Effects of Androgens and Estrogens on the Vasopressin and Oxytocin Innervation of the Adult Rat Brain

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Recently we reported that castration of rats eliminates vasopressin immunoreactivity in the lateral septum and other areas that appear to receive vasopressin innervation from the bed nucleus of the stria terminalis. Testosterone treatment counteracts this effect of castration. In the present study, we investigated whether this action of testosterone depends on its androgenic or estrogenic metabolites by treating long-term castrated rats with estradiol (E) and/or 5α-dihydrotestosterone (DHT) or testosterone. The brains were then processed for immunocytochemistry or radioimmunoassay. DHT did not increase vasopressin staining in the lateral septum, although it fully restored the size of the seminal vesicles. E did restore the original fiber density, but individual fibers stained more weakly than in sham-operated males. Only treatment with both E and DHT fully restored the vasopressin innervation. This pattern was also reflected in the radioimmunoassay data. The vasopressin content of the lateral septum decreased about 90% after castration but was fully restored by either testosterone or E + DHT treatment. E alone, however, was only half as effective as E + DHT. The treatments had no effect on the oxytocin content of the septum, or on the vasopressin or oxytocin content of the dorsal vagal complex. The results suggest that E mediates most of the effects of testosterone on the vasopressin innervation of the lateral septum. DHT enhances the response to E but has little effect on its own.

INTRODUCTION

The role of the hypothalamic vasopressin (VP) and oxytocin (OXT) pathways to the neurohypophysis in osmoregulation, labor and milk ejection is well understood (for recent reviews see ref. 10). There is, however, little knowledge about possible functions of the central VP and OXT pathways that innervate many areas of the central nervous system⁶,⁸,¹⁸. One approach towards filling this gap has been to study how various experimental and physiological conditions affect the peptide content of these pathways. VP pathways, for example, are influenced by dehydration, time of day, pregnancy, hypertension and passive avoidance trials (e.g. refs. 28, 33, 34, 37, 59). The strongest effects described so far, however, are caused by changing gonadal steroid levels¹³,¹⁵,¹⁷,¹⁸. Gonadal steroids affect the VP pathways differentially, depending on the origin of the fibers¹⁴,¹⁵,¹⁸,²⁶. For example, gonadectomy of adult male or female rats does not affect VP immunostaining of the projections of the suprachiasmatic and paraventricular nuclei, nor the staining of the recently detected VP cells in the dorsomedial hypothalamic nucleus and the locus coeruleus⁹,⁵³. In contrast, gonadectomy virtually eliminates VP immunoreactivity of cells and putative projections of the bed nucleus of the stria terminalis (BST) to the lateral septum, lateral habenular nucleus, diagonal band of Broca, midbrain central gray, locus coeruleus and the dorsal raphe nucleus¹⁵,¹⁸. VP fiber staining also disappears from the ventral hippocampus, amygdala and dorsomedial thalamic nucleus after castration¹⁸. The origin of the latter fibers is unknown, but they may be derived from VP cells of the medial amygdaloid nucleus, which show the same changes after gonadecto-

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my\textsuperscript{18}. Disappearance of VP staining was not observed in castrated rats that were treated with testosterone\textsuperscript{15,18}.

Testosterone can act on the brain either directly or after metabolic conversion (for reviews see refs. 27, 35). The two major metabolic pathways are aromatization to estrogen\textsuperscript{32,35} and reduction to the non-aromatizable 5α-dihydrotestosterone (DHT)\textsuperscript{12,30}. Aromatization is, for example, necessary for testosterone effects on masculine sexual behavior\textsuperscript{1,44}, while reduction appears to be involved in the negative feedback of testosterone on gonadotropin release\textsuperscript{4,43}. To determine if metabolism is important for actions of testosterone on central VP pathways, we compared the effects of testosterone with those of estradiol (E) and/or DHT on the VP innervation of the lateral septum, using both immunocytochemistry and radioimmunoassay. We also measured the VP and OXT content in two areas that receive projections from the paraventricular nucleus, viz., the dorsal vagal complex for both peptides and the septum for OXT only (cf. refs. 14, 41, 48).

MATERIALS AND METHODS

Adult male Wistar rats (WU:Cpb) were housed at 21 °C and supplied with food and water ad libitum. Lights were on from 7.00 to 19.00 h.

Immunocytochemical study

Rats were castrated at 3 months of age under Hypnorm (Duphar, Weesp, The Netherlands) anesthesia. Fifteen weeks later, they were implanted subcutaneously, under ether anesthesia, with capsules made of Silastic tubing (Talas, Ommen, The Netherlands) filled with E (1 cm; 1 mm i.d., 2 mm o.d.), DHT (3 cm; 1.5 mm i.d., 2.4 mm o.d.), testosterone (2.5 cm; 1.5 mm i.d., 2.4 mm o.d.), or no hormone (n = 5/group). Five rats received E as well as DHT capsules. The lengths and diameters of the Silastic tubing had been selected for being able to mimic the effects of naturally occurring steroids on the size of the uterus (for E) and seminal vesicles (for DHT and testosterone). After 4 weeks, all castrated rats and 5 sham-operated rats were anesthetized with Nembutal (Ceva, Paris), and 1 ml blood was collected by cardiac puncture. Plasma steroid levels were determined by radioimmunoassay according to Verjans et al.\textsuperscript{54} for DHT and De Jong et al.\textsuperscript{11} for E. The seminal vesicles were dissected and weighed after removing their contents.

The brains were fixed by perfusion with 5% glutaraldehyde in 0.1 M sodium-cacodylate buffer (pH 7.5), dissected, and cut in 50-μm thick transverse sections with a vibrotome (Oxford Instruments). The free-floating sections were stained immunocytochemically for VP (for details about the fixation and staining procedure, see ref. 18). VP fiber density within the lateral septum was estimated in coded sections by making a distinction between 4 categories of grading densities (see Table I) as described in De Vries et al.\textsuperscript{16}.

Radioimmunoassay

Twenty-nine male rats were castrated and subsequently implanted with capsules containing E, E + DHT, testosterone or no hormone as described above (n = 8, 8, 5 and 8, respectively). After 4 weeks, these rats, plus 6 intact controls, were decapitated. The brains were removed and septal tissue was obtained from transverse sections enclosing approximately the area between horizontal planes +1.2 and −0.8 (anterior–posterior positions relative to bregma, see ref. 18) by dissecting the area between the lateral ventricles. Dorsal vagal complex tissue was obtained using sections bordered approximately by horizontal planes −13.0 to −15.0 by dissecting the area lining the dorsal aspect of the medulla and extending 2 mm to ventral and 2 mm to lateral in both directions from the midline. These samples were sonified in 2 ml 0.1 N HCl and the VP and OXT content of these areas was determined by radioimmunoassay according to Dogterom et al.\textsuperscript{20,21}. In the VP and OXT assays crossreactivity with, respectively, OXT and VP was 0.01% in both cases. Intra-assay coefficients of variation were 15.7% (n = 20) for 8 pg VP and 10.2% (n = 20) for 16 pg OXT. The interassay coefficients of variation were 11.6% (n = 20) for 8 pg VP and 8.6% (n = 21) for 32 pg OXT (both peptides extracted from 2 ml). The dissection boundaries were selected so that each sample would include all of the areas of the lateral septum or the dorsal vagal complex that are heavily innervated with VP and/or OXT fibers. However, because the samples were dissected by hand, they would vary in the amount of surrounding tissue, which contains only scattered VP and OXT fibers. Therefore, the data were expressed in
pg peptide per dissected area instead of per mg protein or dry weight.

**Statistics**

The effects of the different hormonal treatments on the content of VP and OXT in the septum and dorsal vagal complex, and the influence of the various hormonal conditions on the weights of the seminal vesicles were tested by means of one-way analysis of variance, followed by the Student–Newman–Keuls multiple-range test for differences between pairs of means. Differences were regarded as significant if $P < 0.05$.

**RESULTS**

The weights of the seminal vesicles in the various hormonal groups are indicated in Table 1. Rats treated with E showed plasma levels of 50–90 pg/ml E, which are comparable to the levels of female rats in proestrus as measured with the same radioimmunoassay. DHT-treated rats had plasma levels of 0.9–1.8 ng DHT/ml. Comparable levels completely suppress LH release in males (Vreeburg, unpublished results). The size of the testosterone capsules yielded plasma testosterone levels of 2.4–3.6 ng/ml in a previous study.

Immunocytochemical staining revealed dense networks of VP immunoreactive fibers in the lateral septum of sham-operated rats, which were virtually absent in rats that had been castrated for 19 weeks. When such rats were treated with testosterone, E or E + DHT, the VP fiber density was generally as high as in sham-operated males (Table 1). Only one of the E-treated rats showed a lower fiber density (comparable to that found in intact female rats in previous studies). In contrast, DHT did not restore VP fiber staining (Fig. 1). These different steroid treatments caused similar effects in other areas where VP immunoreactive fibers disappear after gonadectomy, i.e., in the lateral habenular nucleus and the medial amygdaloid nucleus.

**TABLE 1**

**VP fiber density in the lateral septum of male rats under various hormonal conditions**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>VP fiber density</th>
<th>SV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+ + + + + + + + + 527 ± 28</td>
<td></td>
</tr>
<tr>
<td>Castrated</td>
<td>+ + + + + + + + + + + 45 ± 4*</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>+ + + + + + + + + + + + + 48 ± 3*</td>
<td></td>
</tr>
<tr>
<td>DHT</td>
<td>+ + + + + + + + + + + + 465 ± 27</td>
<td></td>
</tr>
<tr>
<td>E/DHT</td>
<td>+ + + + + + + + + + + + + 490 ± 30</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>+ + + + + + + + + + + + + 546 ± 25</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly lower than all other groups (Student–Newman–Keuls).

sent in rats that had been castrated for 19 weeks.

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Fig. 1. Photomicrographs of the lateral septum of castrated rats treated with 5α-dihydrotestosterone (A), estradiol (B) or a combination of both steroids (C). Note that, although there are no obvious differences in fiber density between B and C, the individual fibers and boutons are more weakly stained in the estradiol-treated rat than in the rat that has been treated with both steroids. Bar = 25 μm.
Although the different hormonal conditions, apart from DHT treatment, did not cause obvious differences in the VP fiber density of the lateral septum, individual fibers stained more weakly in rats treated with E alone (Fig. 1B) than in rats treated with testosterone or E plus DHT (Fig. 1C). On the basis of this feature alone, coded sections of E-treated rats could easily be distinguished from those of the others, which suggests that individual fibers contain less VP in E-treated rats. The radioimmunoassay data confirm a difference between treatment with E versus the other steroids. The different hormonal states significantly affected the VP content of the lateral septum ($F_{4,30} = 20.47, P < 0.0001$; Fig. 2). Castration reduces the VP content of the septum to 12% of the levels found in intact rats. E-treated rats had roughly 4 times higher VP levels than castrated rats but these levels were still only 41% of the levels found in intact rats. VP levels of the septum of rats treated with E plus DHT or with testosterone were not significantly different from those of the intact controls. None of the hormonal conditions significantly affected the VP or OXT content of the dorsal vagal complex or the OXT content of the lateral septum.

DISCUSSION

The major finding of this study is that the VP fibers of the lateral septum were visible in castrated rats treated with E but not in rats treated with DHT alone, even though the levels of the latter steroid were high enough to restore the size of the seminal vesicles. This suggests that under physiological conditions testosterone must be aromatized to influence VP pathways. Yet since rats that had been treated
with E plus DHT had higher VP levels in the lateral septum than did rats treated with E alone, androgen as well as estrogen receptors would seem to be involved in the effects of testosterone on the VP innervation of the brain. It might be that an effect of DHT alone on the VP pathways was prevented by rapid metabolism of this steroid by brain tissue into weaker androgens. A possible explanation for the fact that DHT was effective in the present study when it was given in combination with E could be that E prevents or retards the inactivation of DHT. Another possibility is that DHT stimulates a process that is initiated by E, such as synthesis of VP.

The finding that estrogen and androgen synergize to influence the VP innervation of the lateral septum agrees with the fact that the BST and medial amygdaloid nucleus contain many estrogen and androgen concentrating cells. It is not yet known whether the steroids act directly on the VP cells in these areas. Interestingly, the paraventricular nucleus contains OXT cells, and probably also VP cells, that concentrate estrogen. Although previously no effects were seen on the staining of the VP projections of the paraventricular nucleus, one could argue that possible differences in the levels of VP may not be observed because of ceiling effects in the immunocytochemical reaction. The present study, however, showed that possible actions of gonadal steroids on VP and OXT cells in the paraventricular nucleus are not reflected in dramatic changes in the peptide content of their projections to the septum (OXT fibers only) and dorsal vagal complex. Of course, this does not exclude possible changes in VP and OXT turnover in these projections.

The current findings may be helpful in assessing the physiological role of the VP innervation of the brain. They suggest that the VP projections of the BST, and possibly the medial amygdala, are likely to be involved in functions that are strongly influenced by gonadal hormones. It is therefore interesting to note that the lordosis response is inhibited by intrace-rebroventricular injections of VP and facilitated by VP antagonists. Although the site of action of VP is unknown in this case, it is noteworthy that the lateral septum seems to exert an inhibitory influence on lordosis behavior.

Male sexual behavior may also be influenced by VP. Injections of desglycinamide lysine-vasopressin delay the decrease in intromission and ejaculation patterns that normally occurs after castration of rats. This effect may reflect a physiological action of VP since castration of Brattleboro rats, which do not synthesize VP in their brains, eliminates intromission and ejaculation patterns almost immediately. Since intromission and ejaculatory patterns are also affected by lesioning the BST or the medial amygdala, the putative VP projections of these structures may be involved in these behaviors. Interestingly, these projections lose their immunoreactivity in a time course that is comparable to that of the decrease in male copulatory behavior following castration.

Moreover, the effects of sex steroids on the VP pathways seem to be in line with the effects of these hormones on masculine sexual behavior. This behavior is stimulated in castrated rats by the administration of E, while the effects of E are enhanced by DHT, which by itself only weakly stimulates male sexual behavior. It will, however, be important to assess how changes in immunoreactivity can be explained in terms of activity of the VP pathways.

The suggestion that putative VP projections of the BST and the medial amygdaloid nucleus are implicated in functions that are strongly influenced by gonadal hormones does not necessarily mean that the role of these pathways must be restricted to reproductive processes. Gonadal hormones affect a variety of apparently non-reproductive, neurally regulated functions (for reviews see refs. 3, 24, 51, 52). VP is thought to play a role in some of these functions, e.g., learning and memory, thermoregulation and osmoregulation. The actions of the gonadal steroids described in the present and previous experiments, however, may provide opportunities for manipulating the VP innervation of the brain. This should prove useful in designing experiments to answer questions of how VP is involved in these functions.

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