MOLECULAR PHARMACOLOGY, 17, 156–162

Inhibition of Androgen–Receptor Interaction in Mouse Prostate Gland Cytosol by Divalent Metal Ions

MICHAEL P. DONOVAN,[1] LONNIE G. SCHEIN[2] AND JOHN A. THOMAS[3]

Department of Pharmacology and Toxicology, West Virginia University Medical Center, Morgantown, West Virginia 26506

Received September 7, 1979; Accepted October 11, 1979

SUMMARY

DONOVAN, M. P., L. G. SCHEIN AND J. A. THOMAS. Inhibition of androgen–receptor interaction in mouse prostate gland cytosol by divalent metal ions. *Mol. Pharmacol.* 17: 156–162 (1980).

The binding of [^{3}H]dihydrotestosterone to receptor proteins was studied in cytoplasm of mouse anterior prostate gland. K^+, Ca^{2+}, Mn^{2+}, Mg^{2+}, and Ni^{2+} had little effect upon binding at concentrations up to 10^{-3} M (0.2 M K^+). Pb^{2+}, Fe^{2+}, and Co^{2+} produced substantial inhibition of binding at 10^{-3} M. Cu^{2+}, Hg^{2+}, Zn^{2+}, and Cd^{2+} were very effective inhibitors producing 50% inhibition at 2–3 × 10^{-5} M and nearly 100% inhibition at 10^{-4} M. The inhibition due to Zn^{2+} was competitive. These observations are consistent with the possible involvement of Zn^{2+} in negative feedback control of androgen action in male sex accessory tissues and provide a possible mechanism for weight reduction caused by Cd^{2+} in those organs *in vivo*. Competitive inhibition of binding by *p*-hydroxymercuribenzoate was also observed. Neither this finding nor relative inhibitory effectiveness of the various metals is consistent with involvement of protein sulfhydryl groups at the binding site in the mechanism of inhibition.

INTRODUCTION

Over 25 years ago Mawson and Fischer (1) showed that the male sex accessory organs of mammals contain much higher levels of zinc than other organs. Subsequently, it was demonstrated that zinc has an important role in sexual development (2), and that a relationship exists between the zinc content of the canine prostate and the hormonal status of the animal (3). In the rat, there is a seasonal cycle in the uptake of ^{65}Zn by the prostate gland which correlates with the reproductive cycles (4). Other divalent metal ions like cadmium can affect the male reproductive organs, causing reduction in organ weight without reducing body weight or producing overt signs of toxicity (5).

Since the sex accessory organs are dependent on steroid hormones, it was of interest to determine the effects of various metals on steroid hormone action. An early step in steroid hormone action is the binding of the active hormone to a cytoplasmic receptor molecule (6). Accordingly, these studies were begun by examining the hormone–receptor interaction in the presence of various metal ions.

Earlier reports have been concerned with studies of estradiol binding by uterine cytosols. Emanuel and Oakey (7) first reported that addition of 5 mM $ZnCl_2$ more than doubled the binding of [^{3}H]estradiol to cytosol prepared from endometrium of nonpregnant cows. Calcium (74%), potassium (26%), magnesium (21%), and manganous (20%) ions all increased binding when present at 5 mM. Subsequently, Sanborn *et al.* (8) studied estradiol binding in rabbit uterine cytosol and found that binding was inhibited by 1 mM $ZnCl_2$ (73%), by 10 mM $MgCl_2$ (30%), by 10 mM $CaCl_2$ (27%), and by 300 mM KCl (53%). Very slight increases in binding were noted for 10 mM KCl and for 1 mM EDTA. Inhibition of estradiol binding by a variety of divalent ions at 1 and 5 mM was observed by Young *et al.* (9) who studied human endometrial cytosol. Divalent metal ions also were found to inhibit binding of progesterone by a protein isolated from human myometrium (10). The present paper reports the first study of androgen–receptor interactions in the presence of divalent metal ions.

MATERIALS AND METHODS

Animals and cytosols. Male Swiss–Webster mice weighing 35–40 g (Hilltop Laboratory Animals; Scottsdale, Pa.) were castrated via abdominal incisions under

This study was supported by Department of Energy, Morgantown Energy Technology Center, Contract EY-77-C-21-8087, Task Order 14.

[1] Supported by NIH Training Grant 1-T32 GM 07039-02 and submitted in partial fulfillment of the requirements for Ph.D. in the Department of Pharmacology. Present address: Department of Biology, West Virginia University, Morgantown, WV 26506.

[2] Supported by NIH Training Grant 1-T32 GM 07039-02. Present address: Harvard University, School of Public Health, Department of Nutrition, 665 Huntington Ave., Boston, Mass. 02115.

[3] To whom correspondence should be addressed.

0026-895X/80/020156-08$02.00/0

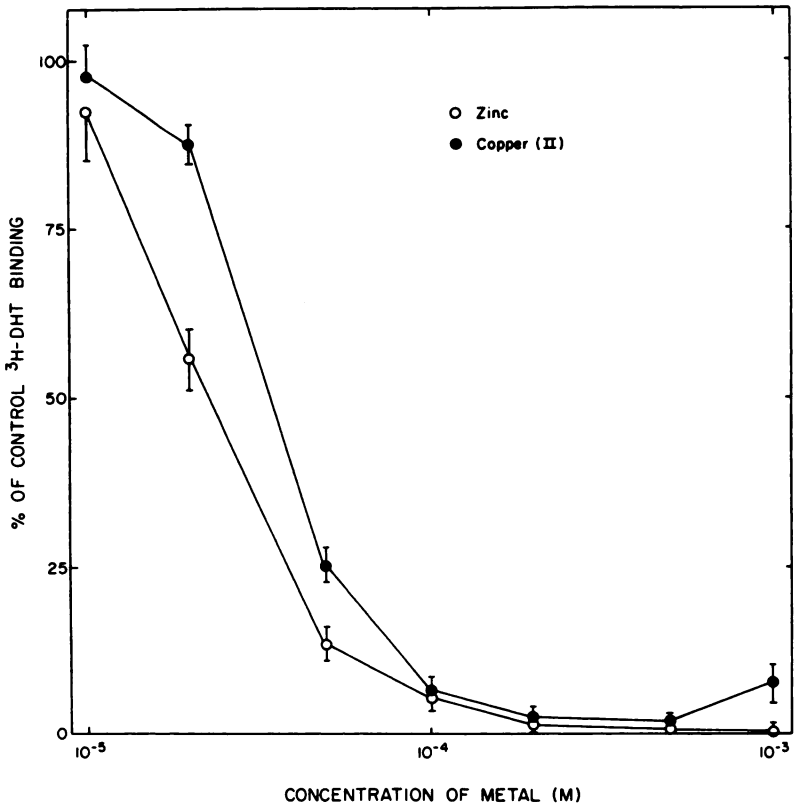

FIG. 1. *Inhibition by Zn^{2+} and Cu^{2+} of specific [^{3}H]DHT binding in mouse prostate cytosol*

Cytosol protein (1–2 mg) was incubated with 5×10^{-9} M [^{3}H]DHT, 0.05 M Tris–HCl, pH 7.2, and metal chloride in a total volume of 0.50 ml for 12 hr at 0–4°. Unbound [^{3}H]DHT was removed by adsorption to 2.5 mg Dextran-coated charcoal. Values were corrected for nonspecific binding determined in identical incubation mixtures to which 5×10^{-7} M DHT was added. Total control binding was 500–1000 cpm of which 2–8% was nonspecific binding. Each point represents the mean of triplicate values determined for each of at least three cytosols. The bars represent the standard error of the mean.

pentobarbital anesthesia. Three days later, anterior prostate glands were excised and homogenized in 10 vol of ice-cold 0.05 M Tris–HCl (pH 7.2). Each preparation contained glands from 30 to 42 animals.

Endogenous metal ions were removed from homogenates by incubation with Dowex chelating resin (Sigma Chemical Co.). The resin was prepared by suspending in 0.05 M Tris–HCl and adjusting to pH 7.2 with 1 M HCl. The resin was filtered, resuspended in Tris buffer, the pH verified at 7.2, and refiltered. One gram of resin was added to each 3 ml of homogenate and the mixture swirled in an ice bath for 30 min. The mixtures were centrifuged at 100,000*g* for 60 min, removing the resin and the particulate organelles. The resulting supernatants were decanted and designated as cytosols or cytoplasmic fractions. Content of zinc ion in cytosols prepared in this way was measured by atomic absorption spectroscopy and was found to be less than 10^{-6} M.

Androgen binding. Steroid–receptor interaction was studied using the method of Schein *et al.* (11). Stock solutions of [^{3}H]DHT,[4] 80 Ci/mmole (New England Nuclear), were dried under N_2 and redissolved in 0.05 M Tris–HCl, pH 7.2, immediately before each experiment. Purity of the radiosteroid was determined by thin-layer chromatography with chloroform:ether (7:3, v/v); solutions were used only if their radiochemical purity exceeded 95%.

The binding reaction was carried out in mixtures containing 1–2 mg cytoplasmic protein, 0.05 M Tris–HCl, pH 7.2, and [^{3}H]DHT. Metal ions and unlabeled DHT were added as appropriate. Incubations were carried out in a total volume of 0.50 ml, at 0–4°, and for 12 hr.

Quantitation of [^{3}H]DHT binding. Dextran-coated charcoal was used to remove free steroid from the incubation mixture (12). A suspension containing 0.25% activated charcoal and 0.025% Dextran T-70 in 0.9% NaCl solution was stirred at 0–4° for at least 1 hr. At the end of the incubation period, 1 ml of charcoal suspension was added to each tube and mixed for 15 min. Charcoal and adsorbed steroid were sedimented by centrifugation at 2000*g* for 10 min. The supernatants were decanted into 10-ml portions of scintillation counting fluid containing 0.05 g POPOP, 4 g PPO, and 200 ml of Beckman BioSolv in 1 liter of toluene. All data were expressed as counts per minute of [^{3}H]DHT per milligram of protein and were corrected for radiosteroid unadsorbed to charcoal by subtracting appropriate blank values derived from incubation mixtures containing [^{3}H]DHT, but no cytosol. Nonspecific binding was determined in incubation mixtures containing unlabeled DHT in 100-fold molar excess over [^{3}H]DHT. Specific binding was then determined by

[4] The abbreviations used are: [^{3}H]DHT, [1,2-^{3}H]dihydrotestosterone; pHMB, *p*-hydroxymercuribenzoate; POPOP, 1,4-bis (2-(5-phenyloxazolyl))benzene; PPO, 2,5-diphenyloxazole.

correction of total binding in tubes containing only radiosteroid (13).

Other procedures. Protein was estimated by the method of Lowry *et al.* (14) using bovine serum albumin as standard. Metal solutions were standardized by titration with 0.100 M Na_2EDTA using murexide or Eriochrome black T as indicator.

Dextran was purchased from Pharmacia Fine Chemicals, $ZnCl_2$ and $CdCl_2$ from Apache Chemicals, charcoal and Tris from Sigma Chemical Corporation, and all other reagents from Fisher Scientific Company.

RESULTS

Both $ZnCl_2$ and $CuCl_2$ were effective inhibitors of specific [^{3}H]DHT binding in mouse anterior prostate cytosol (Fig. 1). Neither metal inhibited binding at 10^{-5} M, but both completely abolished specific binding above 10^{-4} M. Zinc was somewhat more effective than cupric ion. The effects of $CaCl_2$ and $MgCl_2$ on specific [^{3}H]DHT binding are in marked contrast to the effects of Zn^{2+} and Cu^{2+} (Fig. 2). Neither Ca^{2+} nor Mg^{2+} effectively inhibited binding and, in fact, specific binding was slightly increased with Ca^{2+} at concentrations less than 10^{-4} M. On the other hand, magnesium inhibited binding at all concentrations greater than 10^{-5} M, but the maximum inhibition was only 30% at 1 mM $MgCl_2$.

The various metals were distributed into five categories on the basis of inhibitory activity (Table 1). Calcium had no effect upon steroid binding at any concentration while magnesium, manganous and nickel ions exerted only minimal effects, causing at most 41% inhibition at 10^{-3} M $NiCl_2$. Ferric, cobalt, and lead ions were capable of inhibiting binding more than 50%, but only at concentrations near 10^{-3} M. Potassium could also produce up to 67% inhibition, but its IC_{50} was 0.2 M. The most effective category of inhibitors included cadmium, cop-

TABLE 1

Effect of metal ions on specific binding of [^{3}H]dihydrotestosterone to mouse anterior prostate cytosol[a]

Ion	IC_{50} (M)[b]	Maximum inhibition[c]
Ca^{2+}	—	4
Mn^{2+}	—	28
Mg^{2+}	—	31
Ni^{2+}	—	41
K^+	0.2 M	67
Pb^{2+}	9.0×10^{-4} M	53
Fe^{2+}	8.6×10^{-4} M	59
Co^{2+}	7.2×10^{-4} M	62
Cu^{2+}	3.5×10^{-5} M	100
Hg^{2+}	3.5×10^{-5} M	100
Zn^{2+}	2.2×10^{-5} M	100
Cd^{2+}	1.8×10^{-5} M	100

[a] Incubation mixtures contained 1–2 mg cytosol protein, 5×10^{-9} M [^{3}H]DHT, 0.05 M Tris–HCl, pH 7.2, and metal chloride in a total volume of 0.50 ml. Incubations were carried out for 12 hr at 0–4° and were terminated by addition of 2.5 mg dextran-coated charcoal to adsorb free radiosteroid.

[b] IC_{50} is the concentration of metal ion required to reduce specific [^{3}H] DHT binding to 50% of the control level.

[c] Maximum observed percentage inhibition of control binding produced by 1.0 mM metal ion (0.3 M KCl).

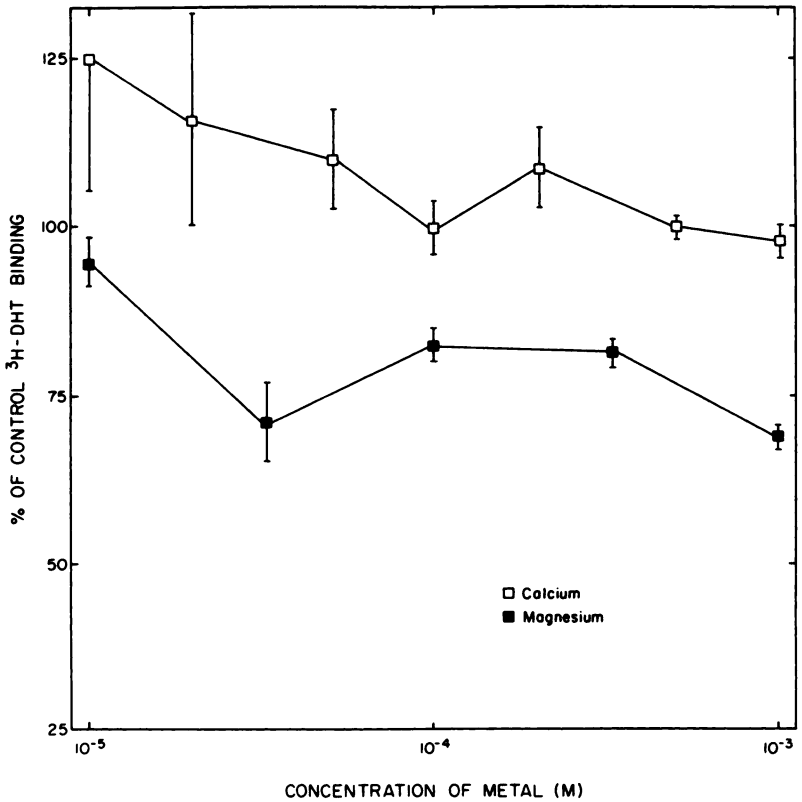

FIG. 2. *Effect of Ca^{2+} and Mg^{2+} on specific binding of [^{3}H]DHT in mouse prostate cytosol*
Conditions were as described in Fig. 1.

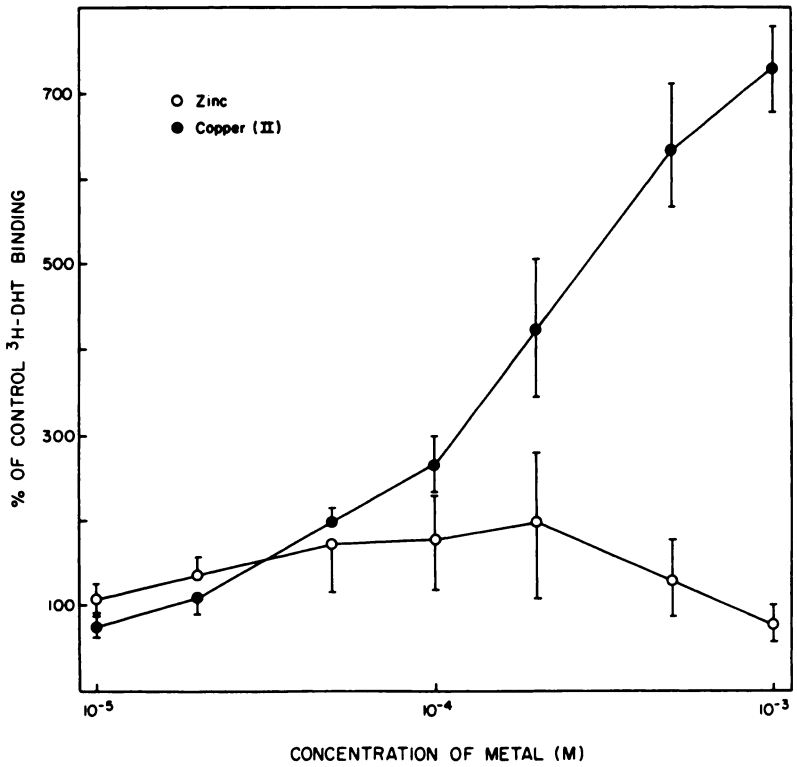

FIG. 3. *Stimulation by Cu^{2+} of nonspecific binding of [^{3}H]DHT in mouse prostate cytosol*
These data represent the nonspecific component of binding in the incubations of Fig. 1.

per, mercuric, and zinc ions. These metals were capable of producing complete inhibition of specific [^{3}H]DHT binding, and had IC_{50} values ranging from 1.75×10^{-5} M (Cd^{2+}) to 3.5×10^{-5} M (Cu^{2+} and Hg^{2+}).

None of the metal ions significantly inhibited nonspecific binding of [^{3}H]DHT. However, cupric ions substantially enhanced nonspecific binding of [^{3}H]DHT (Fig. 3). The increase was more than sevenfold at 10^{-3} M $CuCl_2$ and did not reach a limiting value in the concentration range studied (10^{-5}–10^{-3} M).

Figure 4 is a double reciprocal plot of the specific binding of various concentrations of [^{3}H]DHT in the absence and presence of 2×10^{-5} M $ZnCl_2$. This concentration of Zn^{2+} produced approximately 50% inhibition of binding (Table 1). The presence of zinc ion did not affect the maximum binding of [^{3}H]DHT; it was approximately 14 fmole/mg protein under both conditions (Fig. 4). However, the apparent K_a for binding, which was 5.2×10^9 M^{-1} without zinc, decreased to 2.9×10^9 M^{-1} in the presence of 2×10^{-5} M $ZnCl_2$.

Inhibition of specific [^{3}H]DHT binding by pHMB was also studied (Fig. 5). Complete inhibition of binding was observed at 10^{-4} M pHMB; the IC_{50} was 4×10^{-5} M. Double reciprocal analysis (Fig. 6) showed the same maximum binding (16 fmole/mg protein) in the presence and absence of 4×10^{-5} M pHMB. However, the apparent K_a of [^{3}H]DHT binding was 5.5×10^9 M^{-1} in the absence of pHMB and was lowered to 2.0×10^9 M^{-1} when 4×10^{-5} M pHMB was present.

The presence of 2×10^{-5} M Zn^{2+} did not alter the time course of the binding reaction from that previously reported (11); binding with and without zinc ion reached maximum values by 12 hr of incubation and remained unchanged at least until 24 hr. Similarly, the presence of zinc ion did not affect the time course of removal of free steroid from the incubation mixture by charcoal. In the presence and the absence of 2×10^{-5} M Zn^{2+}, specific binding remained constant from 2 to 20 min of incubation with charcoal while nonspecific binding fell steadily until 15 min and remained unchanged at least until 20 min. [^{3}H]DHT adhering to the glass tubes at the end of the incubation was extracted with scintillation cocktail after pouring out the incubation mixture and without adding charcoal. The radiosteroid remaining was unaffected by the presence of 2×10^{-5} M Zn^{2+} in the incubation and did not differ from the amount left by [^{3}H]DHT in an equal volume of buffer without cytosol.

DISCUSSION

The use of the chelating resin to remove endogenous ions represents a technical advance in studies of the effects of divalent metals on steroid hormone binding and metabolism. Previous studies have either included EDTA in the cytosol medium (8, 15) or they have failed to remove endogenous metals (7, 9, 10, 16). In all of these studies, therefore, the concentration of metal ions in the binding incubation mixture is uncertain. In the present studies the endogenous metal ions are removed with the resin particles and no unsaturated chelating sites are left in the solution. Therefore the concentration of metal ions in the incubation mixture is only the result of added ions.

This study extends knowledge of the interactions of metal ions with steroid hormone receptors to the androgen receptor in male sex accessory organs. The present

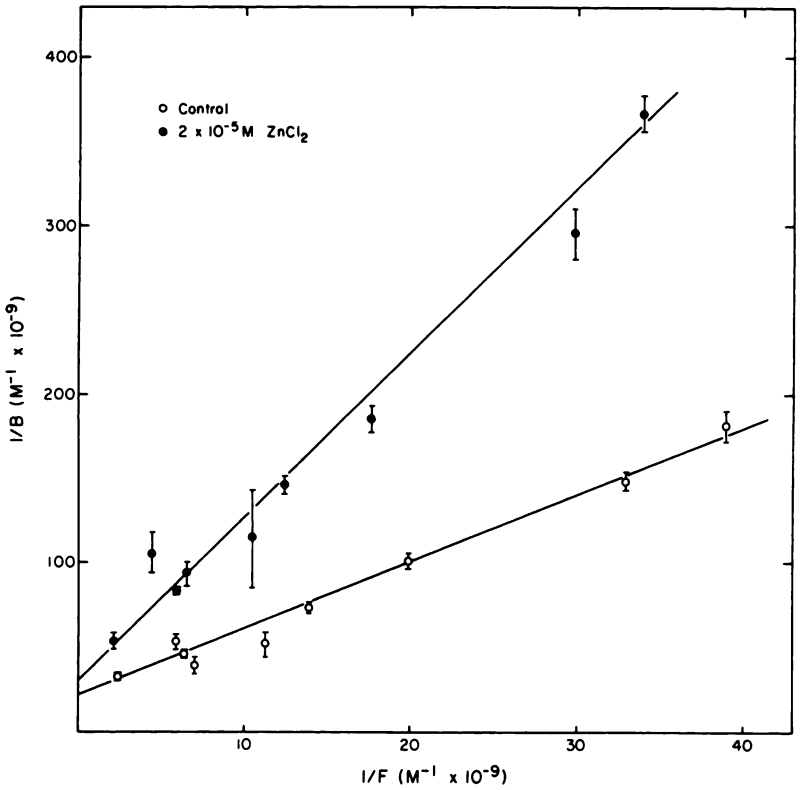

FIG. 4. *Double reciprocal plot of specific binding of [^{3}H]DHT in mouse prostate cytosol in the absence and presence of 2 × 10^{-5} M ZnCl$_2$*
Incubation conditions were as described in Fig. 1. [^{3}H]DHT concentration was varied from 5 × 10^{-10} to 10^{-8} M. Unlabeled DHT in the determinations of nonspecific binding was also varied to maintain a 100-fold molar excess over [^{3}H]DHT and charcoal blanks were evaluated at each concentration of [^{3}H]DHT. Each point represents the mean of at least two determinations on each of at least two cytosol preparations. Bars represent the standard errors of the means. The lines represent linear regression lines calculated on the individual data points. Regression lines were also calculated separately for each set of points determined on each cytosol preparation, generating a sample of estimates for each intercept. These were then compared by Student's *t* test. The mean value for the *x*-intercept in the presence of zinc differed significantly from control ($P < 0.02$) while the means for the *y*-intercept did not differ ($P \simeq 0.45$).

results in the male agree qualitatively with the previous findings of inhibition by metals of binding to estrogen receptors (9) and progesterone receptors (10) in the female. The present findings are also in agreement with the observations of these investigators that Ca^{2+}, Mg^{2+}, Mn^{2+}, and K^+ are relatively weak inhibitors of binding; Fe^{2+} and Pb^{2+} have intermediate potency; and Cu^{2+}, Hg^{2+}, Zn^{2+}, and Cd^{2+} are very effective inhibitors.

These investigators have also noted that inhibition by metals of steroid binding to uterine cytosols could be reversed by addition of dithiothreitol (9, 10). These authors concluded from this observation that sulfhydryl groups of the receptor protein are involved in the processes leading to binding (10) and that the cations interfere with binding by blocking the sulfhydryl groups at the binding sites (9). However, these authors also observed that binding is reversed by EDTA. Furthermore, Wallace and Grant (16) found that inhibition of prostate 5α-reductase by zinc was reversed by dithiothreitol, by EDTA, by *o*-phenanthroline, and by citrate ion. It appears that any chelating agent will reduce the concentration of free cation in the incubation and reverse inhibition. Such studies of chelation provide no information as to the identity of the affected chemical groups on the receptor. Metal ions can form stable complexes with carboxylates (17), imidazoles, terminal amino groups and peptides (18) as well as sulfhydryl groups (19). Therefore, it seems premature to ascribe inhibition of steroid binding to interaction of cations with any one of these chemical groups until the exact nature of the binding site is clarified.

Direct evidence against the involvement of protein sulfhydryl groups in inhibition of steroid binding is derived from the pHMB findings (Fig. 6). This compound forms a stable reaction product with protein sulfhydryls and can only be removed by chemical action (20). If inhibition of steroid binding by pHMB was due to formation of such a product at the binding site, inhibition would be noncompetitive. The Lineweaver-Burk analysis shows that the inhibition by pHMB is competitive and thus is not likely to be mediated by reactivity with sulfhydryl groups (Fig. 6). A final line of evidence against the involvement of sulfhydryl groups in the inhibition of steroid binding by metals comes from the relative inhibitory activity of the metals. Mercuric ion inhibits [^{3}H]-DHT binding with about the same activity as Zn^{2+}, Cu^{2+}, and Cd^{2+} (Table 1). Similar results were found by Kontula *et al.* (10) who found that Hg^{2+} exhibited inhibitory effects comparable to those of Cu^{2+} and Zn^{2+}. Yet the stability constants for mercuric-sulfhydryl binding are as much as 10^{10} times higher than the constants for Zn^{2+} or Cd^{2+} and sulfhydryls (19). Similarly, Pb^{2+} complexes with

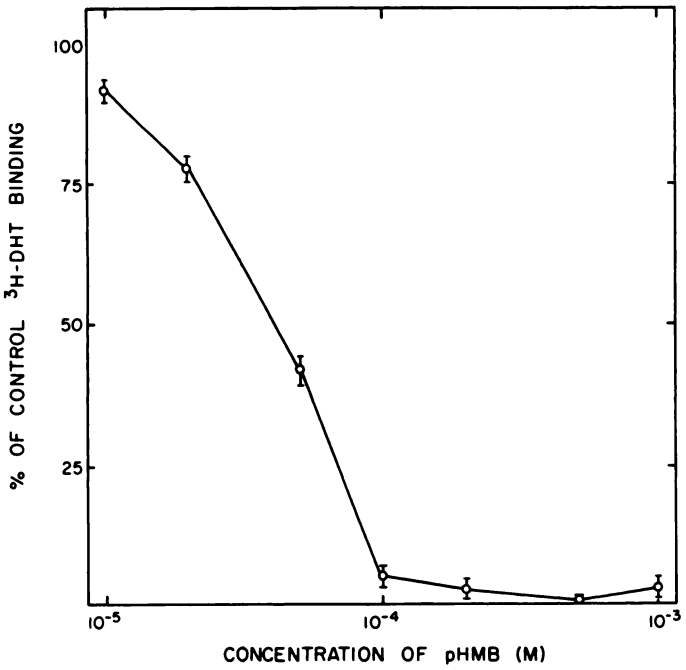

FIG. 5. *Inhibition by pHMB of specific binding of [³H]DHT in mouse prostate cytosol*

Conditions were as described in Fig. 1.

sulfhydryls are much more stable than Co^{2+} and Pb^{2+}, but all three metals are almost equal as inhibitors of binding (Table 1).

The large increase in nonspecific steroid binding resulting from the presence of Cu^{2+} might be due to conformational change induced in some protein by the cation. Such conformational change might create new steroid binding sites on the protein surface. Extensive conformational changes have been detected in several purified proteins as a result of exposure to Cu^{2+} and other ions (21).

Wallace and Grant (16) proposed a model wherein zinc acts as a feedback inhibitor of androgen action in the prostate and they showed that zinc was a competitive inhibitor of 5α-reductase. The present study points out another site at which zinc might function in their model, namely, the steroid-receptor interaction. Inhibition of this interaction could effectively block androgenic action and result in inhibition of secretion and cellular growth of the prostate. This mechanism might also account in part, for the reduction in prostate size observed by Saksena *et al.* (5) in response to administration of Cd^{2+} *in vivo*.

The possibility that zinc might function in a feedback loop in the prostate is further supported by examination of the endogenous levels of zinc in this organ. Various measurements of prostatic zinc content in several species average approximately 7 mmole/g dry wt (1, 3, 22, 23). Assuming that tissue water is 80% and that the zinc is all free and dissolved evenly throughout the tissue water, the concentration of zinc is 1.75 mM. Thus, if as little as 1% of the tissue Zn^{2+} is free, the IC_{50}, 2×10^{-5} M, is achieved. Furthermore, if the Zn^{2+} tends to be sequestered in the same water compartment as the receptor, this concentration can be achieved by an even smaller free/bound fraction.

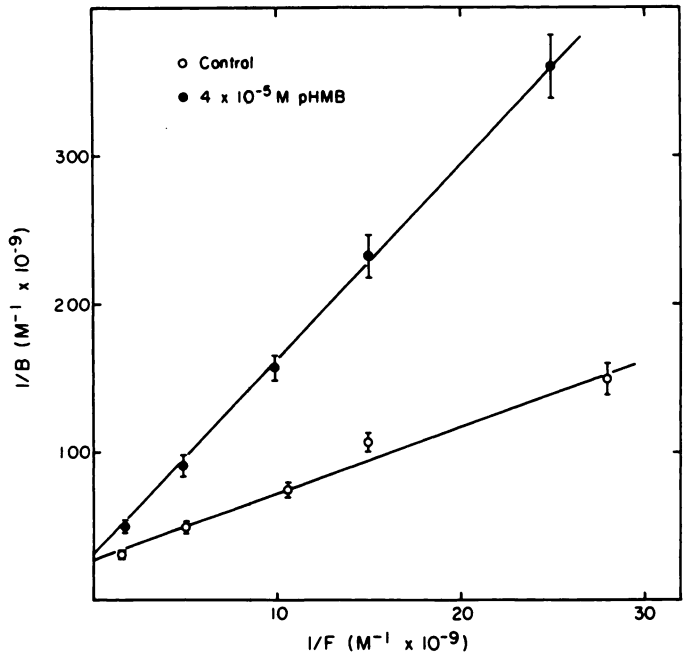

FIG. 6. *Double reciprocal plot of specific binding of [³H]DHT in mouse prostate cytosol in the absence and presence of 4×10^{-5} M pHMB*

Incubations and calculations were as described in Fig. 4. Each point represents the mean of duplicate determinations on each of four cytosol preparations.

REFERENCES

1. Mawson, C. A., and M. I. Fischer. Zinc content of the genital organs of the rat. *Nature (Lond.)* **167:** 859 (1951).
2. Millar, M. J., M. I. Fischer, P. V. Elcoate and C. A. Mawson. The effects of dietary zinc deficiency on the reproductive system of male rats. *Can. J. Biochem. Physiol.* **36:** 557–569 (1958).
3. Mackenzie, A. R., T. Hall, M.-C. Lo and W. F. Whitmore, Jr. Influence of castration and sex hormones on size histology and zinc content of canine prostate. *J. Urol.* **89:** 864–874 (1963).
4. Gunn, S. A., and T. C. Gould. The presence of an inherent reproductive cycle in the male laboratory rat. *J. Endocrinol.* **17:** 344–348 (1958).
5. Saksena, S. K., L. Dahlgren, I. F. Lau and M. C. Chang. Reproductive and endocrinological features of male rats after treatment with cadmium chloride. *Biol. Reprod.* **16:** 609–613 (1977).
6. Liao, S., and S. Fang. Receptor-proteins and androgens and the mode of action of androgens on gene transcription in ventral prostate. *Vitam. Horm.* **27:** 17–90 (1969).
7. Emanuel, M. B., and R. E. Oakey. Effect of Zn^{++} on the binding of oestradiol-17β to a uterine protein. *Nature (Lond.)* **223:** 66–67 (1969).
8. Sanborn, B. M., B. R. Rao, and S. G. Korenman. Interaction of 17β-estradiol and its specific uterine receptor. Evidence for complex kinetic and equilibrium behavior. *Biochemistry* **10:** 4955–4961 (1971).
9. Young, P. C. M., R. E. Cleary and W. D. Ragan. Effect of metal ions on the binding of 17β-estradiol to human endometrial cytosol. *Fertil. Steril.* **28:** 459–463 (1977).
10. Kontula, K., O. Jänne, T. Luukkainen and R. Vihko. Progesterone-binding protein in human myometrium. Influence of metal ions on binding. *J. Clin. Endocrinol. Metab.* **38:** 500–503 (1974).
11. Schein, L., M. P. Donovan and J. A. Thomas. Characterization of cytoplasmic binding of dihydrotestosterone by the prostate gland, seminal vesicles, kidney and liver of the mouse. *Toxicol. Appl. Pharmacol.* **44:** 147–153 (1978).
12. Binoux, M. A., and W. D. Odell. Use of dextran-coated charcoal to separate antibody-bound from free hormone: A critique. *J. Clin. Endocrinol. Metab.* **36:** 303–310 (1973).
13. Robinette, L., and M. G. Mawhinney. The influence of aging on androgen dynamics in the male rat. *Arch. Biochem. Biophys.* **191:** 503–516 (1979).
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–276 (1951).
15. Tamaya, T., Y. Nakata, Y. Ohno, S. Nioka, N. Furuta and H. Okada. The mechanism of action of the copper intrauterine device. *Fertil. Steril.* **27:** 767–772 (1976).

16. Wallace, A. M., and J. K. Grant. Effect of zinc on androgen metabolism in the human hyperplastic prostate. *Biochem. Soc. Trans.* **3:** 540–542 (1975).
17. Katz, S., M. P. Donovan and L. C. Roberson. Structure-volume relationships. Volume effects produced by copper (II) complexing with organic acids. *J. Phys. Chem.* **79:** 1930–1934 (1975).
18. Peters, T., Jr., and F. A. Blumenstock. Copper-binding properties of bovine serum albumin and its amino-terminal peptide fragment. *J. Biol. Chem.* **242:** 1574–1578 (1967).
19. Friedman, M. *The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins.* Pergamon, New York (1973).
20. Hellerman, L., F. P. Chinard and V. R. Deitz. Protein sulfhydryl groups and the reversible inactivation of the enzyme urease. *J. Biol. Chem.* **147:** 443–462 (1943).
21. Katz, S., and L. C. Roberson. Protein-metal ion interaction: Volume effects produced by the interaction of proteins with metal ions. *Bioinorg. Chem.* **6:** 143–154 (1976).
22. Moger, W. H., and I. I. Geschwind. The action of prolactin on the sex accessory glands of the male rat. *Proc. Soc. Exp. Biol. Med.* **141:** 1017–1021 (1972).
23. Habib, F. K., G. L. Hammond, I. R. Lee, J. B. Dawson, M. K. Mason, P. H. Smith and S. R. Stitch. Metal-androgen interrelationships in carcinoma and hyperplasia of the human prostate. *J. Endocrinol.* **71:** 133–141 (1976).

Send reprint requests to: John A. Thomas, Department of Pharmacology, West Virginia University, Morgantown, W. Va. 26506.