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Evidence for the Regulation of Prostatic Oxytocin by Gonadal Steroids in the Rat

LOUISA JENKIN AND HELEN D. NICHOLSON

From the Department of Anatomy, School of Medical Sciences, University of Bristol, Bristol, United Kingdom.

ABSTRACT: Oxytocin and its receptor are present in the mammalian prostate, and the peptide has been shown to increase prostatic growth, 5α-reductase activity, and contractility. This study was performed to investigate whether local concentrations of the peptide were regulated by gonadal steroids in order to establish whether oxytocin has a physiological role in the prostate. Both intact and castrated adult Wistar rats were treated daily for 7 days with either testosterone propionate or the antiandrogen cyproterone acetate. Animals were then killed, and plasma hormone and prostatic oxytocin concentrations were measured. A separate group of rats was treated with the 5α-reductase inhibitor finasteride to investigate whether testosterone or dihydrotestosterone (DHT) was involved in regulating oxytocin concentrations. In a further series of experiments, rats were treated with diethylstilbestrol (DES) or the antiestrogen tamoxifen. Treatment with testosterone significantly decreased prostatic oxytocin, whereas reduction of androgens by castration or by administration of cyproterone acetate increased prostatic peptide concentrations without altering circulating levels of the peptide. Treatment with finasteride increased plasma testosterone but decreased DHT concentrations. Prostatic oxytocin concentrations were higher in finasteride-treated animals than in control animals with comparable testosterone levels. The data suggest that both testosterone and DHT are capable of decreasing prostatic oxytocin concentrations. Treatment with DES did not significantly alter prostatic oxytocin, but administration of tamoxifen decreased concentrations of the peptide, suggesting that low levels of estrogen may be necessary for oxytocin production. These data provide evidence that oxytocin is regulated by androgens, and we hypothesize that this regulatory mechanism may be involved in controlling prostatic growth.

Key words: Testosterone, dihydrotestosterone, 5α-reductase, finasteride, estrogen, tamoxifen.

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There is growing evidence that the traditionally neurohypophysial peptide oxytocin plays a role in the local regulation of prostatic function. Immunoreactive oxytocin is present in prostatic tissue from male rats, dogs, and humans at concentrations higher than those found in the peripheral circulation (Nicholson et al, 1985; Nicholson and Jenkin, 1995). Furthermore, oxytocin messenger ribonucleic acid (mRNA) has been identified in the prostate (Einspanier and Ivell, 1997), suggesting that the peptide may be produced locally. Messenger RNA for the oxytocin receptor has also been identified in the prostate (Ivell et al, 1997), and the translated protein has been localized predominantly to the stromal tissue in the primate gland (Einspanier and Ivell, 1997; Frayne and Nicholson, 1998).

Several functions have been ascribed to oxytocin within the prostate. First, as in other areas of the reproductive tract, oxytocin has been shown to increase contractile activity. The peptide increases smooth muscle contractility in prostatic tissue from various mammalian species (Bodanszky et al, 1992), and thus the peptide may be involved in the maintenance of prostatic tone and in contraction of the gland at ejaculation. Second, oxytocin increases prostatic growth in the rat. Treatment with the peptide promotes epithelial growth in both intact and castrated rats (Popovic et al, 1982; Nicholson and Jenkin, unpublished data). In the castrated animal, this growth is due to an increase in mitotic activity and to decreased apoptosis (Plecas et al, 1992). Furthermore, the effects of the peptide are specific, since treatment with a highly selective oxytocin antagonist significantly reduces epithelial growth (Nicholson and Jenkin, unpublished data). Finally, oxytocin has been shown to modulate testicular testosterone production (Adashi and Hsueh, 1981; Frayne and Nicholson, 1995) and to effect its subsequent conversion to dihydrotestosterone (DHT) by increasing 5α-reductase activity in the testis (Nicholson and Jenkin, 1994). Similar actions have been shown in the rat prostate (Nicholson and Jenkin, 1995), and the resultant increase in local DHT concentrations may be involved in the growth-promoting effects of the peptide.

Although both oxytocin and its receptor have been localized to the prostate and specific effects of the peptide have been demonstrated here, for oxytocin to be considered a true paracrine/autocrine factor, it must be established that local regulation of the peptide occurs. The prostate is an androgen-dependent tissue with DHT as the main biologically active androgen. This androgen is im-

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Correspondence to: Dr. H. D. Nicholson, Department of Anatomy, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK.

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portant in regulating prostatic function and growth. Since oxytocin modulates local DHT concentrations, it might be expected that androgens would be involved in the local regulation of the peptide. Estrogens act synergistically with androgens in the prostate to promote growth (Moore et al, 1979; Trachtenberg et al, 1981) and have also been shown to increase oxytocin secretion from the pituitary (Amico et al, 1981). The aim of this study was to investigate the effects of androgens and estrogens on prostatic and plasma oxytocin concentrations.

Materials and Methods

Adult male Wistar rats, 250–300 g, were used in all experiments. Animals were housed under a controlled temperature (22°C) and light regime (14 hours light : 10 hours dark) and were allowed free access to food and water. All experiments were carried out under the Home Office Animals (Scientific Procedures) Act of 1986, and animals were killed by a Schedule 1-approved method.

Experimental Protocol

Experiment 1: The Effects of Androgens on Prostatic Oxytocin in the Intact Rat—Groups of seven animals were treated by subcutaneous injection daily for 7 days with either testosterone propionate (TP), 4 mg/kg body weight (Sigma Chemical Co., Poole, Dorset, United Kingdom); TP 1 mg/kg; cyproterone acetate (CPA), 4 mg/kg (Schering, Burgess Hill, West Sussex, United Kingdom), or vehicle. Animals were sacrificed on day 8.

Experiment 2: The Effects of Androgens in the Castrated Rat—Groups of seven animals were castrated using a sterile technique, under sodium pentobarbitone anesthetic (Sagatal, May and Baker, Dagenham, United Kingdom). Immediately after surgery, daily treatment with subcutaneous TP (2 mg/kg), CPA (4 mg/kg), or vehicle was commenced and continued for 7 days. A further group of seven animals was treated with oil alone; this group acted as an intact control group. Animals were sacrificed on day 8.

Experiment 3: The Effects of a 5α-Reductase Inhibitor on Prostatic Oxytocin—In order to investigate whether the effects seen in experiments 1 and 2 were caused by testosterone itself or by DHT, groups of both intact and castrated rats were treated with the drug finasteride (Merck, Sharp and Dohme, Hoddesdon, Hertfordshire, United Kingdom), which has been demonstrated to inhibit 5α-reductase activity in the rat (George et al, 1989). The following treatment groups were used: intact rats plus vehicle, intact rats plus finasteride (5 mg/kg), castrated rats plus vehicle, and castrated rats plus finasteride (5 mg/kg). All treatments were given daily for 7 days by subcutaneous injection; in the case of castrated animals, treatment commenced on the day of surgery. Animals were sacrificed on day 8.

Experiment 4: The Effects of Estrogen Treatment on Prostatic Oxytocin in the Intact Rat—Groups of seven animals were treated for 7 days with daily subcutaneous injections of either vehicle (0.1 ml corn oil), diethylstilbestrol (DES) (200 μg/kg; Sigma), or the estrogen antagonist Tamoxifen (200 μg/kg; Sigma). Animals were sacrificed on day 8.

Experiment 5: Effects of Estrogens in the Castrated Rat—Animals were castrated as in experiment 2 and treated daily from the day of surgery with either vehicle, DES (200 μg/kg), or tamoxifen (200 μg/kg). Animals were sacrificed on day 8. In all of the experiments, trunk blood was collected at the time of death for hormone assays. The ventral prostates were removed, snap frozen, and stored at −80°C until weighed, extracted, and assayed for oxytocin.

Hormone Radioimmunoassays

Luteinizing Hormone (LH)—Concentrations of LH were measured in unextracted plasma using reagents provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The tracer was prepared by the chloramine-T method (Greenwood et al, 1963), using peptide r LH-1-9. All samples from each experiment were assayed at one time.

Follicle-Stimulating Hormone (FSH)—Plasma concentrations of FSH were measured using reagents supplied by NIDDK as previously described (Nicholson et al, 1991), and all samples from each experiment were assayed at one time.

Testosterone—Testosterone and DHT were extracted from plasma samples using diethyl ether, and testosterone concentrations were measured as described by Yeung et al (1988). The limit of detection of the assay was 40 pg/ml.

DHT—Plasma DHT concentrations were measured in ether-extracted samples using a kit supplied by Amersham (Bucks, United Kingdom). The limit of detection was 62.5 pg/ml.

Oxytocin—Oxytocin was extracted from plasma and prostatic tissue using C18 ODS cartridges (SepPak, Waters Corporation, Milford, Massachusetts) (Nicholson et al, 1991). Peptide concentrations were measured as described by Nicholson et al (1994). All samples from each experiment were measured in one assay, and the limit of detection was 5 pg/ml.

Statistics

Data were analyzed by analysis of variance. If interaction was found, differences between the means were tested for significance by use of Student's *t*-test.

Results

Plasma oxytocin concentrations did not vary between groups in any of the experiments.

Experiment 1: The Effects of Androgens on Prostatic Oxytocin in the Intact Rat

Treatment with TP produced an increase in prostatic weight (TP 1 mg/kg, $P < 0.01$; TP 4 mg/kg, $P < 0.001$) (Fig. 1a). A fall in weight was observed following administration of CPA, but this decrease was not significant. As expected, plasma testosterone levels rose following administration of TP and fell after treatment with CPA (Fig. 1b). Plasma DHT concentrations were not affected by treatment with TP or with CPA (data not shown). TP treatment reduced prostatic oxytocin concentrations in a dose-related fashion (Fig. 1c). Conversely, administration

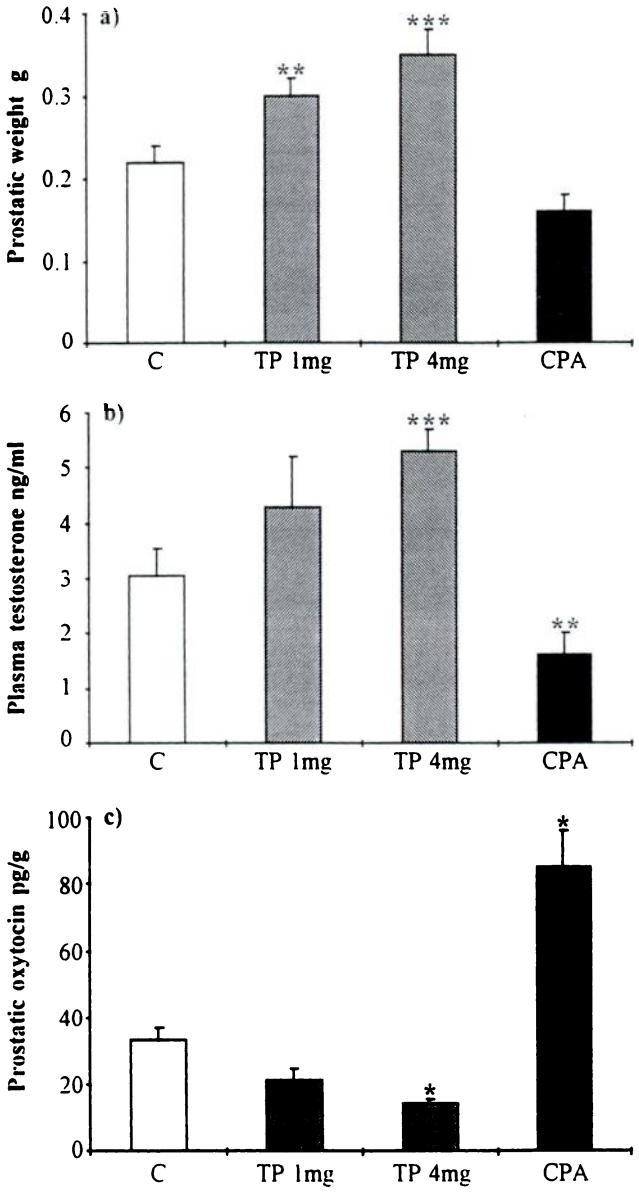

FIG. 1. The effects of treatment with testosterone propionate (TP) or with cyproterone acetate (CPA) on **(a)** prostatic weight, **(b)** plasma testosterone, and **(c)** prostatic oxytocin concentrations. In each panel, C represents control animals, TP 1mg represents the group that received testosterone propionate 1 mg/kg, TP4 mg represents the group that received testosterone propionate 4 mg/kg, and CPA represents the group that received cyproterone acetate 4 mg/kg. All treatments were given daily for 7 days. Values represent mean ± SEM, $n = 7$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared to control group.

of the antiandrogen CPA significantly increased prostatic oxytocin ($P < 0.05$) (Fig. 1c).

Experiment 2: The Effects of Androgens in the Castrated Rat

Castration significantly reduced prostatic weight, plasma testosterone, and DHT concentrations (Fig. 2a–c). As in experiment 1, the fall in androgens was accompanied by an increase in prostatic oxytocin concentrations (Fig. 2d). Treatment with TP restored plasma androgen levels and prostatic weight but significantly decreased prostatic oxytocin. Cyproterone acetate treatment did not reduce prostatic weight or plasma DHT concentrations when compared with the castrated controls. However, prostatic oxytocin concentrations were highest in this group.

Experiment 3: The Effects of a 5α-Reductase Inhibitor on Prostatic Oxytocin

Treatment of intact rats with finasteride resulted in a decrease in plasma DHT concentrations and prostatic weight and an increase in plasma testosterone concentrations (Fig. 3a–c). Administration of finasteride did not alter plasma LH or FSH levels or prostatic oxytocin concentrations (Fig. 3d).

Castration significantly reduced prostatic weight and plasma androgen levels and increased prostatic oxytocin concentrations, as in experiment 2 (Fig. 3). Administration of finasteride did not alter plasma gonadotrophin concentrations or prostatic weight compared with the castrated controls. However, plasma gonadotrophins were significantly elevated compared with the intact control animals (LH in intact controls, 1.3 ± 0.2 ng/ml; LH in castrated controls, 17.7 ± 3.4 ng/ml; FSH in intact controls, 6.9 ± 0.4; FSH in castrated controls, 20.8 ± 1.1 ng/ml). Plasma DHT concentrations were below the level of detection in both groups. The addition of finasteride increased plasma testosterone to values similar to those seen in the intact control animals (Fig. 3). Although prostatic oxytocin concentrations were higher than those seen in intact animals ($P < 0.05$), they were lower than those seen in the castrated control group ($P < 0.05$) (Fig. 3).

Experiment 4: The Effects of Estrogen Treatment on Prostatic Oxytocin in the Intact Rat

Treatment with DES significantly decreased prostatic weight ($P < 0.001$), but prostatic weight was unaffected by the administration of tamoxifen (Fig. 4). Both treatments reduced plasma LH levels (control group, 1.72 ± 0.19 ng/ml; DES group, 0.95 ± 0.03 ng/ml; tamoxifen group, 1.33 ± 0.12 ng/ml), but the reduction was only significant in the DES group ($P < 0.001$). Plasma FSH concentrations were also reduced in DES-treated ($P < 0.01$) and tamoxifen-treated ($P < 0.05$) animals (control group, 2.10 ± 0.14 ng/ml; DES group, 1.36 ± 0.19 ng/ml; tamoxifen group, 1.42 ± 0.26 ng/ml). Diethylstilbestrol significantly reduced plasma testosterone and DHT concentrations, but no changes in androgen levels were seen following treatment with tamoxifen (Fig. 4a,b). Despite the changes in androgen levels seen following DES administration, prostatic oxytocin concentrations were not significantly different from those of control animals. Treatment with tamoxifen significantly reduced prostatic

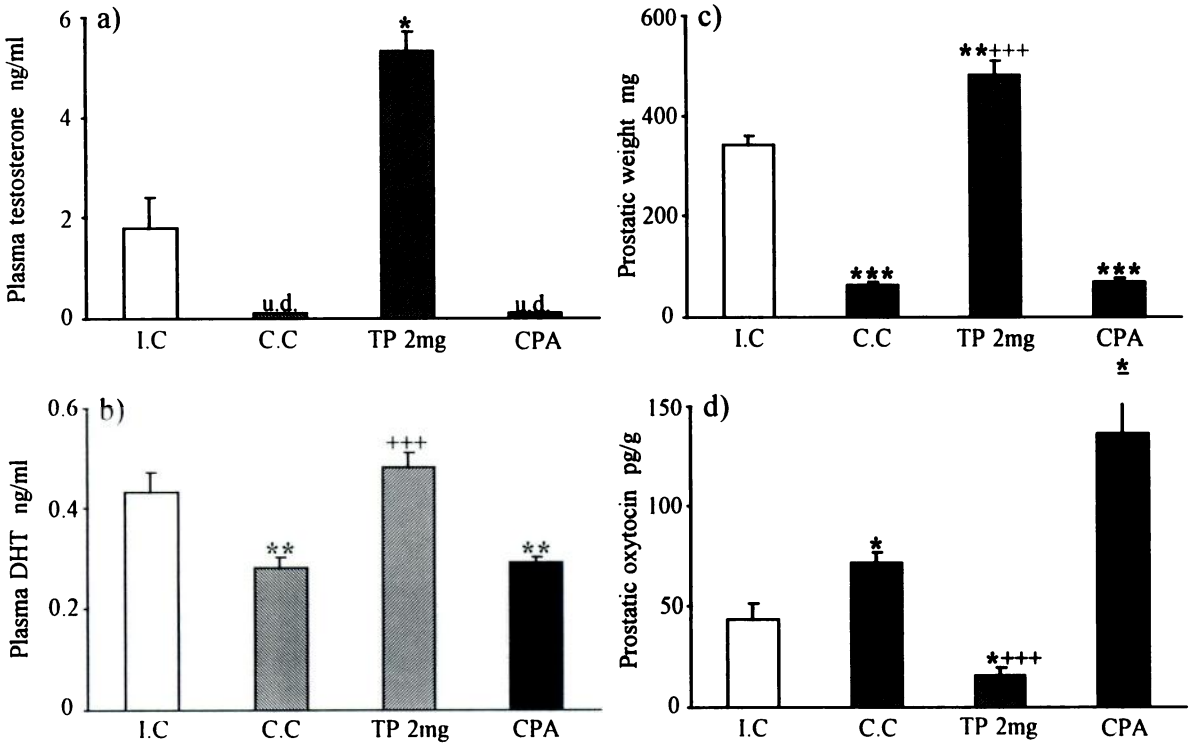

FIG. 2. The effects of administration of testosterone or cyproterone to castrated rats on **(a)** plasma testosterone, **(b)** plasma dihydrotestosterone, **(c)** prostatic weight, and **(d)** prostatic oxytocin concentrations. In each panel, I.C. represents intact control animals, C.C. represents castrated controls, TP 2mg represents castrated rats that received testosterone propionate 2 mg/kg, and CPA represents castrated animals that received cyproterone acetate 4 mg/kg. Values represent mean ± SEM, $n = 7$. * $P < 0.05$; ** $P < 0.01$; $P < 0.001$ when compared with intact control animals; +++ $P < 0.001$ when compared with the castrated control group; u.d. signifies a quantity below the level of detection.

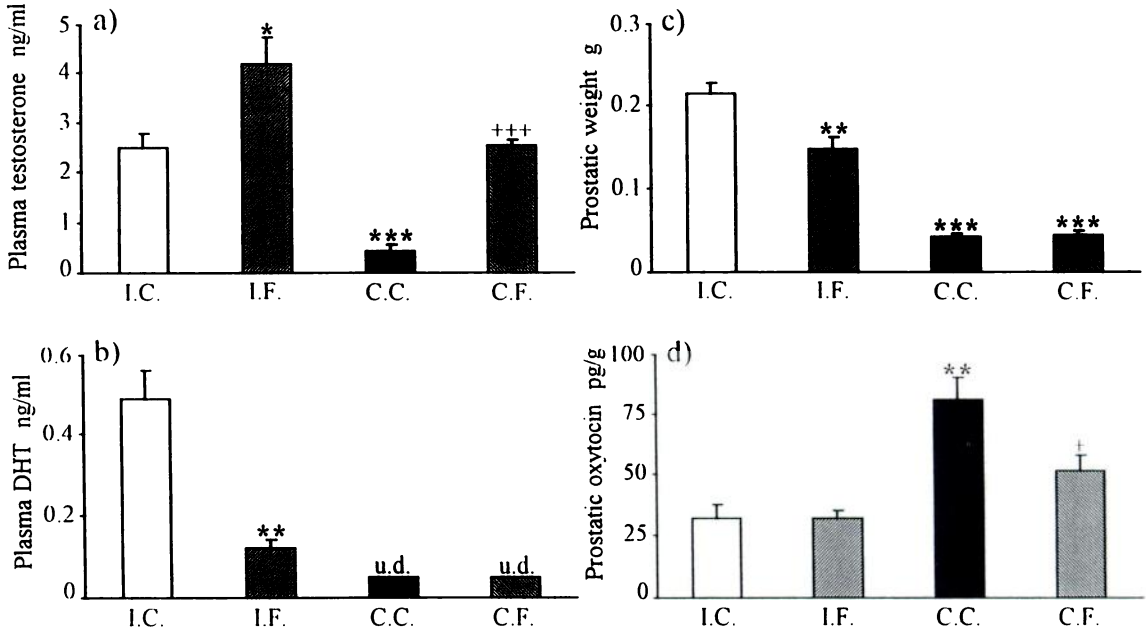

FIG. 3. The effects of finasteride treatment to intact and castrated rats on **(a)** plasma testosterone, **(b)** plasma dihydrotestosterone, **(c)** prostatic weight, and **(d)** prostatic oxytocin concentrations. In each panel, I.C. represents intact control animals, I.F. represents intact animals that received finasteride 5 mg/kg, C.C. represents castrated controls, and C.F. represents castrated animals that received finasteride 5 mg/kg. Values represent mean ± SEM, $n = 7$. * $P < 0.05$ when compared with intact control group; + $P < 0.05$ when compared with castrated control group.

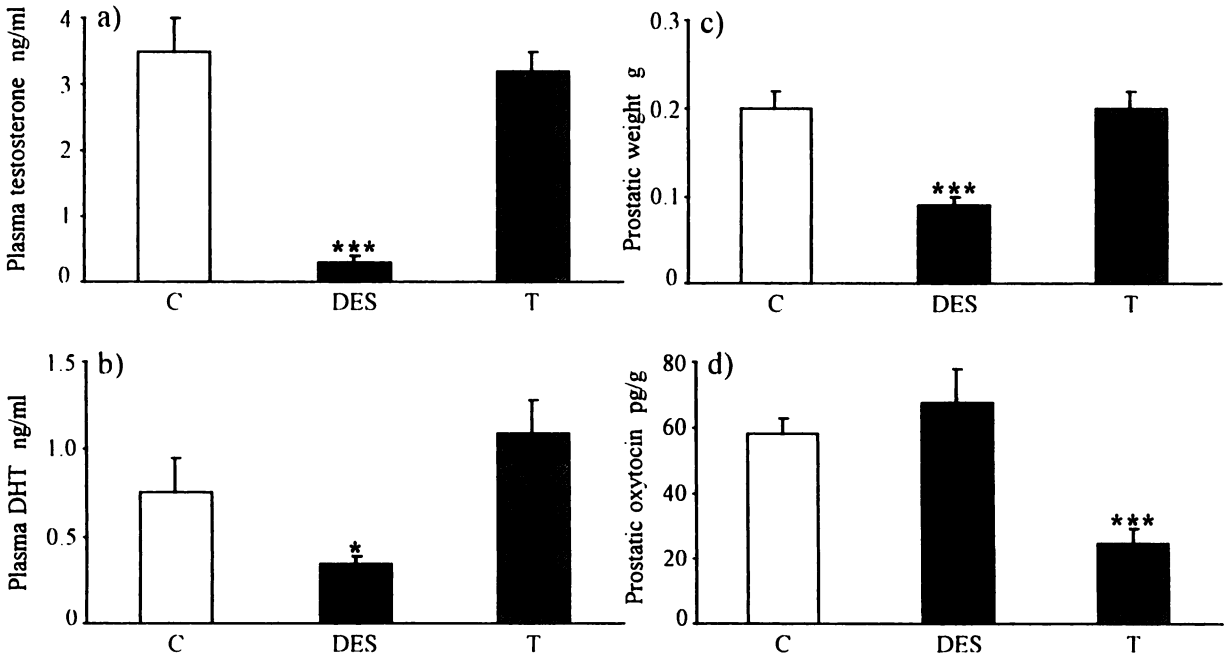

FIG. 4. The effects of estrogen treatment on **(a)** plasma testosterone, **(b)** plasma dihydrotestosterone, **(c)** prostatic weight, and **(d)** prostatic oxytocin concentrations. In each panel, C represents control animals, DES represents animals that received diethylstilbestrol 200 μg/kg, and T represents animals that received tamoxifen 200 μg/kg. Values represent mean ± SEM, $n = 7$. * $P < 0.05$; *** $P < 0.001$ when compared with control group.

oxytocin concentrations ($P < 0.001$) (Fig. 4d) but did not result in any alterations of plasma androgens.

Experiment 5: Effects of Estrogens in the Castrated Rat

Castration produced a similar pattern of changes to those observed in experiments 2 and 3, including a fall in plasma testosterone and a rise in prostatic oxytocin concentrations. Concomitant treatment with DES did not affect prostatic weight or prostatic oxytocin but significantly decreased plasma LH (control group, 11.7 ± 1.4; DES group, 1.16 ± 0.06), FSH (control group, 21.0 ± 1.1; DES group, 5.6 ± 0.24) and testosterone concentrations (Fig. 5). In all three groups of animals, plasma DHT concentrations were below the level of detection. Treatment with the antiestrogen tamoxifen almost doubled prostatic weight but decreased plasma FSH (16.9 ± 0.5), testosterone, and prostatic oxytocin concentrations (Fig. 5). No effect on plasma LH was seen.

Discussion

This study confirms our preliminary data showing that prostatic oxytocin concentrations are regulated by androgens (Nicholson and Jenkin, 1995). Thus, testosterone decreases oxytocin concentrations, whereas removal of androgens by castration or by treatment with an antiandrogen increases prostatic concentrations of the peptide. These changes are likely to reflect local changes in peptide production, since circulating levels of oxytocin were unaffected by androgens. These changes are caused by the androgen rather than by changes in gonadotrophins. Androgen deprivation, either by castration or by administration of an antiandrogen, increased oxytocin concentrations, even though castration increases gonadotrophin levels, and CPA decreases or has no effect on plasma LH (Neumann and Schenck, 1976). Similarly, the changes in oxytocin are not caused by alterations in prostatic weight. Although changes in weight occurred following castration, androgen deprivation induced by cyproterone produced similar effects on prostatic oxytocin without any alteration in prostatic weight. Changes in prostatic weight may reflect a changed relationship between the stromal and epithelial components of the prostate, since androgen withdrawal was accompanied by a decrease in epithelial cell height. The data suggest that testosterone alone is able to modulate prostatic oxytocin, because in intact animals both testosterone and CPA produce alterations in peptide concentrations without altering plasma DHT. There was insufficient tissue to measure prostatic DHT concentrations in this study, but these would provide further evidence to support this hypothesis.

The results from the experiments with finasteride are not definitive and are complicated by the fact that although finasteride decreases DHT concentrations, it also produces a concomitant increase in testosterone. However, the results do suggest that both DHT and testosterone are capable of reducing prostatic oxytocin. In intact

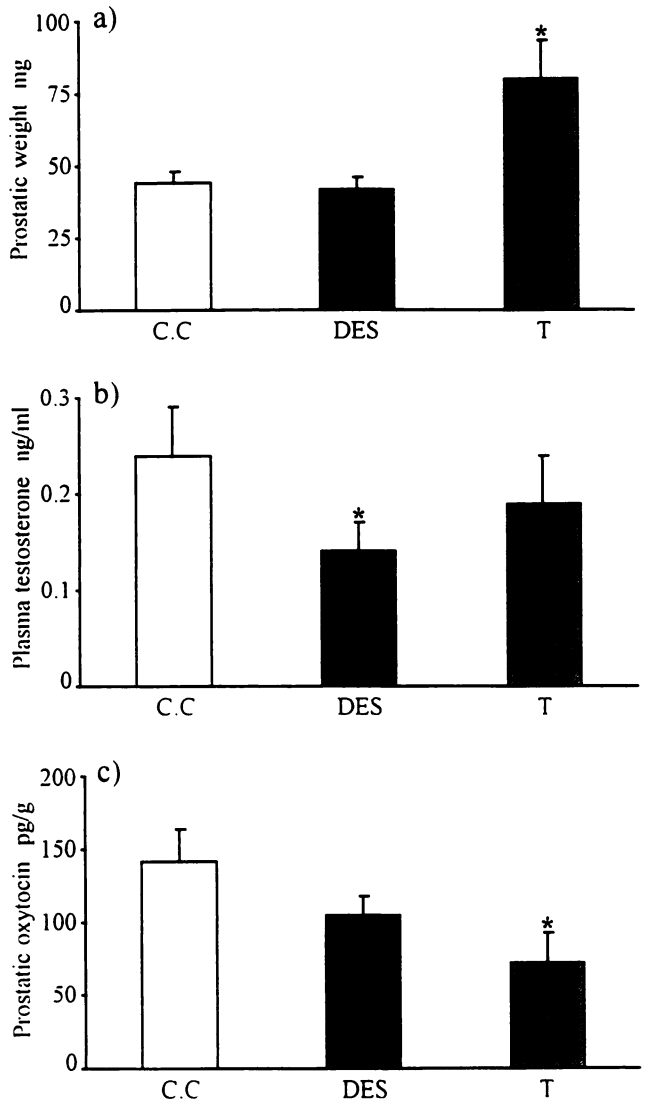

FIG. 5. The effects of estrogen treatment in the castrated rat on **(a)** plasma testosterone, **(b)** plasma dihydrotestosterone, **(c)** prostatic weight, and **(d)** prostatic oxytocin concentrations. In each panel, C.C. represents castrated control animals, DES represents castrated animals that received diethylstilbestrol 200 μg/kg, and T represents castrated animals that received tamoxifen 200 μg/kg. Values represent mean ± SEM, $n = 7$. * $P < 0.05$ when compared with castrated control group.

animals treated with finasteride, the rise in testosterone concentrations would have been expected to decrease oxytocin if DHT had no effect on the peptide. However, oxytocin concentrations in these animals were not altered, suggesting that DHT has an inhibitory effect on peptide production. Similarly, in castrated animals, finasteride treatment returned testosterone levels to control values, yet prostatic oxytocin concentrations were still elevated. Thus, although further studies are necessary, these data imply that both DHT and testosterone are involved in the regulation of prostatic oxytocin.

Although it is known that oxytocin may modulate testosterone secretion (Adashi and Hsueh, 1981; Frayne and Nicholson, 1995) and that androgens can regulate oxytocin receptor expression (Tribollet et al, 1990; Bale and Dorsa, 1995), there is surprisingly little evidence concerning the effects of androgens on the peptide. It has been shown that neither testosterone nor DHT affect the oxytocin content of the brain (DeVries et al, 1986). In the male reproductive tract, Frayne and Nicholson (1994) were unable to demonstrate a specific effect of testosterone on oxytocin secretion by isolated Leydig cells. However, in the rat epididymis, androgens have been reported to inhibit oxytocin production in a similar manner to that seen in the prostate (Harris et al, 1996).

Conversely, the effects of estrogen on oxytocin are well documented. The rat oxytocin gene contains a consensus sequence for the estrogen response element (Burbach et al, 1990), and estrogen, especially when administered sequentially with progesterone, has been shown to increase oxytocin expression in the brain (Caldwell et al, 1989; Amico et al, 1995). Estrogen also increases peptide expression in peripheral tissues such as the ovary (Shukovski et al, 1990) and the uterus (Chibbar et al, 1995), and it also increases expression of the oxytocin receptor (Bale and Dorsa, 1995). Though DHT has no effect on oxytocin receptor expression, administration of estrogen and DHT together decreases expression of the receptor (Bale and Dorsa, 1995). In the male, the majority of estrogens are produced by the aromatization of testosterone. Within the prostate, estrogen acts synergistically with androgens to increase androgen receptor content (Trachtenberg et al, 1981) and to enhance binding of DHT to androgen receptors (Moore et al, 1979). The present study demonstrates that although treatment with the antiestrogen tamoxifen decreases prostatic oxytocin concentrations in both the intact and castrated rat, DES has little effect on the peptide. These findings are in agreement with *in vitro* evidence using choriodecidua explants, where low levels of estradiol increased oxytocin synthesis, but higher levels tended to reduce peptide concentrations (Chibbar et al, 1995). The data raise the possibility that a low level of estrogen is necessary to maintain oxytocin secretion, but that further increases in estrogens do not continue to stimulate peptide secretion.

What significance do these present findings have on the regulation of prostatic function and growth in the rat? Dihydrotestosterone and the ratio of androgens to estrogens are known to be important for normal prostatic function. Benign prostatic disease is associated with an increased estrogen/androgen ratio (Coffey and Walsh, 1990). Oxytocin has also been shown to increase prostatic growth (Popovic et al, 1982); this effect may be a direct action of the peptide or may be mediated by oxytocin's effects on 5α-reductase activity. Within the prostate, DHT formation is brought about by the action of the enzyme

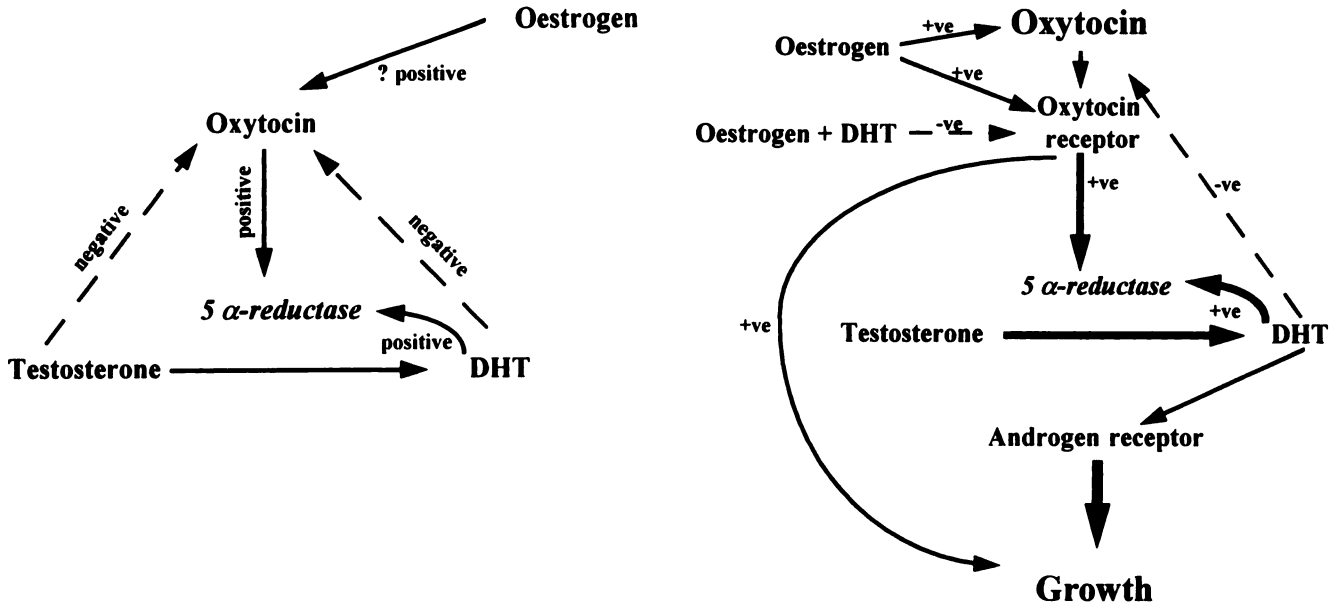

FIG. 6. **(a)**Diagram to show possible interactions between androgens, estrogen, and oxytocin within the rat prostate. Solid lines indicate stimulatory effects, and dotted lines indicate inhibitory actions. Thus both oxytocin and dihydrotestosterone (DHT) act to stimulate 5α-reductase activity and to increase local DHT concentrations. Raised concentrations of DHT or testosterone feed back and reduce prostatic concentrations. **(b)** Diagram to show possible interactions between gonadal steroids and oxytocin and its receptor in the prostate. Solid lines indicate stimulatory actions, and dotted lines indicate inhibitory effects. +ve indicates stimulatory action, and −ve represents inhibitory action. The effects of testosterone on oxytocin (see **a**) have been omitted for the sake of clarity. Thus, DHT may act with estrogen to inhibit oxytocin receptor production as well as synthesis of the peptide.

5α-reductase. Dihydrotestosterone increases expression of this enzyme in a feed-forward mechanism (George et al, 1991), and oxytocin also increases 5α-reductase activity (Nicholson and Jenkin, 1994). Whether oxytocin increases activity by interacting directly with the enzyme (Nicholson and Jenkin, 1995) or by increasing synthesis has not yet been established. Both DHT and oxytocin thus act to increase local DHT concentrations and to promote prostatic growth. Though this situation may be appropriate in the prepubertal animal, further growth is not required once sexual maturity is reached. Therefore, the presence of a regulatory system in which raised concentrations of both testosterone and DHT reduce local oxytocin concentrations, and thus 5α-reductase activity, would act to control prostatic growth (Fig. 6a). Estrogen may have a permissive role in this process to maintain adequate oxytocin and androgen receptor concentrations. However, in the presence of high concentrations of DHT, estrogen may also act at the level of the oxytocin receptor (Bale and Dorsa, 1995) to reduce peptide activity (Fig. 6b). Thus, in the absence of DHT, oxytocin receptor levels are increased, but the addition of DHT down-regulates receptor number. Disruption of this feedback mechanism may result in abnormal growth of the prostate.

In conclusion, this study demonstrates that in the rat, prostatic oxytocin concentrations are regulated by gonadal steroids, providing further evidence that oxytocin may have a physiological role within the gland.

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