Castration increases and androgens decrease nitric oxide synthase activity in the brain: Physiologic implications

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Sex differences in nitric oxide synthase (NOS) activity in different regions of the rat brain and effects of testosterone and dihydrotestosterone (DHT) treatment in orchidectomized animals were investigated. Regional but no sex differences in NOS activity were detected in gonadectomized animals. Orchidectomy significantly increased NOS activity in the hypothalamus, ''amygdala,'' and cerebellum but not in the cortex. In the hypothalamus, the increase in NOS activity after castration and its reversal by androgen treatment was mimicked by changes in neuronal NOS mRNA level. In contrast, androgen receptor (AR) mRNA level in the hypothalamus was slightly reduced by castration and increased by treatment with DHT. Again in the hypothalamus, the increase in NOS activity in castrated rats was accompanied by an increase in the number of neuronal NOS+ cells determined immunohistochemically, whereas androgen treatment prevented this increase. The changes in NOS+ neurons correlated with the changes in the number of AR+ cells to a degree. Overlap of AR in NOS+ cells was not present in the regions of the hypothalamus analyzed. These results indicate that testosterone or, most likely, its metabolite DHT down-regulates NOS activity, mRNA expression or stabilization, and the number of neuronal NOS+ neurons.

Nitric oxide (NO) synthesized by the enzyme neuronal nitric oxide synthase (nNOS) plays an important role in many brain functions. These include effects on long-term potentiation (1), gonadotropin secretion (2–10), and sexual behavior (11–13). NO functions as a neurotransmitter (2–10) and nNOS is present near gonadotropin-releasing hormone terminals (14) and in regions of the brain that appear to regulate emotional behaviors (15, 16). Estradiol (E_2) up-regulates endothelial NOS in endothelial cells (17, 18) and nNOS in the brain (18–21), and many of the actions of E_2 on the brain have been suggested to be through a NO-mediated mechanism (2–10, 18–22). Administration of E_2 and progesterone to ovariectomized rats leads to an increase in the luteinizing hormone surge, the magnitude of which is significantly attenuated after administration of nNOS antisense oligonucleotides into the third ventricle (22), suggesting an intermediary role of NO in modulating some actions of E_2 by up-regulating nNOS activity.

However, the actions of androgens on NOS activity in the brain are not clear. Male mice with targeted disruption of the nNOS gene ($nNOS^-$) display a great increase in aggressive behavior and excessive and inappropriate mounting behavior (11). Because androgens also promote aggressive behavior (23, 24), facilitate mounting behavior (25, 26), and decrease gonadotropin release (27, 28), we wanted to elucidate whether the androgens, testosterone (T) and its 5α reduced metabolite, dihydrotestosterone (DHT), modulate nNOS activity in the brain and the potential mechanism(s) by which this may occur.

Therefore, sex-related and region-specific distributions of NOS in select regions of the rat brain of both sexes were assessed as well as the effects of castration and hormone replacement in the male. Our primary focus was on evaluating the effects of androgens on nNOS in the brain of male animals, as there is already literature on the effects of E_2 on nNOS in females (18–21). Four regions of the brain were evaluated for NOS enzymatic activity. They were the cerebellum because it is rich in nNOS (15, 18) but has relatively few androgen receptors, and a section of the hypothalamus including the posterior component of the sexually dimorphic nucleus of the preoptic area (SDN-POA), a markedly sexually dimorphic nucleus of the rat hypothalamus (29), which presumably plays an important role in the regulation of both masculine and aggressive behavior (30). Also included was the region of the brain lateral to the hypothalamus, which included part of the amygdala, which also has been implicated in the control of emotional behavior (31). The dorsal cerebral cortex, although implicated in the control of sexual behavior (32), has few gonadal hormone receptors and served as a control region. We also evaluated whether the effect of DHT on nNOS activity was evident at the level of mRNA expression and finally whether it was exerted directly on nNOS+ neurons via the androgen receptor (AR).

Although no $nNOS^-$ model is currently available in rats, this species was studied as numerous investigators have elucidated the actions of gonadal hormones on rat brain function. In addition and perhaps surprisingly, the mouse does not have a demonstrable SDN-POA, at least in thionin-stained sections (33).

Materials and Methods

Chemicals. The following chemicals were purchased from Sigma: L-arginine HCl, L-citrulline, pepstatin A, leupeptin, sodium acetate, calcium chloride, sodium chloride, magnesium chloride, tris-base, sodium citrate, PMSF, EDTA, EGTA, NADPH, FMN, FAD, DL-DTT, triethanolamine hydrochloride, tetrahydrobiopterin, and calmodulin. Dowex AG 1-X8 and Dowex AG 50WX8 resins were purchased from Bio-Rad. [^{3}H] testosterone and [^{3}H] DHT were purchased from NEN. Biotinylated anti-rabbit and anti-mouse IgG were from Vector Laboratories. Oligonucleotide primers were obtained from GIBCO/BRL. Polyclonal and monoclonal nNOS antibodies were purchased from Transduction Laboratories (Lexington, KY), and the AR antibody (monoclonal PG21–25A) was obtained from Gail Prins, University of Illinois, Chicago.

Animals. Adult Sprague–Dawley rats weighing 180–200 g were purchased from Charles River Breeding Laboratories. They

Abbreviations: NOS, nitric oxide synthase; nNOS, neuronal NOS; E_2, estradiol; T, testosterone; DHT, dihydrotestosterone; SDN-POA, sexual dimorphic nucleus of the preoptic area; pSDN-POA, posterior SDN-POA; AR, androgen receptor; IHC, immunohistochemistry; RT-PCR, reverse transcription–PCR.

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were housed under conditions of controlled temperature and light cycle (12/12) and provided free access to food pellets and water. All experimental procedures were approved by the University of California, Los Angeles Animal Use Committee. Male and female rats were gonadectomized under ether anesthesia. In some cases, at the time of gonadectomy male rats were implanted s.c. on the back with 20- or 60-mm silastic capsules filled with crystalline T or DHT or left empty (blank) as a control procedure. Before surgical implantation, the capsules were incubated overnight in physiological saline. These males were sacrificed 20 days after implantation of the capsules at which time 2–3 ml of blood was obtained for the measurement of plasma T and DHT levels by RIA.

Brain Studies. At the time of sacrifice, animals were anesthetized with ether and subsequent procedures varied depending on whether the brain samples were to be assayed for NOS activity or mRNA level or to be subjected to immunohistochemical analysis.

For the enzymatic assays the brains were removed after euthanasia of the animals and a 1-mm thick slice was obtained as described (34). This slice then was dissected on a slide on ice into five fragments: a horizontal cut was placed below the anterior commissure extending to its lateral extent. Two vertical cuts at that point created a bilateral hypothalamic fragment containing the SDN-POA including the central component of the medial preoptic nucleus (35). The horizontal cut then was extended to the lateral margin of the brain creating left and right fragments that were combined and labeled "amygdala" although they contained other regions such as the pyriform cortex. A horizontal cut above the corpus callosum produced bilateral samples labeled cortex that also were combined. The remaining tissue was discarded. The samples were frozen in crushed dry ice and stored at −70°C until assayed. In some cases the entire cerebellum also was collected and frozen. Three assays of nNOS activity were conducted on different pools of samples, each from three animals of the same treatment group. For mRNA evaluation additional animals were treated as described above but only the hypothalamic fragment was studied.

For the immunohistochemical studies, deeply anesthetized rats were perfused via the left ventricle with PBS followed by 10% formalin in PBS. The brains were embedded in paraffin and sectioned at 16 μm in the plane described by Dodson and Gorski (34). The paraffin ribbons were dried at 70°C for 10–15 min and stored at room temperature. Every fifth section was removed from the paraffin ribbon and stained with thionin by routine histological procedures. These stained sections then were examined to identify the cell dense posterior SDN-POA (pSND-POA). Depending on the location of the pSDN-POA, two adjacent sections from the paraffin ribbon were mounted on slides coated with poly-L-lysine either anterior or posterior to the reference section and used for immunohistochemistry (IHC).

Assay for NOS. Homogenates of the different regions of the brain were prepared in 50 mM triethanolamine-HCl (pH 7.4) containing 0.1 mM EGTA, 0.1 mM EDTA, 0.5 mM DTT, 1 μM pepstatin A, and 2 μM leupeptin at 4°C. The homogenates were centrifuged at 20,000 *g* for 60 min at 4°C, and the supernatants were assayed for [^{3}H]-L-arginine to [^{3}H]-L-citrulline conversion and calcium-dependent NOS activity was measured as described (36).

RIA for T and DHT. Known amounts of [^{3}H]-T and [^{3}H]-DHT were added to 0.6–0.8 ml of plasma, and the assays were performed as described (37, 38).

Reverse Transcription–PCR (RT-PCR) and Southern Blot Analysis. Total cellular RNA (3 μg) was used for RT-PCR analysis of nNOS and AR by using the set of primers and conditions as described in Table 2, which is published as supplemental data on the PNAS web site, www.pnas.org. PCR amplifications were performed in a 100-μl vol for 35 cycles for nNOS and AR and 30 cycles for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). PCR products (nNOS, 602 bp; AR, 524 bp; GAPDH, 558 bp) were size-fractionated on 1.5% agarose gel and transferred to nitrocellulose membranes. Southern blots were hybridized with [α-^{32}P]dCTP-labeled respective cDNA probes.

IHC. Stored brain sections containing the SDN-POA were rehydrated in distilled water and placed in citrate buffer (10 mM, pH 6.0) and cooked in an 800-W microwave at high power for 5 min twice and allowed to cool for at least 20 min in citrate buffer and then washed in distilled water. Slides were placed in 1% H_2O/methanol for 20 min in citrate buffer (10 mM, pH 6.0) and washed in running tap water for 2–3 min and placed in phosphate buffer (pH 7.4). Polyclonal nNOS antibody (dilution 1:20) was placed in normal goat serum for 20 min; AR antibody (dilution 1:50) was placed in normal horse serum for 20 min. The horse and goat serum were decanted, and sections were covered with primary antibody, diluted in PBS, incubated overnight at room temperature in a humidity chamber, and then washed with PBS. For nNOS immunostaining, the slides were incubated in biotinylated anti-rabbit IgG for 30 min. The slides were incubated with antibody conjugated for 45 min followed by avidin-biotin complex for 30 min. The slides then were incubated in diaminobenzidine for 2–3 min, washed in running tap water for 2 min, stained with hematoxylin, washed in tap water, dehydrated, and mounted on slides.

Quantitative analysis of the IHC slides was performed by one investigator blind to the treatment groups. Samples from only one side of the brain were first drawn on specially constructed graph paper by using an overhead projector. The graph paper consisted of rectangles, which corresponded to the grid on the eyepiece of a Zeiss microscope at ×200. In the initial tracings, obvious landmarks (blood vessels, artifacts, and neuronal structures) were included to minimize the possibility of counting labeled cells twice. The counting field was always started at the base of the third ventricle and moved up one grid at a time until the base of the anterior commissure was reached. The microscope stage then was moved one grid lateral, and counts were continued to the base of the brain and in this manner ultimately extended to the lateral margins of the brain. These data represent labeled cell counts for one-half of the hypothalamus and

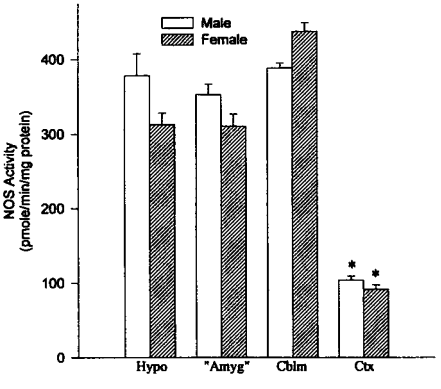

Fig. 1. Regional difference in NOS enzymatic activity (expressed as the amount of L-citrulline produced in pmol/min per mg protein) in the hypothalamus (Hypo), amygdala ("Amyg"), cerebellum (Cblm), and cerebral cortex (Ctx) of adult male and female rats 20 days after gonadectomy. *, Significantly different from other regions, same sex ($P < 0.0001$).

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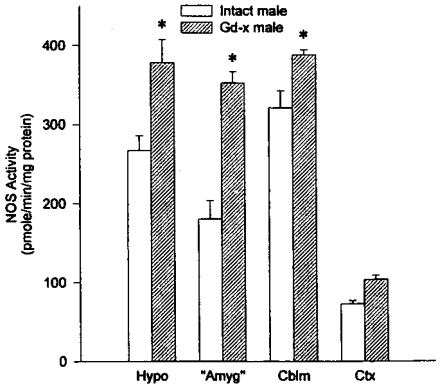

Fig. 2. Levels of NOS enzymatic activity (expressed as the amount of L-citrulline produced in pmol/min per mg protein) in four regions of the brain in sham-operated (Intact) or gonadectomized (Gd-x) male rats sacrificed 20 days after surgery. Brain samples were hypothalamus (Hypo), amygdala (''Amyg''), cerebellum (Cblm), and cerebral cortex (Ctx). *, Significantly greater than intact, same region ($P < 0.05$).

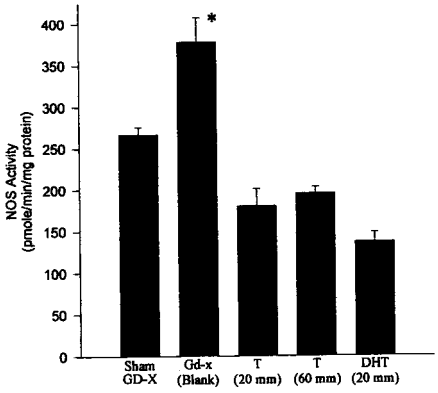

Fig. 3. Influence of empty (blank) and silastic implants filled with T (20 or 60 mm) or DHT (20 mm) on NOS enzymatic activity in the hypothalamus 30 days after gonadectomy (Gd-x) or sham surgery in adult male rats. *, Significantly increased over sham-operated controls ($P < 0.05$).

amygdala samples assayed for nNOS enzymatic activity. For the sample of cortex, the area analyzed consisted of four vertical grids descending from the dorsal surface of the cortex and extending from midline to the lateral margin of the brain.

For nNOS IHC, cells were subjectively labeled as darkly, moderately, or lightly stained, but in the final analysis the lightly stained cells were disregarded. For AR IHC cells were considered either stained or unstained.

Statistics. All values are expressed as mean ± SEM. Regional and sex differences as well as the effect of castration on NOS enzymatic activity were analyzed by two-way ANOVA. The effects of castration and hormone replacement were analyzed by one-way ANOVA. Although samples from a total of nine rats were assayed, the number of assays (three) was used for statistical analysis. Androgen levels were measured in four assays/group in pooled samples from two animals, except for the intact group in which three assays were performed. The number of assays were used for statistical analysis. The number of positive cells in the immunohistochemical evaluations of the hypothalamic samples were subjected to one-way ANOVA. The number of animals in these groups ranged from three to five. Post hoc pairwise comparisons were made by the Bonferroni method (SIGMASTAT for Windows). P values equal to, or less than, 0.05 were considered significant.

Results

Regional and Sex Differences in nNOS Activity and Effect of Orchidectomy. To avoid any potential residual activational effects of gonadal hormones, calcium-dependent NOS activity was measured 2 weeks after gonadectomy. The levels of NOS activity varied in different regions of the brain (Fig. 1). Enzymatic activity was highest in the cerebellum although not significantly different from that of the hypothalamus or amygdala. The levels were significantly lower in the cerebral cortex. No sex differences were observed (Fig. 1). Castration of male rats significantly increased nNOS activity in the hypothalamus, amygdala, and cerebellum but not in the cortex when compared with that of intact animals (Fig. 2).

Table 1. Plasma levels of T and DHT in gonadectomized (Gd-x) males after silastic implants of T or DHT

Treatment	T, pg/ml	DHT, pg/ml
Intact	2,856 ± 48	128 ± 10
Gd-x	188 ± 46*	23 ± 17†
Gd-x + 20 mm T	2,267 ± 55	104 ± 17
Gd-x + 60 mm T	4,598 ± 602‡	215 ± 29
Gd-x + 20 mm DHT	192 ± 55*	325 ± 65‡

*Significantly less than intact, same hormone.
†Significantly less than intact, same hormone (*t* test only).
‡Significantly greater than all other groups, same hormone.

Effect of Castration and Replacement with T or DHT on Hypothalamic NOS Activity and Correlation with Plasma T and DHT Levels. Castration significantly reduced plasma levels of both T and DHT. Implants of T significantly increased both T and DHT levels whereas implants of DHT significantly increased only plasma DHT levels (Table 1). The 60-mm T implants produced significantly higher than normal serum levels of T. Castration led to an increase in NOS activity in the hypothalamus whereas both T and DHT led to a significant decrease in NOS activity (Fig. 3). In the cerebellum there was no effect of 20-mm implants of T or DHT on NOS activity in castrated males (data not shown).

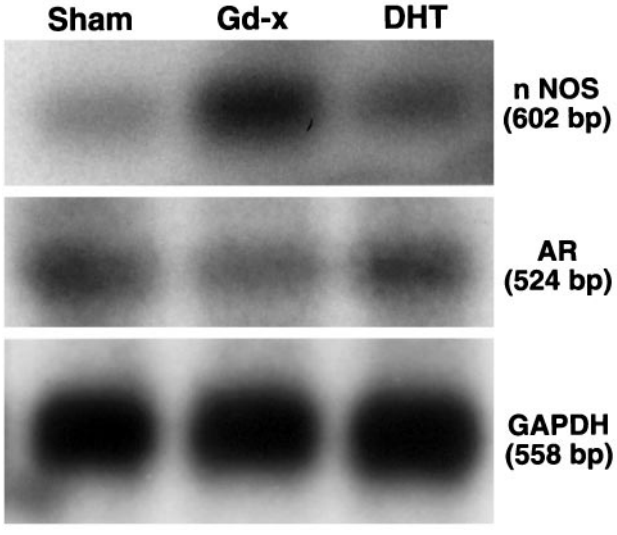

Fig. 4. Southern blot analysis of RT-PCR products in the hypothalamus of male rats subjected to sham surgery, castration (Gd-x), or castration plus s.c. implants of DHT. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

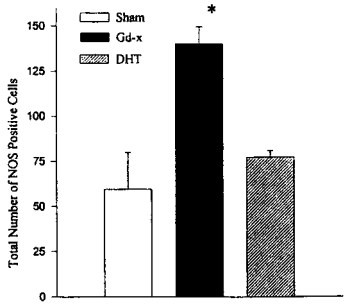

Fig. 5. Influence of gonadectomy (Gd-x) and hormone replacement with 20-mm silastic implants of DHT for 20 days on the total number of nNOS+ cells in the male hypothalamus. *, Significantly increased over sham-operated controls and DHT-treated group ($P < 0.05$).

RT-PCR Analysis. Fig. 4 clearly shows that castration led to an increase whereas DHT treatment led to a decrease in nNOS mRNA levels. Castration decreased AR mRNA level whereas DHT treatment increased it.

Quantification of nNOS+ Cells. Although orchidectomy increased NOS activity in the hypothalamus, the increase could be the result of an increase in the expression of nNOS by the same number of neurons and/or by "recruitment" of new neurons expressing NOS enzyme activity. In an attempt to differentiate between these two alternatives, the number of nNOS+ cells was determined by IHC. As shown in Fig. 5, there was a significant increase in the number of nNOS+ cells in the hypothalamus after castration and a significant decrease in the DHT-treated group. There were no changes in the samples of cortex, and no statistically significant changes in the amygdala samples although castration did increase the number of nNOS+ cells (data not shown).

Possible Overlap of nNOS+ and AR+ Cells. We next attempted to determine whether nNOS and the AR overlapped in cells by IHC in adjacent brain sections. As shown in Fig. 6, castration essentially completely down-regulated AR's expression because none were detected in the hypothalamus. Implants of DHT fully restored the number of AR+ cells.

IHC offers the opportunity for a cell-by-cell analysis unlike the enzymatic assay for NOS or mRNA expression. In an attempt to correlate these two differing levels of analysis, two regions within

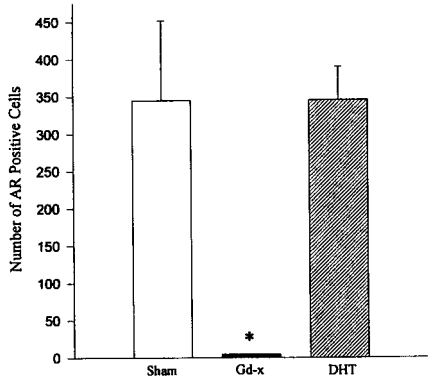

Fig. 6. Influence of gonadectomy (Gd-x) and hormone replacement with 20-mm silastic implants of DHT on the number of AR+ cells in the male rat's hypothalamus. *, The number of AR+ cells in the castrate was zero and significantly different from the other groups ($P < 0.001$).

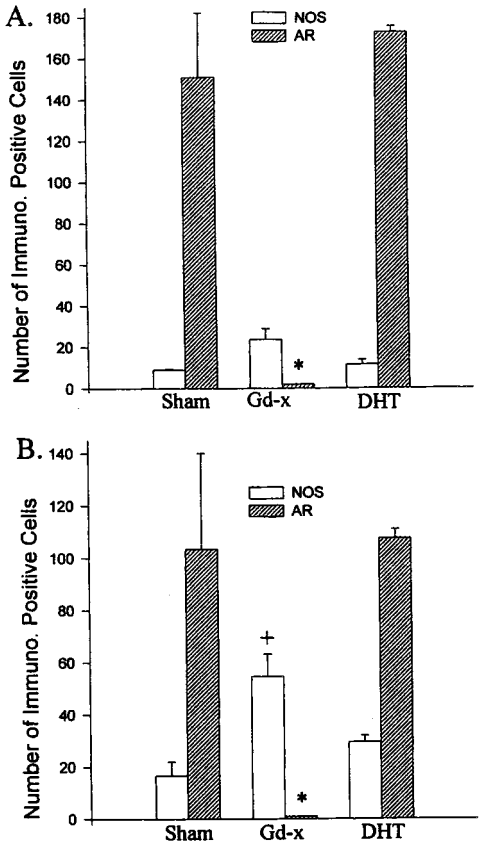

Fig. 7. Influence of gonadectomy (Gd-x) and hormone replacement with 20-mm silastic implants of DHT on the number of nNOS and AR+ cells in the pSDN-POA (*A*) and the strial region (*B*) in the male rat. *, Significantly different from the Sham and DHT groups ($P < 0.05$). +, Significantly different from the sham group ($P < 0.03$).

the hypothalamic fragment analyzed for NOS enzyme activity were subjected to a finer analysis of the IHC results. We chose two regions known to be rich in AR+ cells, which were present in the slides processed for IHC, namely the pSDN-POA and the region immediately below the anterior commissure as it crosses midline, which we labeled the strial region. The strial region included the septohypothalamic and parastrial nuclei and the ventral part of the bed nucleus of the stria terminalis (39). It was hypothesized that these regions were most likely to reflect the DHT-induced changes observed in NOS enzymatic activity in the hypothalamic tissue samples.

Three grids just below the anterior commissure extending laterally from the third ventricle were evaluated. For the pSDN-POA four grids that included these regions were evaluated for the number of nNOS+ and AR+ cells on adjacent sections. As shown in Fig. 7, there was a highly significant change in the number of AR+ cells, castration down-regulating them to zero, and a significant change in the number of nNOS+ cells but only in the sample labeled stria. (The increase in the pSDN-POA approached significance [$P < 0.08$].) It should be noted, however, that the selection of regions for analysis was made before obtaining the IHC results, and it turned out that the pSDN-POA, although quite rich in AR+ cells, had few nNOS+ cells as illustrated in Fig. 8. Because the failure to find evidence for overlap of nNOS+ and AR+ cells could have been caused by technical difficulties, we also examined the ventral premamillary nucleus where colocalization of nNOS+ and AR+ cells has been reported to be

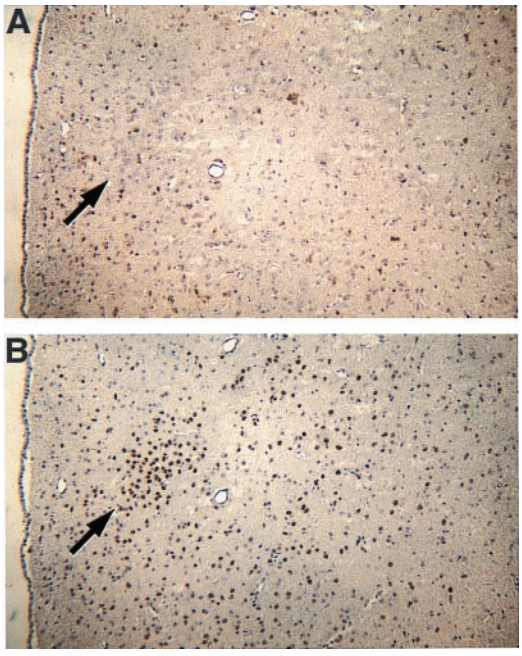

Fig. 8. Evidence for the lack of possible colocalization of nNOS (*A*) and AR (*B*) positive cells in the pSDN-POA (arrows).

extensive (40, 41). The numbers of AR+ and NOS+ cells were both high in the ventral premammilary nucleus in our tissue samples (data not shown). This supports the existence of colocalization in this region and reinforces the absence of overlap in the hypothalamus.

Discussion

NOS enzymatic activity varied with the tissue sampled. However, no sex differences were observed in NOS activity in the different regions of the brain sampled. The results indicated that NOS in gonadectomized males and females was high in the cerebellum, the hypothalamus, and the amygdala with very low amounts in the cerebral cortex. These observations are similar to those reported by other investigators for male rats and mice (42).

Orchidectomy led to a significant increase in NOS activity in the cerebellum, hypothalamus, and amygdala but not in the cortex. The increase in NOS activity was greatest in the hypothalamus and amygdala with only a slight increase in the cerebellum. These results suggested that circulating levels of androgens most likely inhibited NOS activity, and the magnitude of which varied in different regions of the brain. The magnitude of change was greatest in samples of the amygdala and the hypothalamus. Because the $nNOS^{-}$ mouse displays markedly enhanced aggressive and mounting behavior and because androgens regulate these behaviors, the observed increase in NOS activity after castration was expected. The hypothalamus and amygdala both are involved in the regulation of emotional behavior and the smaller change in NOS activity in the cerebellum and its lack of response to T or DHT may reflect its nonsexual functions and a relative paucity of ARs.

The effect of androgen is opposite to that of E_2, which increases nNOS in various regions of the brain (18–21). Immunohistochemical localization has revealed that nNOS represents the major form of NOS in the hypothalamus and is especially dense in the organum vasculosum of the lamina terminalis, medial preoptic area, and supraoptic nucleus and is more moderate in the arcuate nucleus and median eminence (16). Many of the actions of T in certain areas of the brain are mediated by its conversion by the enzyme aromatase to E_2 (43, 44) followed by binding to the estrogen receptors. Testosterone also can be converted in certain areas of the brain by the enzyme 5α reductase to DHT that combines with the ARs to exert its actions (45, 46). We therefore elected to study the effect of both T and the nonaromatizable androgen, DHT, on NOS in the hypothalamus and the cerebellum to assess whether reduction of T to DHT by 5α reductase could explain the actions of T.

Implants of T in gonadectomized males increased plasma levels of both T and DHT whereas implants of DHT only increased plasma levels of DHT but not T. Both hormones reduced NOS activity in the hypothalamus, which is very rich in ARs (47), but neither had any effect in the cerebellum. The decrease in NOS activity in the hypothalamus was similar with 20- and 60-mm implants of T, indicating that maximum inhibition was achieved by the shorter capsules. The effect of 20-mm capsules of DHT was slightly greater, although not significantly so, than the same or even higher doses of T. The similarities in the effects of T and DHT indicate that this action of T most likely is mediated by the conversion of T to DHT. Moreover, the results of the RT-PCR and Southern blot analysis of the RT-PCR products indicate that at least one mechanism of DHT action is to decrease nNOS mRNA level either by decreasing its expression or its stability.

Analysis by IHC revealed that in parallel with changes in NOS activity, the number of detectable nNOS+ cells in the hypothalamus was increased by castration and reduced by DHT replacement. A similar pattern was seen in the amygdala but did not reach statistical significance, perhaps because of the small number of animals evaluated. Orchidectomy did not increase NOS activity in the sample of cerebral cortex assayed, nor did it change the number of nNOS+ cells. Although these data do not rule out the possibility that individual neurons produce more nNOS, they are consistent with the interpretation that castration causes the recruitment of neurons to the active nNOS pool.

The present data suggest that nNOS and the AR most likely do not colocalize in the pSDN-POA but may in the strial region where AR+ cells are abundant. On the other hand, there was a high degree of overlap in the ventral premammilary nucleus similar to that reported by other investigators (40, 41). There was essentially a complete down-regulation of ARs in the hypothalamus after castration, although the effect on AR mRNA was modest. It had been reported previously that 4 days after castration AR mRNA remained elevated but decreased by 2 months after castration (48). The present result of only a modest decrease in AR mRNA level 20 days after castration is consistent with this report.

The interrelationship between the AR and nNOS containing neurons after castration and after replacement with DHT in selected areas of the hypothalamus is complex. Our results indicate that in the pSDN-POA, the number of nNOS containing neurons did not change after castration or replacement with DHT in spite of marked changes in the number of AR+ cells. Therefore, the hypothesis that changes in these specific regions at the level of IHC would reflect changes in nNOS enzymatic activity in the hypothalamic fragment was not supported by the data. Although changes in other regions contained within the hypothalamic sample could have occurred and dominated the enzymatic or IHC results for the total tissue sample, it remains possible that androgens may modulate nNOS activity by a direct action of T or DHT on nNOS+ cells, indirectly through androgen-responsive interneurons or possibly by a receptor-independent mechanism in a regionally specific manner. It is also possible that like the newly discovered estradiol β receptor (49), another subtype of AR is present that may be responsible for modulating nNOS activity by androgens in areas of the brain where the conventional AR is absent.

Our results demonstrating that DHT decreases NOS activity, nNOS mRNA, and the number of nNOS+ neurons in the hypothalamus may have important physiological implications. T is the main circulating androgen and can be aromatized in various parts of the hypothalamus to estrogens or can act directly on the AR or be converted by 5α reductase to DHT. Thus, T may act like E_2 to increase nNOS in some areas of the body and the brain where aromatase is present, whereas it would decrease nNOS in other areas where 5α reductase is present. This could explain the discrepancies observed in the actions of T in regulating NOS activity in different organs of the body. This differential regulation by androgens on NOS has been reported in rats where castration led to a decrease in NOS activity in the caput, corpus, cauda epididymidis, and penis whereas NOS activity was increased from nondetectable levels in the seminal vesicles and lateral prostate (50).

Administration of either T propionate or DHT propionate to either the wild-type or estrogen receptor α knockout male mice with very little sexual experience could induce mounting behavior (51), suggesting that this action is mediated by the AR and not via the estrogen receptor α after conversion of T to E_2. This androgen elicited sexual behavior is accentuated in nNOS$^-$ male mice in the presence of T where the males display excessive and inappropriate mounting behavior (12). In contrast, NO appears to facilitate female sexual behavior in rats (13), which suggests a possible sex difference in the role of NO in the control of sexual behavior.

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