tissue (*B*) of rat vas deferens. Values of sham-operated rats are shown for norepinephrine (●) and tyrosine hydroxylase (▲); and for castrated animals for norepinephrine (○) and tyrosine hydroxylase (△). Results represent mean values ± SEM of 4–6 experiments; ANOVA analysis and test post hoc indicate a significant difference: *a*, compared with all the values obtained from sham-operated rats; *b*, from sham-operated animals, after 40 days post-operation.

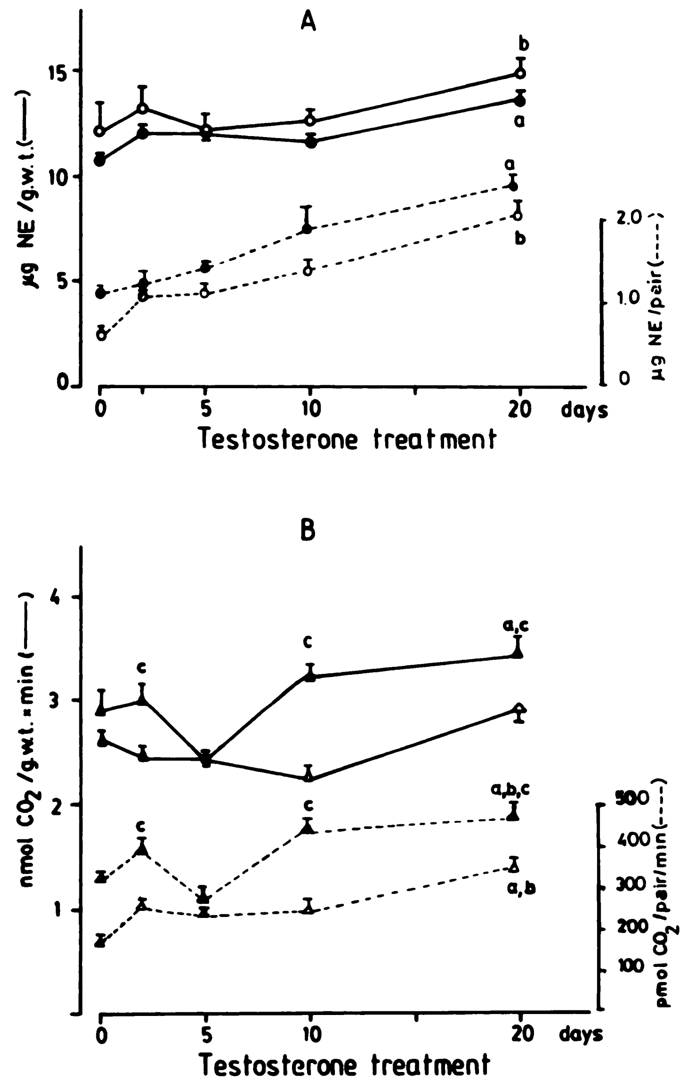


FIG. 3. (*A*) Effect of testosterone on norepinephrine (NE) concentration (*continuous lines*), on norepinephrine content per pair of organs (*discontinuous line*), in vas deferens of sham-operated rats (*black circles*) and castrated rats 10 days after operation (*open circles*). (*B*) Effect of testosterone on tyrosine hydroxylase activity per gram of tissue (*continuous line*) and per pair of organs (*discontinuous line*) in vas deferens of sham-operated rats (*black triangles*) and castrated rats 10 days after operation (*open triangles*). Results are expressed as the mean ± SEM of 4–6 experiments; ANOVA analysis and test post hoc indicated: *a*, significant difference from sham-operated rats after 10 days of the operation; *b*, significant difference from castrated rats after 10 days of operation; *c*, significantly different from castrated rats (10 days of castration) and after 2, 5, 10, and 20 days of testosterone treatment.

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trated-treated animals were under the values of sham-operated-treated animals (Fig. 3B). In both groups of animals, the enzyme activity per pair of organs was slightly increased after 2 days of T treatment. In castrated animals, this activity remained unchanged, to the end of the treatment period. However, in sham-operated animals, the hormone induced an increase of the enzyme activity per pair of organs and, on Day 20, the TH activity was higher than that observed in castrated rats.

### *Effect of Castration and T Treatment on the Kinetic Properties of TH*

To study the apparent kinetic parameters of TH, the Lineweaver and Burk linearization method (Cantarow and Schepartz, 1962) was used. The results obtained after 10 days of castration and 10 days of hormone treatment to castrated rats (10 days of castration) are shown in Figure 4. They are compared with those in sham-operated animals. The apparent $K_m$ values in the experimental groups were similar. Apparent $V_{max}$ values of sham-operated rats were higher than those from castrated animals injected with T.

These results and those obtained from groups studied at different periods are summarized in Table 1. The apparent $K_m$ was not modified by castration. In contrast, the apparent $V_{max}$ value obtained for castrated animals decreased up to 10 days but increased after 20 days of castration. T administration to castrated animals did not modify the apparent $K_m$ values, except after 20 days where they were significantly lower than those found in sham-operated animals. After 5 days of T administration to castrated rats, a significant decrease of apparent $V_{max}$ was observed as compared with the 15-day-castrated rats. After 5 additional days of T treatment, the apparent $V_{max}$ was significantly decreased as compared with the equivalent group of castrated rats (20 days after castration). At Day 20 of hormone treatment, the apparent $V_{max}$ values were even lower than those found in the corresponding sham-operated rats

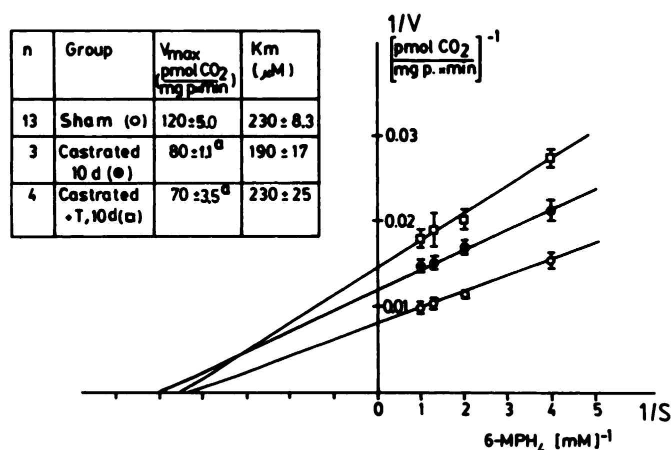


FIG. 4. Tyrosine hydroxylase activity, with respect to 6-$MPH_4$, in the vas deferens of sham-operated rats (*open circles*), castrated rats after 10 days of the operation (*black circles*), and castrated rats treated during 10 days with testosterone (*open squares*) (L-tyrosine concentration of 0.06 mM). *V* represents pmoles $CO_2$/min/mg protein (*P*) of the organs. The *inset* shows the kinetic parameters calculated from the *figure*. The *number* of experiments is indicated between *brackets*. Data from the *table* and *figure* represent mean values ± SEM, *a*, $p<0.05$ for the difference from sham-operated rats.

TABLE 1. Kinetic properties of tyrosine hydroxylase from rat vas deferens of sham-operated, castrated, and castrated rats treated with testosterone (T) (values represent mean ± SEM).

| Experimental group | Cofactor (6-$MPH_4$)* Apparent $V_{max}$ (nmoles $CO_2$/mg P/min) | Apparent $K_m$ (μM) |
|---|---|---|
| Sham-operated | 120 ± 4.99 (13)§ | 230 ± 8.32 (13) |
| Castrated | | |
| 5 days | 94 ± 1.15[b] (3) | 190 ± 11.54 (3) |
| 10 days | 80 ± 1.15[b] (3) | 190 ± 17.32 (3) |
| 15 days | 104 ± 4.47[b] (5) | 330 ± 31.30 (5) |
| 20 days | 150 ± 1.50[d] (4) | 250 ± 25.00 (4) |
| 40 days | 110 ± 4.61[b] (3) | 260 ± 46.18 (3) |
| Castrated† + T | | |
| 2 days of T | 100 ± 6.50[b] (4) | 200 ± 10 (4) |
| 5 days of T | 85 ± 3.00[bc] (4) | 200 ± 10 (4) |
| 10 days of T | 70 ± 3.50[abcd] (4) | 230 ± 25 (4) |
| 20 days of T | 86 ± 6.50[bcd] (4) | 190 ± 15[d] (4) |

*Kinetic constants with respect to 6-$MPH_4$ were determined using concentrations of the cofactor of 0.25–1 mM at a tyrosine concentration of 0.058 mM.

†Animals were used after 10 days of castration.

§Number in parentheses = number of experiments.

[a–e]ANOVA analysis followed by the Duncan test post hoc gives the following significant differences ($p<0.05$): a, from 15-day-castrated rats; b, from 20-day castrated rats; c, from 40-day-castrated rats; d, from sham-operated rats.

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TABLE 2. Effect of castration and testosterone (T) on norepinephrine level, tyrosine hydroxylase and dopamine-β-hydroxylase activities and weight of the rat vas deferens (values represent mean ± SEM of 4 experiments).

| Experimental group | Vas deferens weight (mg) | Norepinephrine (μg/pair) | Dopamine-β-hydroxylase (pmoles/pair/min) | Tyrosine hydroxylase (pmoles/pair/min) |
|---|---|---|---|---|
| Sham, 10 days | 112 ± 3.5 | 1.17 ± 0.02 | 63 ± 6.5 | 438 ± 15.5 |
| Castrated* | 62 ± 1.0[a] | 0.68 ± 0.12[a] | 40 ± 3.5[a] | 177 ± 16.5[a] |
| Castrated* + 10 days of T | 107 ± 1.0 | 1.41 ± 0.18[b] | 330 ± 21.5[b] | 277 ± 17.6[b] |

*Ten days post-operation.

[a,b]ANOVA analysis followed by the Duncan test post hoc gives a significant difference for *a* compared with sham-operated and testosterone-treated rats and for *b* compared with sham-operated rats.

(117.0 ± 4 pmoles/mg protein/min, mean value ± SEM, n=4; $p<0.01$) or those of sham-operated rats treated with T (130.0 ± 4.5 pmoles/mg protein/min, mean value ± SEM; n=4, $p<0.01$).

### *Comparative Effects of Castration and T Treatment on NE Content, TH, and DβH Activities*

To compare the effects of castration and T treatment on NE and on the enzymes of its biosynthesis, an experimental period of 10 days was used. Besides the marked atrophy of the vas deferens observed after 10 days of castration, NE content decreased. The activities of both TH and DβH were also decreased (Table 2). In castrated animals, after 10 days of T treatment, vas deferens weight and NE levels returned to control values, but TH activity was less than half that of the controls. However, DβH activity was markedly increased and reached values 3.3 times over those of castrated and 5.2 times over those of sham-operated animals.

## DISCUSSION

In this work, we describe the effect of endocrine manipulations on the activities of the enzymes that regulate the levels and biosynthesis of NE in the rat vas deferens. These parameters were studied throughout 40 days of castration and during 20 days of T treatment. The dose of T used was similar to that we have previously shown to produce substantial changes in the storage and release of NE from vas deferens of castrated rats (Lara et al., 1985). The dose administered for 5 days resulted in plasma testosterone levels slightly higher than those of control noncastrated rats.

Since it is generally accepted that NE levels are regulated by TH activity, we were interested in examining the changes induced by endocrine manipulation on both NE levels and enzyme activity. DβH activity was also determined to compare and evaluate the specificity of the hormonal effect on the neuronal enzyme that catalyzes the last step in NE biosynthesis. We have found no data in the literature describing the temporal course of castration and T effects on TH activity in the reproductive organs of the male. Some authors have described a decrease of TH activity in the rat hypogastric ganglia after a month of castration (Hammill et al., 1984). Another group (Rastogi et al., 1977) reported a decrease in TH activity in the rat ventral prostate 7 days after castration. This effect was reversed by T. In the present work, no clear correlation was found between changes in NE content and TH activity. Although both parameters had a biphasic profile of decrease after castration, NE levels initially decrease faster than TH activity. In addition, after a longer time of castration (40 days), NE content had decreased only to 55% of control values compared with a 73% decrease in TH activity. This suggests that NE content is not simply regulated by TH activity. Indeed, castration may affect the storage and/or release of the transmitter, thereby regulating its cytoplasmic pool and, indirectly, affecting TH activity via a negative feedback mechanism. If this is the case, the T administration to castrated animals would increase TH activity only if the NE cytoplasmic pool were diminished.

T effects on peripheral reproductive organs are always related to general trophic effects that affect the mass and proteins of the tissue. As can be seen from Figure 1, castration resulted in atrophy of the organ; thus the decrease in NE content and TH

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activity associated with castration might be related to trophic effect withdrawal of this steroid-dependent trophic effect rather than to a specific alteration in catecholamine biosynthesis. To verify if this trophic effect of T alters the NE content or TH activity generally or specifically, we also studied the relationship of NE content and TH activity with changes in the weight of the vas deferens. NE concentration ($\mu$g/g wet tissue) was higher in castrated than in sham-operated rats, but TH activity, expressed per gram of tissue or per pair of organs, was not increased. On the contrary, by Day 40 after castration, its concentration was significantly lower than that of sham-operated rats. Even though NE synthesis may be decreased or unchanged, the more pronounced decrease in organ weight may explain the apparent increase of NE concentration. There are two possible effects of T on vas deferens: a) general trophic effects of testosterone affecting the mass, size, or structure including the innervation, and b) a more specific effect related to the neurotransmitter and to TH activity. In support of the first possibility, we found that NE increased and/or decreased in direct correspondence with the mass of tissue; TH activity also decreased after castration. In support of the second possibility, we found that T administration did not increase TH activity, as a general trophic effect would predict. The enzyme activity was clearly inhibited up to 10 days of T treatment. This inhibitory effect of T on TH has also been described in the hypothalamic arcuate nucleus (Brawer et al., 1986). The possibility also exists that the decreased amount of enzyme molecules present in vas deferens after castration cannot be recovered by T until new enzyme molecules arrive—by axoplasmic flow—to the nerve terminal. Whatever the case, if the castration- and steroid-induced changes in NE levels do not parallel a similar alteration in TH activity, an additional intraneuronal mechanism must exist that regulates NE levels in the vas deferens. This mechanism may involve the storage and the release of the neurotransmitter. In support of this view, we found that although T inhibited TH activity, D$\beta$H, the enzyme that catalyzes the final step in NE biosynthesis, was greatly stimulated. This may have allowed a partial recovery of NE content (independent of TH activity) because D$\beta$H not only participates in NE biosynthesis but also is a constitutive protein of the membrane of NE storage vesicles. Using D$\beta$H activity as a biochemical vesicle marker, we previously demonstrated that T induces, in castrated rats, an increase in the storage capacity and in the number of storage vesicles (Lara et al., 1985), both of which result in an increase in the endogenous NE pool. It is noteworthy that NE content (see Fig. 1) after castration exhibits the same pattern of decrease observed by Glowinsky et al. (1971) in the central nervous system after blockade of TH activity with alfa-methyl-p-tyrosine. These authors concluded that catecholamines are stored in two pools with different turnover rate, the smaller pool being faster. If this is the case for the rat vas deferens, we can also hypothesize that castration preferentially decreases the pool of NE with a higher turnover rate. Conversely, T would increase the same storage pool. This would mean that, in a first stage following T treatment, the storage of NE rather than its synthesis would be increased as we have previously demonstrated (Lara et al., 1985).

The inhibitory effect of T on TH activity was also evident when we studied the kinetic properties of the enzyme. The calculated apparent $K_m$ for the cofactor 6-methyl-tetrahydrobiopterin (6MPH4) was similar to that obtained in rabbit oviduct, guinea pig brain, and vas deferens from rats and mice (Ames et al., 1978; Kennedy and Marshall, 1978). Since the affinity of the enzyme for the cofactor did not differ between the different groups examined, changes in TH activity obtained after the hormonal manipulation could be best explained in terms of the apparent $V_{max}$, suggesting a change in the number of enzyme molecules. This change was similar to that obtained by Kennedy and Marshall (1978) in oviducts from ovariectomized rabbit. These authors found a clear increase in apparent $V_{max}$ after administration of estrogen or estrogen-plus-progesterone; unfortunately, since these authors expressed their results as activity per pair of oviducts, a general trophic effect of the hormone cannot be ruled out. Our results with a T treatment beginning after 10 days of castration showed no stimulatory effect of the hormone on TH activity. Instead, this activity was inhibited since the apparent $V_{max}$ values after 5 days of treatment were even lower than those of control, castrated rats. This decreased TH activity could explain why, after 20 days of hormone treatment, NE levels were still lower than those observed in sham animals (Fig. 1B). Should TH activity correspond to a decreased number of active enzyme molecules, and high D$\beta$H activity to

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an increased number of NE storage vesicles, a normalization of the release process would tend to occur.

Altogether these data further support the view (Belmar et al., 1983; Lara et al., 1985) that in organs with low turnover rate, such as the rat vas deferens, the mechanism regulating NE levels does not depend exclusively on the regulation of TH. A comparative analysis of T effect of NE, TH activity, and DβH activity reinforces the idea that there would be a differential hormone effect on the biosynthetic enzymes of the neurotransmitter: while TH is inhibited, DβH is strongly stimulated by the steroid. As a result of this balance, NE levels can be maintained in relation to the mass of tissue. On physiological grounds, the availability of NE for release and for subsequent action on postsynaptic receptors can be maintained by at least two mechanisms, one dependent on the biosynthetic pathway regulated by TH activity and the other (which can be stimulated preferentially by testosterone) dependent on the presence of enough vesicles to store NE (resulting from biosynthesis and/or uptake) necessary for the releasing process. The occurrence of both mechanisms could explain why the rat vas deferens presents one of the highest levels of NE when compared to other organs receiving sympathetic innervation. Because of its low turnover rate, only a fraction of the total NE (the portion affected by T) would be necessary to maintain normal noradrenergic neurotransmission, thus adding a high degree of control to the sympathetic regulation of the rat vas deferens.

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