Induced Hypoprolactinemia and Testicular Steroidogenesis in Man

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The effects of short-term hypoprolactinemia on the pituitary-gonadal axis were evaluated in a group of patients with untreated prostatic carcinoma. Each patient was studied prior to and during 7-day oral administrations of bromocriptine (2.5 mg q.i.d.). Serum LH, prolactin (PRL), androst-4-ene-3,17 dione (androstenedione), testosterone, and 5α-androstane-3α,17β-diol (5α-Diol) levels, as well as intra-testicular testosterone, dihydrotestosterone (DHT), 5α-Diol and zinc (Zn) concentrations, were determined. Daily administration of bromocriptine caused a marked supression of serum PRL (mean ± SEM, 23.8 ± 2.5 vs. 6.4 ± 1.0 ng/ml) without concomitant changes in serum LH levels (mean ± SEM, 8.3 ± 1.6 vs. 8.9 ± 2.1 ng/ml). Hypoprolactinemia induced a significant decrease ($P < 0.05$) in the mean peripheral testosterone levels; but 5α-Diol and androstenedione remained unchanged. However, in testicular tissues, bromocriptine treatment resulted in significant increases in mean concentrations of total androgens ($P < 0.001$), testosterone ($P < 0.001$) and DHT ($P < 0.02$). Testicular levels of 5α-Diol were not significantly altered. There was no change in Zn levels in basal conditions and during bromocriptine administration. These results indicate that short-term suppression of serum PRL levels in man affects basal testicular function without altering serum LH. However, a direct action of bromocriptine on the human gonad cannot be excluded.

Key words: prolactin, testes, bromocriptine, androgens.

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Prolactin (PRL) plays a role in the regulation of testicular steroidogenesis (Bartke, 1971; Hansson et al, 1976). In man, PRL exerts effects that can either inhibit or facilitate Leydig cell function.

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While chronic hyperprolactinemia is associated with hypogonadism (Koening et al, 1977; Perryman and Thorner, 1981), hyperprolactinemia induced for short time periods enhanced serum testosterone levels after hCG stimulation (Rubin et al, 1975; Ambrosi et al, 1976). Furthermore, experimental short-term hyper- and hypoprolactinemia induced by sulpiride and bromocriptine, respectively, did not significantly change basal serum steroid levels in normal men (Martikainen and Vihko, 1982). However, in a recent study of male patients with prolactinomas, it was reported that bromocriptine treatment restored low TeBG levels (Vermeulen et al, 1982).

A high concentration of zinc (Zn) has been described in various male accessory organs. In the human testis, a positive correlation between this metal and androgen content was also reported (Suescun et al, 1981).

The present study was designed to elucidate more clearly the influence of PRL levels on gonadal function by examining serum LH, PRL, androst-4-ene-3,17-dione (androstenedione), testosterone, and 5α-androstane-3α,17β-diol (5α-Diol) levels, as well as intra-testicular testosterone, dihydrotestosterone (DHT), 5α-Diol and Zn concentrations in a group of patients both basally and during short-term bromocriptine treatment.

Materials and Methods

Twelve patients 52 to 73 years old with stage C and D prostatic carcinomas were studied after being fully

informed. No subject had received any previous hormonal or neuroleptic therapy, and none had any indication of other disease. Each patient was studied prior to and during bromocriptine administration (Parlodel, Sandoz, Switzerland, 2.5 mg q.i.d.) for 7 days, followed by orchidectomy. Initial blood samples were drawn for hormone measurement between 8:00 and 10:00 AM by venipuncture from the antecubital vein, concomitantly with a needle biopsy for histologic confirmation of the prostatic carcinoma. Sera were obtained by clotting and centrifugation and then stored at −20 C. Simultaneously, a testis biopsy was taken from each patient and divided into three pieces: 1) an aliquot for androgen radioimmunoassay was plunged into cold acetone and stored at −20 C, 2) an aliquot for Zn determination was stored at −70 C, and 3) a small portion was fixed in Bouin's fluid for determination of testicular morphology. At the time of orchidectomy, additional blood samples and a piece of central testicular tissue were taken as before. All operations were performed using spinal anesthesia and the average time between the injection of the anesthetic and the removal of the specimen was approximately 30 minutes.

The sera were assayed for LH using an antiserum provided by the National Pituitary Agency (batch #2). The hormone used for labeling and standards was highly purified hLH (31079; 20000 IRP-2HMG/mg) from The Hormone and Isotope Laboratory, Aker Hospital, Oslo, Norway. PRL was determined using a double antibody RIA with kits supplied by the National Pituitary Agency (NIH-NPA, batch h-PRL-I-6). Iodination was performed by the lactoperoxidase method (Thorell and Johansson, 1971). The procedures for these RIAs have been previously described (Chiauzzi et al, 1982).

Serum androgen (testosterone + DHT and 5α-Diol) determinations were performed following a validated procedure reported previously from this laboratory (Suescun et al, 1981), with the exception that 5α-Diol was measured using a highly specific antibody (androstane-3α, 17β-diol-15-carboxymethyloxime-HSA) from Immunotech Diagnostic, Montreal, Canada. Serum androstenedione was analyzed with a specific antibody (androstenedione-6-hemisuccinate-BSA) generously provided by Dr. K. M. Pirke (Munchen, West Germany). The lower limit of detection was 12.5 pg, and the intra-assay and interassay coefficients of variation were less than 10%. Individual intra-testicular tissue androgens (testosterone, DHT and 5α-Diol) were also measured as previously described (Suescun et al, 1981). Briefly, after the extraction and purification, the combined ether extracts were resuspended in 1.0 ml isooctane and chromatographed on celite glass microcolumns washed with 10 ml of isooctane. The fractions containing the three androgens were eluted as follows: DHT with 6 ml of the solvent mixture isooctane-toluene (80:20 v/v), testosterone with 6 ml of isooctane-toluene (60:40 v/v) and 5α-Diol with 6 ml of cyclohexane-ethylacetate (85:15 v/v). Details of the chromatography and RIA are given elsewhere (Suescun et al, 1981), with the exception that the antiserum mentioned above was used for androstanediol. Recovery of this androgen in the testis was 64.1 ± 9.1% (mean ± SE). The intraassay coefficient of variation was less than 12%, and the interassay coefficient of variation was less than 14.5%. Tests for parallelism between standard curves and unknowns were satisfactory and calculated as for DHT and testosterone (Suescun et al, 1981).

Aliquots of testicular tissue were submitted to the wet ashing procedure, and Zn was determined by flame atomic absorption spectrophotometry (Dawson and Walker, 1969). Protein was measured by a conventional method (Lowry et al, 1951), using BSA as the standard protein.

The paired Student's *t* test was used for the statistical evaluation.

Results

During the 7-day bromocriptine treatment, PRL levels decreased from 23.8 ± 2.5 to 6.4 ± 1.0 ng/ml. No significant changes were observed in serum LH levels (8.3 ± 1.6 vs. 8.9 ± 2.1 mIU/ml) and these data are depicted in Fig. 1 (upper and lower panel, respectively).

The total peripheral levels of testosterone, 5α-Diol and androstenedione are shown in Fig. 2. It can be seen that the mean serum 5α-Diol and androstenedione levels did not differ significantly prior to or following bromocriptine treatment, but the main androgen, testosterone + DHT, showed a significant decline ($P < 0.05$) during hypoprolactinemia. It should be noted that, in five out of 12 patients, there was a clear fall in testosterone levels.

Intra-testicular androgens, following individual

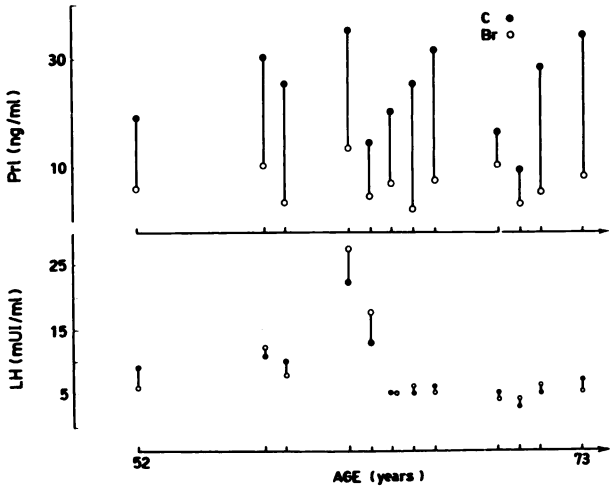

Fig. 1. Individual serum levels of prolactin (PRL, upper panel) and LH (lower panel) in basal (dotted circle) conditions and during (open circle) bromocriptine administration (2.5 mg q.i.d., for 7 days), in a group of patients with carcinoma of the prostate.

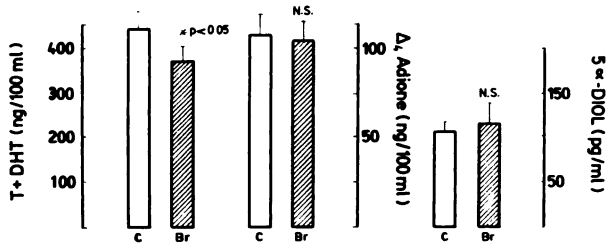

Fig. 2. Peripheral serum testosterone (T) plus dihydrotestosterone (DHT), androstenedione (Δ_4-Adione) and 5α-androstane-3α, 17β-diol (5α-Diol) levels in basal conditions (open column □ C) and during bromocriptine administration (hatched column ▨ Br). Vertical lines represent mean values (± SEM) and the asterisk indicates the level of significant difference, compared to the control. N.S. = non-significant differences. For further details, see legend to Fig. 1.

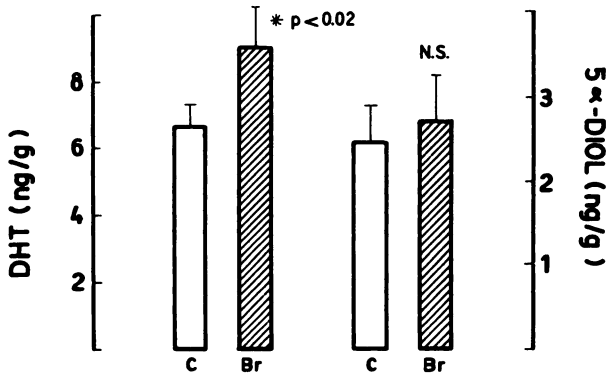

Fig. 3. Intra-testicular dihydrotestosterone (DHT) and 5α-androstane-3α, 17β-diol (5α-Diol) concentrations in basal conditions (open column □ C) and during bromocriptine administration (hatched column ▨ Br). Vertical lines represent mean values (± SEM) and the asterisk indicates the level of significant difference compared to the control. N.S. = non-significant differences. For further details, see legend to Fig. 1.

separation on celite micro-columns, are shown in Figs. 3 and 4. Total androgens, testosterone, and DHT, but not 5α-Diol, increased significantly during bromocriptine administration (mean ± SE, $P < 0.001$, $P < 0.001$, and $P < 0.02$, respectively).

There was no significant difference in gonadal levels of Zn before and during bromocriptine treatment (data not shown). No differences in the mean protein concentrations per gram of testis wet weight were observed. The results, therefore, are similar when expressed per milligram of total protein or per gram of tissue.

Histologic examination of the testis showed individual variations in the appearance of the seminiferous tubules, from intact spermatogenesis to age-correlated processes of organ involution, including varying degrees of hypospermatogenesis, "Sertoli cell only" tubules, or spermatogenic arrest, and small foci of tubular hyalinosis. In every case, mature Leydig cells could be observed with eosinophilic cytoplasm and sometimes vacuolization. They were organized in small clumps related to the capillary system. Even though the number of Leydig cells seemed to be directly related to intratesticular androgen levels in different patients, no variations could be observed in the seminiferous tubules or interstitial cells before and during treatment in individual cases.

Discussion

We have investigated the effect of hypoprolactinemia induced by bromocriptine in the pituitary-gonadal axis of a group of men with untreated cancer of the prostate.

As expected, the present results showed a marked suppressive effect of bromocriptine on normal PRL levels, whereas serum LH remained unchanged. These results are in agreement with previous findings in either healthy men under oral administration of bromocriptine for 13 days (Martikainen and Vihko, 1982), or in patients with prostatic carcinoma (Jacobi et al, 1978).

During short-term hypoprolactinemia, we found a significant decrease of total testosterone (plus DHT) in the peripheral circulation, a finding that is in accord with what has been reported previously for patients with prostatic carcinoma (Jacobi

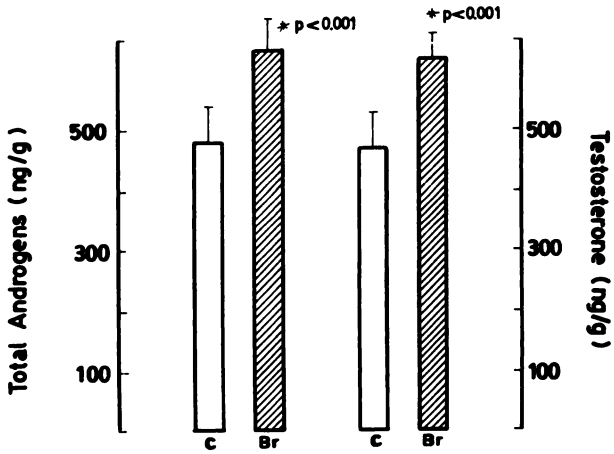

Fig. 4. Intra-testicular testosterone and total androgen concentrations in basal conditions (open column □ C) and during bromocriptine administration (hatched column ▨ Br). Vertical lines represent mean values (± SEM) and the asterisks indicate the level of significant differences compared to the control. N.S. = non-significant differences. For further details, see legend to Fig. 1.

et al, 1978). In contrast, Martikainen and Vihko (1982) could not find any significant change in several steroids measured during short-term bromocriptine administration prior to and after a single dose of 5000 IU of hCG. On the other hand, Nakagawa et al (1982) reported high serum testosterone levels in healthy men after 2.5 mg of bromocriptine given daily for only 3 days. These conflicting results might be ascribed, at least in part, to a difference in testicular responsiveness to LH during short- and long-term hypoprolactinemia (Martikainen and Vihko, 1982; Martikainen et al, 1983). However, in this study we did not investigate the gonadal response to gonadotropin stimulation. Furthermore, it should be recalled that bromocriptine is known to reduce the number of LH binding sites on rodent Leydig cells (Bohnet and Friesen, 1976; Aragona et al, 1977; Purvis et al, 1979; Bartke 1982; Calandra et al, 1982). Notwithstanding, Huhtaniemi and Catt (1981) have observed that, in the adult rat, treatment with bromocriptine affects only the binding of LH, and not testicular responses. Whether or not a similar effect occurs in man needs to be studied.

The present results showed that there were highly significant increases in testosterone and DHT tissue levels, as well as in total androgens, though no changes could be detected in testicular 5α-Diol. It is difficult to speculate about the apparent discrepancies between total peripheral and testicular androgen levels. However, a possible explanation may be the bromocriptine-induced elimination of ^{3}H-testosterone from the circulation that has been reported by others (Jacobi et al, 1978).

Previous studies (Jacobi et al, 1978; Martikainen and Vihko, 1982), as well as our own data, have demonstrated that induced hypoprolactinemia in men with normal serum prolactin does not modify LH levels. Therefore, we can only suggest that this dopaminergic agent possibly exerts a direct effect on testicular steroidogenesis. Similar results of a stimulatory role of bromocriptine on local androgen production have been demonstrated in decapsulated mouse and immature rat testes *in vitro* (Bartke and Lackritz, 1981; Suescun et al, in preparation). A direct suppressive effect of the same agonist on androgen production by rat Leydig cell preparations has also been reported (Vermes and Telegdy, 1978).

We have previously given evidence of a positive correlation between intratesticular androgens and gonadal Zn concentrations (Suescun et al, 1981). In the present study, however, there was no difference in Zn content before and during bromocriptine administration.

In summary, the induction of hypoprolactinemia by short-term bromocriptine treatment in men with normal serum PRL appears to influence testicular function at several levels. In this study, we have shown a previously unreported incremental effect on testosterone and DHT tissue levels. It is difficult to fully ascertain whether the observed effects are due to changes in PRL release, a direct effect on the gonads, or both. The precise mechanism of bromocriptine action within the human gonad remains to be established.

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